Original article

Multidrug resistance and extended spectrum betalactamase producing strains causing lower respiratory tract and urinary tract infection

B. M. Pokhrel, J. Koirala, S. K. Mishra, R. K. Dahal, P. Khadga N. R. Tuladhar

T.U. Teaching Hospital, Maharajgunj, Kathmandu, Nepal, Southern Illinois University, School of Medicine, Springfield, USA

Correspondence to: Prof. Dr. Bharat Mani Pokhrel, Head of Microbiology Department, TU Teaching Hospital and Maharajgunj Campus, Kathmandu, Nepal

e-mail: bmp268@hotmail.com

Background: Multidrug resistance (MDR) and Extended spectrum beta-lactamase (ESBL) producing strains are becoming world-wide problem. With a view to determine prevalence of MDR and ESBL strains causing lower respiratory tract infection (LRTI) and urinary tract infection (UTI), a pilot study was conducted in Microbiology Laboratory of TUTH from April-September 2004.

Material and Methods: Sputum and urine samples were collected, cultured and the bacterial isolates were identified with the use of standard method as described by American Society for Microbiology. These bacterial isolates were then subjected for antibiotics-sensitivity test with the use of disc diffusion method as described by Kirby Bauer. ESBL production was tested as described by National Committee for Clinical Laboratory Standards (NCCLS).

Results: Of the total bacterial isolates from sputum samples, 47.57% were MDR. The ESBL producing isolates were 24.27%. In case of urinary isolates, 60.40% and 16.00%. were found to be MDR and ESBL respectively.

Conclusion: These strains should be subjected for genetic study to acquire their detail genetic make-up to characterize the mechanism of drug resistance. This type of study should be continued throughout the year in order to acquire exact status of MDR and ESBL in Nepal.

Key words: MDR, ESBL, LRTI, UTI

Introduction

In modern medical practice, newer antimicrobial drugs have been used extensively resulting in emergence and rapid dissemination of resistant bacterial strains[1]. Since one of the mechanisms of bacterial resistance to ?-lactam antibiotics is the production of ?-lactamase enzyme that breaks down the structural ?-lactam ring of penicillin and its synthetic derivatives[1], the property of stability to many bacterial ?-lactamase was increased with the later generations of cephalosporins. However, the persistent exposure of bacterial strains to a multitude of ?-lactams has induced a dynamic and continuous production and mutation of ?-lactamase in many bacteria, expanding their activity even against the third and fourth generation Cephalosporins. These new ?-lactamases are called *extended spectrum beta lactamases* (*ESBL*s)[2] which were first reported in 1983 in Germany[3,4,5].

The ESBL enzymes are mutant, plasmid-mediated beta lactamases derived from older, broad-spectrum ?-lactamases (e.g., TEM-1, TEM-2, SHV-1)[5]. Thus, they mediate resistance to extended spectrum (third generation) cephalosporins (e.g.

ceftazidime, cefotaxime, ceftriaxone), i.e., they are specific to third and fourth generation cephalosporins but not to cephamycins (e.g. cefoxitin and cefotetan) or carbapenems (e.g. meropenem or imipenum). These enzymes are most commonly produced by *Klebsiella* spp and *Escherichia coli* but may also occur in other gram negative bacteria, including *Enterobacter, Salmonella, Proteus, Citrobacter, Morganella morganii, Serratia marcescens, Shigella dysenteriae, Pseudomonas aeruginosa, Burkholderia cepacia, Capnocytophaga ochracea.* [5,6,7]

The infection due to ESBL- producing organisms can cause the failure of treatment if one of the above classes of drugs is used. Besides, ESBL producing bacteria are typically associated with multidrug resistance (MDR)[2]. Antibacterial choice is often complicated by multi-resistance[8,9]. Thus infection due to ESBL-producing bacteria can result in avoidable failure of treatment and increased cost in patients who have received inappropriate antibiotic treatment[1]. Colonization and infection with these bacteria have also been associated with indiscriminate use of antibiotics, prolonged hospitalizations, increasing numbers of immunocompromised patients, and medical progress resulting in increased use of invasive procedures and devices.[10,11]. Updated knowledge of the susceptibility pattern of bacteria is important for the proper selection and use of antimicrobial drugs and for the development of an appropriate prescribing policy[12]. Without gathering the information about the existing MDR strains, we cannot reduce the morbidity and mortality due to infections caused by MDR pathogens; reduce the rate of emergence and spread of antimicrobial resistance[13].

Lower respiratory tract infection (LRTI) and the urinary tract infection (UTI) are the commonest domiciliary and nosocomial infections caused by different organisms, therefore the study on the prevalence of MDR and ESBL producing strains causing clinical infections is of utmost importance.

