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High MDR and ESBL Producing *Escherichia coli* and *Klesbiella pneumoniae* from Urine, Pus and Sputum Samples

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was done to assess the prevalence of multidrug resistance and extended spectrum β-lactamase producing *E. coli*, *K. pneumoniae* in urine, pus and sputum.
Place and Duration of Study: This study was done to assess the prevalence of MDR and ESBL producing *E. coli* and *Klebsiella* in urine, pus and sputum from March 2013 to April 2014 at KIST Medical College, Lalitpur, kathmandu, Nepal.
Methodology: *E. coli* and *K. pneumoniae* were isolated from urine, pus and sputum samples in KIST Medical College, Lalitpur, Nepal. Antibiotic susceptibility test was performed by using disk diffusion method. MDR isolates which were suspected as ESBL producers were confirmed by using double disk synergy test and combined disk diffusion test for same isolates.
Results: Out of 580 urine samples, (87/580) 15% showed significant growth of *E. coli* and *K. pneumoniae* while in 97 pus and 124 sputum (16/221) 7% showed significant growth of *E. coli* and *K. pneumoniae*. From the sputum among 9 isolates, 3 were *E. coli* and 6 were *K. pneumoniae* whereas in pus among 7 isolates, 6 were *E. coli* and one was *K. pneumoniae*. Out of *E. coli* (77)

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isolates from urine, (74/77) 96.10% were MDR and of *K. pneumoniae* (10) isolates from urine 90% were MDR. Among *E. coli* (74) MDR isolates 52/74 (70.27%) were ESBL producers whereas all MDR *K. pneumoniae* isolates from urine were ESBL producers. All the isolates of *E. coli* and *K. pneumoniae* from pus and sputum were MDR which were resistant to tested third generation cephalosporins. Among the isolates *E. coli* (55.55%) and *K. pneumoniae* (42.85%) isolates were ESBL producers.

Conclusions: The high prevalence of MDR *E. coli* and *K. pneumoniae* was observed in urine, pus and sputum. The resistance pattern was alarmingly higher to all the antibiotics used except imipenem and amikacin. The prevalence of ESBL was higher so necessary step should be taken to prevent the spread and emergence of resistance.

Keywords: MDR; ESBL; E. coli; K. pneumoniae.

1. INTRODUCTION

From the discovery of antimicrobial agents, the rate of survival from infection is high, however, with introduction of new antibiotics; microorganisms learned to live in its presence and developed resistance [1]. The increase in resistance pattern of microbes is demanding new regimens for therapy. The antimicrobial resistance is one of the main problems in clinical as well as public health view points. Prolonged use of antibiotics, antibiotic selection pressure, overstay in hospitals, severe illness, unpredicted use of cephalosporins, use of intravenous devices or catheters are most important risk factors which increases the multi-drug resistance [2]. In developing countries, drug resistance to pathogenic bacteria is alarming due to indiscriminate use of antibiotics. Antimicrobial resistance is also increasing the healthcare cost as well as severity and deaths [3]. The ability of pathogens to survive in the presence of antibiotic that kills the counterparts is antimicrobial resistance. The antimicrobial resistance is not only increasing morbidity and mortality but also great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them [4].

Those bacteria, which showed resistance to at least one agent in three or more antimicrobial categories, are considered multidrug resistant bacteria. European Centre for Disease Prevention and Control (ECDC) and Centers for Disease Control and Prevention (CDC) have defined for Multidrug resistance (MDR), Extensively drug resistance (XDR) and pandrug resistance (PDR) to enhance the comparability of data comprehension of problem regarding drug resistance bacteria. They defined MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as susceptible to one or two categories and PDR as non-susceptible to all agents in all microbial categories [5].

β-lactamase are enzymes produced by microorganisms which hydrolyze the β-lactam ring of β-lactam antibiotics rendering them ineffective. β-lactam antibiotics include penicillins, cephalosporins, carbapenems and monobactams. β-lactamase are plasmid mediated as well as chromosomally mediated [6].