Material and methods

Hospital setting: This study was carried out in Tribhuvan University Teaching Hospital, which is a referral center with 450 beds, located in Kathmandu, the capital city of Nepal.

Bacterial isolates: A total of 338 sputum samples and 1766 urine samples were investigated during 5 months period beginning from April to September to find out the prevalence of MDR and ESBL-producing isolates from in-patients and out-patients, clinically suspected of LRTI or UTI. Duplicate isolates from the same patient were excluded in the study[14,15]. The methods for the collection, isolation and identification were followed as described by American Society for Microbiology.

Collection and Culture of urine samples: Midstream urine was collected from a patient with full aseptic precautions and the sample was processed within half an hour. Culture of each uncentrifuged urine sample was done by semiquantitative method on 5% Blood agar (BA) and Mac Conkey agar (MA) (Oxoid, UK) plates. An inoculating loop of standard dimension was used to take up approximately fixed and a known volume (0.001ml) of mixed uncentrifuged urine for inoculation. After incubating the plates aerobically (Yamato Model IL-81, Japan) at 37^0C for 24 hours, colonies were counted[16]. Samples showing ? 10^5 colony forming unit (CFU) per milliliter (ml) was taken as significant. Low count significant bacteriuria was also taken into consideration if there was any indication which could lower the concentration of bacteria in the urine, e.g., patient under treatment, patient with certain endocrine disorder such as diabetes, chronic kidney disease where concentrating power of kidney is low, obstruction in the ureter due to tumor or stone, etc. Identification of significant isolates were done by using standard microbiological techniques which involved morphological appearance of the colonies, Gram's staining reactions, catalase test, oxidase test with other biochemical properties and also serotyping if required in specific cases[17,18,19]. The biochemical media employed were Triple Sugar Iron agar (TSI), Sulphide Indole Motility (SIM) media, Simmon's citrate media and Christensen's urease media (Oxoid, UK).

Collection and Culture of sputum samples: Early morning expectorated sputum sample was used for the culture. The sputum samples were first digested with commercially available sputalysin containing 0.01% Dithiothreitol, and then it was microscopically evaluated for its real representation of LRT as described by the American Society for Microbiology. The samples not satisfying the criteria were rejected while those satisfying the criteria were subjected for culture. The accepted samples showed WBCs > 25 per low power field (LPF) and epithelial cells <10/LPF. The digested sputum samples were cultured in Chocolate agar (CA), BA and MA. On the CA, optochin disk (5 µg) and bacitracin disk (10 Unit) (Oxoid, UK) were placed at primary and secondary inoculation to screen *Streptococcus pneumoniae* and *Haemophilus influenzae* respectively. The CA plate was incubated in CO_2 incubator (10% CO_2) at 37^0C for 24 hours while BA and MA plates were incubated at 37^0C for 24 hours in aerobic atmosphere. Significant bacterial isolates were identified by standard laboratory methods as described above[20].

Antimicrobial susceptibility testing: The antibiotic sensitivity tests of the pathogens isolated from the clinical

specimen against different antibiotics were determined by Kirby-Bauer method[21] of disk diffusion technique as recommended by NCCLS using Mueller Hinton agar (MHA) (Oxoid, UK). At least three to five well isolated colonies of the same morphological types were selected from the MHA plate. The base of each colony was touched with a inoculating wire and the growth was transferred into a tube containing 5ml of nutrient broth and was incubated at 37°C (usually 2 to 6 hours) until it achieved the McFarland tube number 0.5. In case overgrowth, the broth was diluted with sterile physiological saline to match with McFarland tube number 0.5. For testing the fastidious organisms, *Haemophilus* spp, *Streptococci*, direct colony suspension method was employed by making a direct broth or saline suspension of isolated colonies selected from a 16 to 24 hour growth on culture plate. The suspension was then adjusted to match with McFarland tube number 0.5. A sterile cotton swab was dipped into the broth and the swab was rotated several times and pressed firmly on the inner side wall of the tube above the fluid level to remove excess inoculum from the swab. Then the dried surface of a MHA plate was inoculated by streaking the swab over the entire agar surface three times, turning the plate 60° between streaking. In case of H. influenzae, CA was used to do sensitivity test. Finally the inoculum was left to dry for a few minutes at room temperature with the lid closed. The predetermined battery of antimicrobial disks was placed on the surface of the prior inoculated agar plate such that there was 25mm distance from disk to disk. The disks were pressed down to ensure complete contact with the agar surface[22]. For about 15 minutes of applying the disks, the plates were left at room temperature to allow antimicrobials to diffuse from the disk. Then they were incubated aerobically at 37°C overnight. After overnight incubation, the diameter of zone of inhibition (ZOI) of each disk was measured (including the diameter of the disk) and recorded in millimeter. It is then compared with Standard Chart developed by Kirby-Bauer to determine bacterial susceptibility towards different antimicrobial agents in terms of 'sensitive', 'resistant' and 'moderately sensitive (intermediate)'. The measurements were made with a ruler on the under surface of the plate without opening the lid[21]. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853 were also tested, in every set of experiment, in parallel, as a part of quality control[13].

Tests for ESBL-production in Gram negative isolates: The initial screen test for the production of ESBL was performed by using both ceftazidime (CAZ) (30µg) and cetotaxime (CTX) (30µg) disks (Oxoid, UK). If the zone of inhibition was ? 22mm for CAZ and/or ? 27mm for CTX, the isolate was considered a potential ESBL-producer as recommended by NCCLS. Isolates those were suspected as ESBL-producer by screen test were tested further by double-disk synergy test (DDST). In DDST method[2], amoxicillin-clavulanic acid (AMC) disk (20/10µg) was placed at the center and disks containing the 30µg of CAZ and CTX were placed separately beside 15mm distance (edge to edge), away from the central disk, in a horizontal manner,[22,23]. Any enhancement of the ZOI between the disks (either of the cephalosporin disks and clavulanate containing disk) indicated the presence of ESBL[1]. Isolates with this pattern were recorded as DD positive[22]. Combination disk method[2] was also used for the confirmation of ESBL-producing strains in which CTX and CAZ (30µg), alone and in combination with clavulanic acid (CA) (10µg) were used (Becton Dickinson, USA). An increase ZOI of 5mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirmed ESBL[24] (Photograph no. 2,3,4). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls respectively.

Results

LRTI Cases

Out of total 338 sputum samples, 30.47% showed significant bacterial growth, of which 47.57 % and 24.27% were MDR and ESBL producer respectively *(Table 1)*

Table 1: Distribution Pattern of Positive & Negative Cases from sputum samples

Significant positive culture	103/338 (30.47%)
Gram negative isolates	94/103 (91.26%)
Gram positive isolates	9/103 (8.74%)
Total MDR isolates	49/103 (47.57%)
Total ESBL producer	25/103 (24.27%)

Among total gram negative isolates, *Haemophilus influenzae*(31.07%) was the commonest isolate followed by *Pseudomonas (Fig.1).*

Fig. 1: Total Gram negative bacterial isolates from sputum samples

Majority of *Staphylococcus aureus* were MDR *(Table 2).*

Table 2: Total Gram positive bacterial isolates from sputum samples

Isolates	Number	MDR
Staphylococcus aureus	5 (4.85%)	3
Streptococcus pneumoniae	4(3.88%)	0

Among the Gram negative isolates, the highest number of MDR and ESBL producer was seen in *Klebsiella* and *Pseudomonas (Fig. 2).*

For testing ESBL producing strain, Combination disk method was found to be superior to Double disk synergy test *(Table 3).*

Table 3: Comparison of methods for the phenotypic confirmation of ESBL strains

No of suspected ESBL cases	Double Disk Synergy Test (AMC / CAZ)	Combination Disk Method (CAZ / CAZ+CA)	True ESBL
33	19	25	25

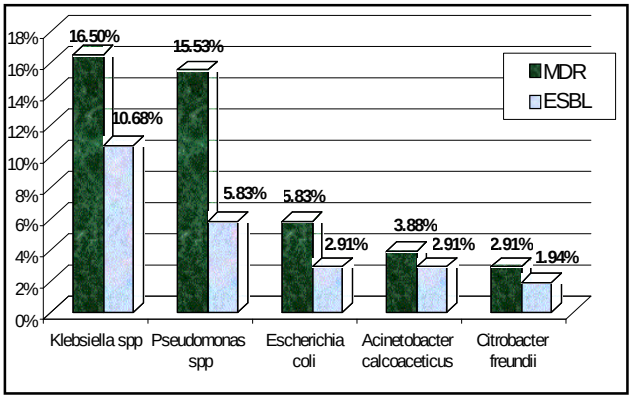

Fig 2: MDR versus ESBL Strain

UTI Cases

Amikacin (35.29%) was found to be effective to MDR *Klebsiella* spp followed by Ciprofloxacin and Ceftazidime*(Table 4).*

MDR *Pseudomonas* was found to be sensitive to Amikacin (56.25%) followed by Ceftazidime *(Table 5).*

Table 4: Antibiotic Sensitivity Profile of MDR *Klebsiella* spp.