Extended spectrum β-lactamases (ESBLs) are those enzymes produced by bacteria which can hydrolyze oxyimino-β-lactams, that include cefotaxime, ceftriaxone, ceftazidime and aztreonam (but not the cephamycins and carbapenems) and are inhibited by clavulanic acid [7]. ESBLs are chromosomal or plasmid mediated β-lactamases which have mutated from the pre-existing broad specturm β-lactamases TEM-1, TEM-2 and SHV-3 [7]. The high prevalence of ESBL have been found in *E. coli*, *Klebsiella* spps, *Citrobacter* spps, *Enterobacter* spps, *Proteus* spps, and *Pseudomonas* spps [8]. Among the above mentioned bacteria, *E. coli* and *K. pneumoniae* were Screened for ESBL production and those which are resistant to third generation cephalosporins were considered as probable ESBL producers. The confirmation of ESBL production is done by double disk confirmation method and combine disk diffusion method [9].

Since, there are few publications on ESBL producing gram-negative organisms, thus the study was aimed to study the antibiotic susceptibility pattern, MDR and ESBL prevalence in *E. coli* and *K. pneumoniae* isolated from urine, pus and sputum.

2. MATERIALS AND METHODS

The study was approved by ethical review board of Nepal Health Research council (NHRC), and supported by NHRC Nepal. The study was conducted in Microbiology Laboratory of KIST Medical College, Lalitpur from March 2013-December 2014. Written informed consents were obtained from patients prior to their inclusion in study. A total of 801 samples including 580 urine, and 221 (97 pus and 124 sputum) were processed.

Urine, sputum and pus samples were collected from both inpatients and outpatients. Urine samples were inoculated aseptically on blood agar and MacConkey agar whereas pus and sputum was inoculated at blood agar, chocolate agar and Mac-Conkey agar at 37℃ for 24 hours. In urine bacteria (10^5 CFU/ml) were regarded as significant bacteriuria. The isolates from urine, pus and sputum were characterized by cultural, morphological characters, Gram's stain and biochemical tests Indole, Methyl red, Voges-Proskauer and citrate, TSI [Triple sugar iron], O/F [Oxidation/fermentation], and urease. The mucoid and smooth colonies were stained by using India ink for the presence of capsule [10].

2.1 Antimicrobial Susceptibility Tests

Antimicrobial susceptibility testing was performed on *E. coli* and *K. pneumoniae* following guidelines of Clinical and Laboratory Standard Institute 2012 [11]. The inoculums used for susceptibility testing was prepared in nutrient broth by touching colony of *E. coli* and *Klebsiella pneumoniae* that matched to 0.5 McFarland standard. Within 15 minutes, a sterile cotton swab was dipped into the inoculums suspension and pressed inside the wall of tube above the fluid level and inoculated at 60° over the dried surface of Muller-Hilton agar (MHA) plate. After 3-5 minutes antibiotic disc were applied and gently pressed down to ensure complete contact with agar. Organisms which showed resistance to at least one agent in three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [5,12]. The antibiotic disc used for urine were ampicillin (10 mcg/disc), cotrimoxazole (25 mcg/ disc), norfloxacin (10 mcg/disc), imipenem (10 mcg/disc), amikacin (30 mcg/disc), cefazoline (30 mcg/disc), nitrofurantoin-(300 mcg/disc), cefotaxime (30mcg/disc), ceftriaxone (30 mcg/disc), ceftazidime, gentamycin (10 µg), and nalidixic acid (30 mcg/disc). For pus and sputum ampicillin (10 mcg/disc), cotrimoxazole (25 mcg/disc), amikacin (30 mcg/disc, imipenum (10 mcg/disc), cefotaxime-(30 mcg/disc), ceftriaxone (30 mcg/disc), ceftazidime, gentamycin (10 µg), were used. Plates were incubated at 37℃ for 18-24 hrs and zone size in mm was measured which was compared with zone interpretive criteria of Clinical and Laboratory Standards Institute (CLSI). Isolates were identified as susceptible, resistant and intermediate. *Escherichia coli* strain ATCC 25922 and *K. pneumoniae* ATCC 62003 was used as control strains [13].

2.2 Screening of ESBL

Isolates resistant to 3rd generation cephalosporins: Ceftazidime, Cefotaxime and Ceftriaxone in antibiotic susceptibility test on MHA media were identified as possible ESBL producers [14,15]. ESBL productions by these isolates were confirmed by confirmatory tests following Clinical and Laboratory Standards Institute (CLSI).

2.3 Confirmatory Test for ESBL

The confirmation of ESBL production was done by two phenotypic confirmation methods.

2.3.1 Double disk diffusion approximation/synergy test (DDST)

Dried MHA plates were inoculated with 0.5 McFarland matched test microbial inoculums. On inoculated plate) amoxicillin-clavulanate (20 µg/10 µg was placed at center) and 30 µg of ceftazidime and cefotaxime were placed on either side of 30mm apart from center to center. Plates were incubated at 37℃ for 18-24 hrs. Those inoculums which exhibited an enhanced zone of inhibition (ZOI) in between Amoxacillin/clavulanic acid and ceftadizime and cefotaxime were identified as confirmed ESBL producers. In doubtful cases distance between amoxicillin-clavulanate and cefotaxime and ceftadizime were decreased to 20mm apart from center to center [16,17].

2.3.2 Combined disk method

On an inoculated MHA plate ceftazidime30 µg alone and in combination with clavulanic acid (30 µg/10 µg) and cefotaxime alone and with clavulanic acid (30 µg/10 µg) were placed. Plates were incubated at 37℃ for 18-24 hrs. An increase in zone of inhibition by ≥ 5 mm to

ceftazidime clavulanic acid with ceftazidime alone and cefotaxime clavulanic acid with cefotaxime alone was interpreted as confirmed ESBL producers [18]. The quality assurance was performed by using *K. pneumoniae* (ATCC) 700603 positive control and *E. coli* (ATCC) 25922 negative control.

2.4 Statistical Analysis

The chi-square was used for statistical analysis of data. A '*P*' value less than 0.05 was considered as statistically significant.

3. RESULTS

A total of 801 samples were collected in which 580 were urine, 97 were pus and 124 were sputum. These samples were tested for antibiotic susceptibility profile. Samples were collected from patients between 6 months to 90 years of both sexes. *E. coli* (77) and *K. pneumoniae* (10) were isolated from urine, whereas from pus and sputum (16) isolates of *E. coli* and *K. pneumoniae* were isolated. Among the urine isolates 85.5% (77/87) were *E. coli* and 11.5% (10/87) were *K. pneumoniae*. Most of the *E. coli* isolates from urine were resistant to ampicillin, Nalidaxic acid, cefazoline, and cotrimoxazole and used cephalosporins: cefotaxime (77.9%), ceftriaxone (71.4%) and ceftazidime (65.8%) indicating that these are possible ESBL producers (Table 1).

Similarly, most of the *K. pneumoniae* isolates were resistant to ampicillin (100%), cefazoline (70%), nitrofurantoin (70%), ofloxacin (60%), cotrimoxazole (40%), nalidixic acid (40%), cefotaxime (90%), ceftriaxone (90%) and ceftadizime (80%). Most sensitive was imipenem (90%) followed by amikacin (70%) Isolates resistant to third generation cephalosporins were suspected as possible ESBL producers (Table 2).

Out of 221 pus and sputum samples 16/221 (7.2%) showed growth of *E. coli* 9/16 (56.29%) and *K. pneumoniae* 7/16 (43.8). All *E. coli* were found 100% resistant to ampicillin, cefazoline, cefepime, cefotaxime, ceftadizime followed by ciprofloxacin (88.9%), and cotrimoxazole (88.9%), ceftriaxone (88.9%). Isolates resistant to used third generation cephalosporins were considered as possible ESBL producers (Table 3).

Among the (16) isolates of *E. coli* and *K. pneumoniae* from pus and sputum, (7) were found to be *K. pneumoniae* which showed following antibiotic susceptibility pattern (Refer Table 4).

Among the 87 total isolates from urine, 83/87 (95.4%) isolates were multi-drug resistance. *E. coli* (74/77) 96.1% *E. coli* and (9/10) 90% *K. pneumoniae* isolates were MDR (Table 5).

3.1 Confirmatory Test for ESBL

3.1.1 Double disk approximation test (DDST)

The Double disk diffusion synergistic test was performed by using amoxycillin-clavulanic acid, cefotaxime and ceftadizime. Among 83 isolates of MDR *E. coli* (74) and *K. pneumoniae* (9) from urine, 60 of the isolates showed perfect synergism, 18 samples did not showed synergism and 5 isolates gave doubtful result. Doubtful samples were repeated by reducing distance between disks to 20 mm. Among them only one showed ESBL production. Hence, 61 isolates showed ESBL production. (52/74) *E. coli* 52/74 (70.27%) and all MDR *K. pneumoniae* were ESBL producers. The non-MDR isolates were also tested for ESBL production however were found negative (Fig. 1).

3.1.2 Combined disk diffusion test in urine isolates

Among 83 MDR isolates, 61 of the isolates were found ESBL positive, of which 52 were *E. coli* and 9 were *K. pneumoniae*. Combined disk diffusion test was performed by using ceftadizime and ceftadizime/clavulanic acid, and cefotaxime, and cefotaxime/clavulanic acid individually. Among the total MDR isolates, 100% of the *K. pneumoniae*, and 70.27% (52/74) *E. coli* were ESBL Producers (Fig. 2).

The comparative results obtained by DDST and Combined disk diffusion test shows that combined disk diffusion test is better as there were no possibility of doubtful case as compared to DDST and experiment need not to be repeated.

Out of 221 samples of pus and sputum, 16 isolates were found of which 9 were *E. coli* and 7 were *K. pneumoniae*. Among 16 isolates of *E. coli* and *K. pneumoniae*, 50% (8/16) of the isolates were ESBL producers of these 55.6% (5/9) of *E. coli* and 42.9% (3/7) of *K. pneumoniae* were ESBL producers (Fig. 3).

Table 1. AST pattern of *E. coli* isolated from urine

Susceptibiliy rate (%)	Antibiotic susceptibility pattern % / (no)												
***E. coli* (77)**	**AMP**	**COT**	**NX**	**NA**	**CZ**	**NIT**	**IPM**	**AK**	**OF**	**CTX**	**CTR**	**CAZ**	**GEN**
Sensitive	1.3 (1)	29.9 (23)	66.2 (51)	11.7 (9)	10.4 (8)	42.9 (33)	96.1 (74)	92.2 (71)	33.8 (26)	22.1 (17)	28.6 (22)	34.2 (26)	57.1 (44)
Intermediate	-	-	-	1.3 (1)	2.6 (2)	13 (10)	-	-	-	-	-	-	2.6
Resistant	98.7 (76)	70.1 (54)	33.8 (26)	87 (67)	87 (65)	44.1 (34)	3.9 (3)	7.8 (6)	66.2 (51)	77.9 (60)	71.4 (55)	65.8 (51)	40.3 (31)

Note: *Amp:-Ampicillin, COT:-Cotrimoxazole, NX:-Norfloxacin, NA:-Nalidixic Acid, CZ:-Cefazoline, NIT:-Nitrofurantoin, IPM: - Imipenem, Ak:- Amikacin, OF:- Ofloxacin, CTX:-Cefotaxime, CTR:-Ceftriaxone, CAZ:-Ceftazidime, GEN:-Gentamycin.*

Table 2. AST pattern of *Klebsiella pneumoniae* isolated from urine

Susceptibiliy rate (%)	Antibiotic susceptibility pattern % / (no)												
***Klebsiella pneumoniae* (10)**	**AMP**	**COT**	**NX**	**NA**	**CZ**	**NIT**	**IPM**	**AK**	**OF**	**CTX**	**CTR**	**CAZ**	**GEN**
Sensitive	-	60 (6)	50 (5)	60 (6)	30 (3)	30 (3)	90 (9)	70 (7)	40 (4)	20 (2)	50 (5)	40 (4)	60 (6)
Intermediate	-	-	-	-	-	-	-	-	-	-	-	-	-
Resistant	100 (10)	40 (4)	50 (5)	40 (4)	70 (7)	70 (7)	10 (1)	30 (3)	60 (6)	90 (9)	90 (9)	80 (8)	40 (4)

Table 3. AST pattern of *E. coli* isolated from pus and sputum

***E. coli* (9)**	Antibiotic susceptibility pattern (%) / no.										
	AMP	**CIP**	**COT**	**CZ**	**CPM**	**IPM**	**Ak**	**CTX**	**CTR**	**CAZ**	**GEN**
Sensitive	-	11.1 (1)	11.1 (1)	-	-	88.9	88.9	-	11.1 (1)	-	55.5 (5)
Intermediate	-	-	-	-	-	-	-	-	-	-	11.1 (1)
Resistant	100 (9)	88.9 (8)	88.9 (8)	100 (9)	100 (9)	11.1 (1)	11.1 (1)	100 (9)	88.9 (8)	100 (9)	33.3 (3)

Table 4. AST pattern of *K. pneumoniae* isolated from pus and sputum

***K. pneumoniae* (7)**	Antibiotic susceptibility pattern (%)										
	Amp	**CIP**	**COT**	**CZ**	**CPM**	**IPM**	**Ak**	**CTX**	**CTR**	**CAZ**	**GEN**
Sensitive	-	14.3	14.3	-	-	28.2	28.2	-	-	-	57
Intermediate	-	-	-	-	-	-	-	-	-	-	-
Resistant	100	85.7	85.7	100	100	71.8	71.8	100	100	100	43

Table 5. Distribution of MDR *E. coli* and *K. pneumoniae* isolated from urine

Organisms	Total isolates	MDR in urine	MDR in percentage (%)	Among MDR percentage (%)
E. coli	77	74	96.10%	89.2 (74/83)
K. pneumoniae	10	9	90%	10.81 (9/83)
Total	87	83		

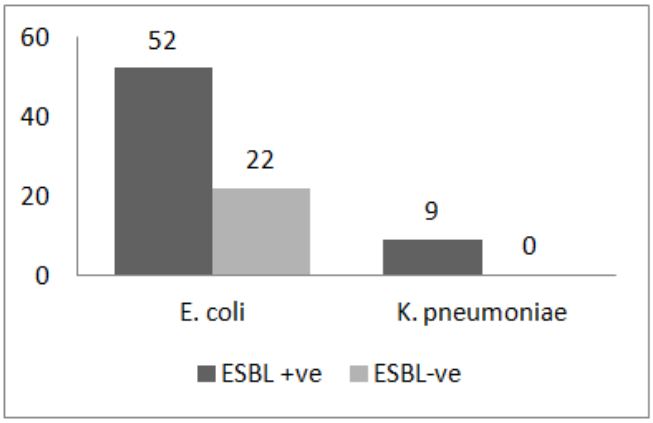

Fig. 1. ESBL isolates among *E. coli* and *K. pneumoniae* isolates from urine

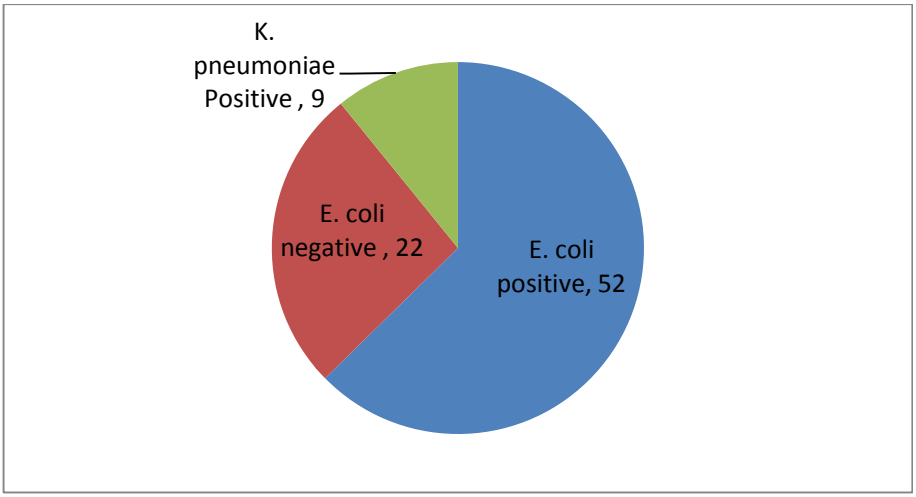

Fig. 2. ESBL pattern among MDR isolates isolated from urine

In combined disk diffusion test performed by using Ceftadizime and Ceftadizime/clavulanic acid, and Cefotaxime, with Cefotaxime/clavulanic acid. Same result was obtained as that of DDST (Fig. 4).

4. DISCUSSION

The present study provides the information about situation of highly diverse antibiotic resistance pattern of *E. coli* and *K. pneumoniae* pathogens isolated from urine, pus and sputum from KIST Medical college, Nepal. *E. coli* and *K. pneumoniae* are the most common organisms encountered in clinical medicine causing wide range of diseases from mild to serious, sometime life threatening condition up to death [2,3]. All age groups male and female were tested for antibiotic susceptibility. *E. coli* and *K. pneumoniae* showed great extent of

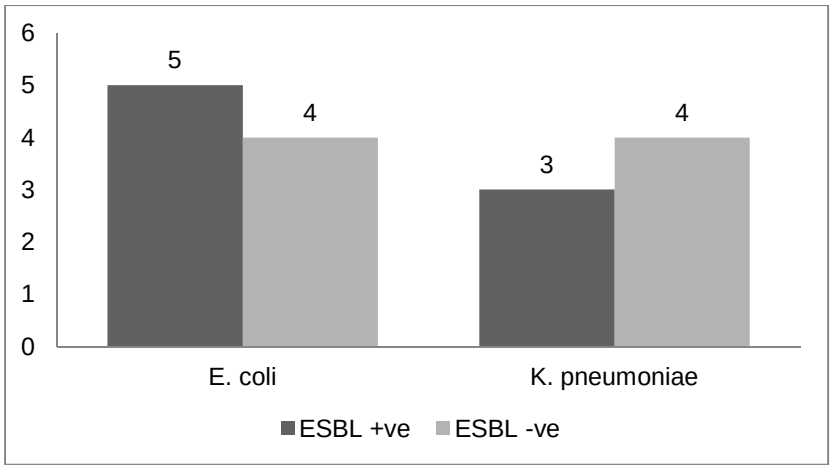

Fig. 3. Synergism test by DDST of *E. coli* and *K. pneumoniae* isolated from pus and sputum

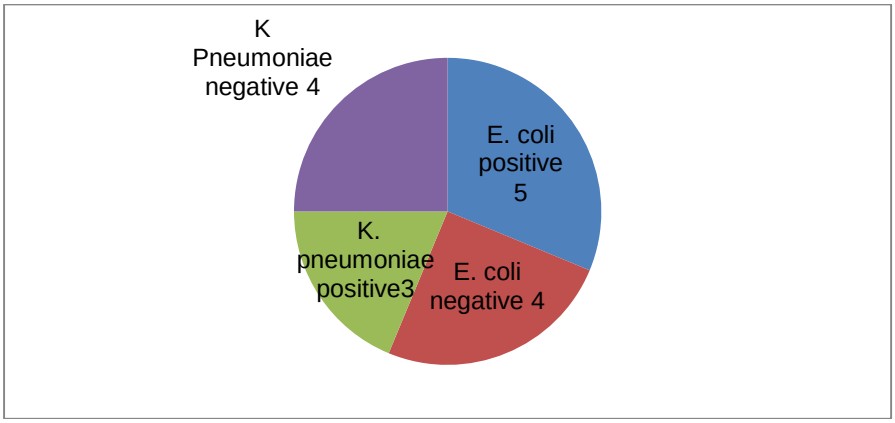

Fig. 4. Combined disk diffusion test of *E. coli* and *K. pneumoniae* from pus and sputum

resistance to ampicillin, nalidixic acid, fluroquinolones, cotrimoxazole and third generation cephalosporins. The MDR occurrence was higher as compared to that of previous report in Nepal. This study showed that 96.10% of the isolated *E. coli* from urine was MDR whereas 90% of the *K. pneumoniae* were MDR. Those *E. coli* and *K. pneumoniae* isolated from pus and sputum were 100% MDR showing their higher resistance pattern compared to that of urine. In a similar study conducted in Kathmandu Model Hospital, Nepal *E. coli* (81.25%) isolates were MDR [19].

Many factors may have contributed to high rates of resistance such as misuse of antibiotics by health care professionals or non-skilled practitioners, misuse of antibiotics by the general public and inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing [2]. The Extended-spectrum β-lactamases are one of the most alarming groups of β-lactamases in clinical practice. The prevalence of ESBL producing *E. coli* and *Klebsiella* spps varies from country to country and even among two different institutions in the same country and that continuously changes over time [2,3,19]. The data obtained on this ESBL study do not match with other data published in Nepal and other countries except a few. In this study, ESBL producing *E. coli* and *K. pneumoniae* was phenotypically characterized. *E. coli* showed 77.9% resistance to cefotaxime, 71.4% to ceftriaxone and 65.8% to ceftadizime from urine. Similarly, *K. pneumoniae* isolated from urine showed 90% resistance to cefotaxime, 90% to ceftriaxone and 80% to ceftadizime respectively. Similarly in case of sputum and pus *E. coli* showed 100% resistance to cefotaxime, 88.9% to ceftriaxone and 100% to ceftazidime. The resistance of *K. pneumoniae* was 100% to the used third generation

cephalosporins. Upon repetition of antibiotic susceptibility test *E. coli* and *K. pneumoniae* from urine, pus and sputum, the susceptibility pattern to cefotaxime, ceftriaxone and ceftadizime remained same with slight variation in some cases. The antibiotic resistance pattern of pus and sputum isolates of *E. coli* and *K. pneumoniae* was found higher as compared to urine isolates. In the present study (52/77) 67.53% of the *E. coli* isolates among total *E. coli* isolates from urine were found ESBL positive. Among the total MDR *E. coli,* 70.27% of *E. coli* were ESBL producers. Among the total *K. pneumoniae* isolated from urine 90% of the isolates were MDR in which all were ESBL producers. In case *of K. pneumoniae* all MDR isolates were ESBL producers which indicates that *K. pneumoniae* in future can be an organism of problem during treatment. In a similar study conducted in Sudan showed that 92.2% *E. coli* isolates were MDR and 32.7% were ESBL producers [20]. In the similar study conducted in China, all *K. pneumoniae* isolated were MDR among which 89.5% were ESBL producers [21]. Similar, type of study was conducted by Omar et al. [22] in which 65% of the *E. coli* isolates and 68.8% of the *K. pneumoniae* isolates were ESBL producers.

In this study, isolates from pus and sputum showed high resistance to used antibiotics. They were resistant to all used third generation cephalosporins but the prevalence of ESBL was low. Only 55.55% of *E. coli* isolates were ESBL producers whereas 42.85% *K. pneumoniae* were ESBL producers. Similar result has been reported in 2005 in Institute of Medicine and Teaching hospital where 48% of the isolates were ESBL positive [23]. Furthermore, in the study conducted in Kathmandu Model hospital 55% of the *E. coli* isolates were ESBL positive [24]. In this research both DDST and combined disk diffusion test was used. It has been stated that the sensitivity and specificity of DDST is upto 97% and 100% if cefodoxime is used as oxyimino-cephalosporins. Sensitivity and specificity of combined disk diffusion method has been claimed to be 96% and 100% [16,25,26]. Doubtful synergism result in DDST was again repeated by reducing the distance which was tedious than combined disk diffusion test although both were effective in diagnosing the ESBL production [18].

The most effective antibiotics found during this study were imipenem and amikacin. *E. coli* isolated from urine was 96.1% sensitive to imipenem whereas 92.2% sensitive to amikacin. In urine isolates, 90% *K. pneumoniae* were sensitive to imipenem, 70% to amikacin and 60% to gentamycin. In case of pus and sputum 88.9% *E. coli* isolates were sensitive to imipenem and amikacin whereas only 28.2% *of K. pneumoniae isolated from* pus and sputum isolates were susceptible. Similar study conducted in India showed that 95.3% *E. coli* isolates from urine, blood, pus and sputum were sensitive to imipenem and 27.23% to amikacin whereas 97.46% of *K. pneumoniae* were sensitive to imipenem and 34.78% to amikacin [27].

In this study, the majority of the bacteria producing ESBL were resistant to common antibiotics used to treat UTI, pulmonary infections and wound infections. The indiscriminate use of antibiotics is leading the world toward great health problem, morbidity, mortality, treatment failure and great economic loss [2,3,4]. The overuse of antibiotics, random use of antibiotics as heat and trial methods by clinicians without proper sensitivity test, unawareness of people about emergence of antibiotics, random use of antibiotics without advice of physicians, prolonged intensive care unit (ICU) stay, nursing home residency, severe illness, use of instrumentation or catheterization etc are the major causes of drug resistance in our country. Thus, by reducing the inappropriate antibiotic use is the best way to control resistance.

Hence, microbiology laboratory plays a key role in the decision to choose a particular antimicrobial agent. Once the bacteria causing the disease have been identified, the rational choice of the class of antibiotics can be used. Therefore, continuous increasing antimicrobial resistance pattern can be regulated by continuous updated data on antimicrobial susceptibility profile through which provision of safe and empirical therapies can be ensured.

5. CONCLUSION

Based on the findings of this study, it can be concluded that

- Prevalence of ESBL producing *E. coli* and *K. pneumoniae* in urine, pus and sputum is increasing.
- Urine sample had a higher prevalence of ESBL as compared to pus and sputum although screening showed higher in pus and sputum.

- Imipenem is choice of antibiotic followed by amikacin in urine and gentamycin in pus and sputum.

CONSENT AND ETHICAL APPROVAL

This research was approved by Nepal Health Research Council ethical review board Nepal. Written consent was taken from the patients during this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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