Antibiotics	No. of isolates on which each antibiotic was used	*Klebsiella* spp. (N=17) S	R	I
Ampicillin	17	0 (0.0%)	17 (100.0%)	-
Ciprofloxacin	17	4(23.53%)	13 (76.47%)	-
Cotrimoxazole	14	2 (14.29%)	12 (85.71%)	-
Cephalexin	17	0 (0.0%)	17(100.0%)	-
Gentamicin	16	1(6.25%)	14 (87.5.0%)	1 (6.25%)
Amikacin	17	6 (35.29%)	9 (52.94%)	2 (11.76%)
Ceftazidime	17	4 (23.53%)	13 (76.47%)	-

Table 5: Antibiotic Sensitivity Profile of MDR *Pseudomonas* spp.

Antibiotics	No. of isolates on which each antibiotic was used	Pseudomonas spp (N=16) S	R	I
Amikacin	16	9 (56.25%)	5 (31.25%)	2 (12.50%)
Carbenicillin	10	3 (30.00%)	7 (70.00%)	-
Ciprofloxacin	16	5 (31.25%)	11 (68.75%)	-
Gentamicin	14	4 (28.57%)	10 (71.43%)	-
Ceftazidime	16	7 (43.75%)	8 (50.00%)	1(6.25%)
Ceftriaxone	12	3 (25.00%)	9 (75.00%)	-

Out of total 1766 urine samples, 28.31% showed significant bacteriuria, of which 60.40 % and 16.00% were MDR and ESBL producer respectively *(Table 6)*.

Table 6: Distribution Pattern of Positive & Negative Cases from urine samples

Significant bacteriuria	500/1766 (28.31%)
Gram negative isolates	436/500 (87.20%)
Gram positive isolates	64/500 (12.80%)
Total MDR isolates	302/500 (60.40%)
Total ESBL producer	80/500 (16.00%)

Escherichia coli (64.6%) was found to be the most common bacterial isolate to cause UTI followed by *Klebsiella*. *(Fig. 3)*

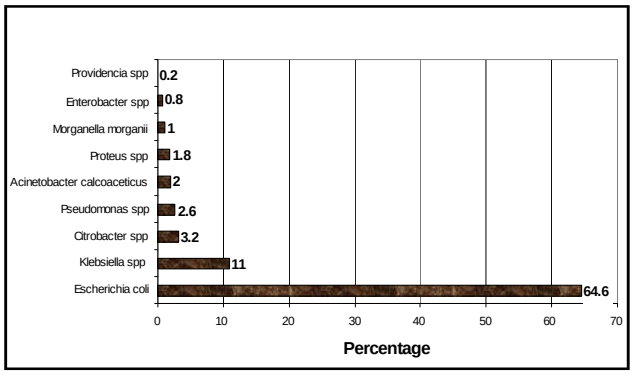

Fig. 3: Total Gram negative bacterial isolates from urine samples

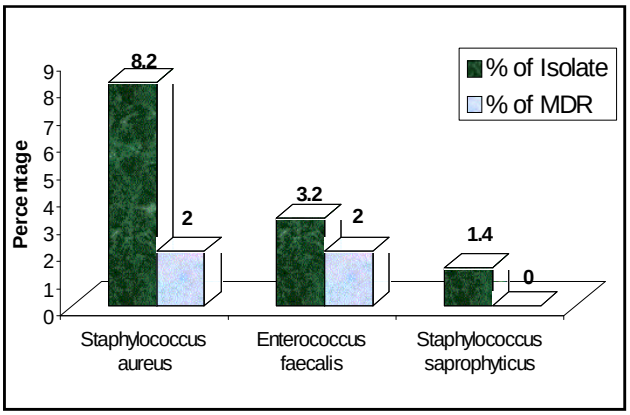

Fig. 4: Gram positive bacterial isolates from urine samples

Among the Gram negative isolates those causing UTI, the highest number of MDR and ESBL producer was seen in *Escherichia coli* and *Klebsiella (Table 7, Fig. 5).*

Table 7: MDR versus ESBL Strain

Isolates	MDR	ESBL
Escherichia coli	199(39.80%)	58 (11.60%)
Klebsiella spp	48 (9.60%)	14 (2.80%)
Citrobacter freundii	11 (2.20%)	5 (1.00%)
Pseudomonas spp	7 (1.40%)	1 (0.20%)
Acinetobacter calcoaceticus	6 (1.20%)	1 (0.20%)
Enterobacter spp	4 (0.80%)	1 (0.20%)
Proteus spp	4 (0.80%)	0 (0.00%)
Morganella morganii	3 (0.60%)	0 (0.00%)

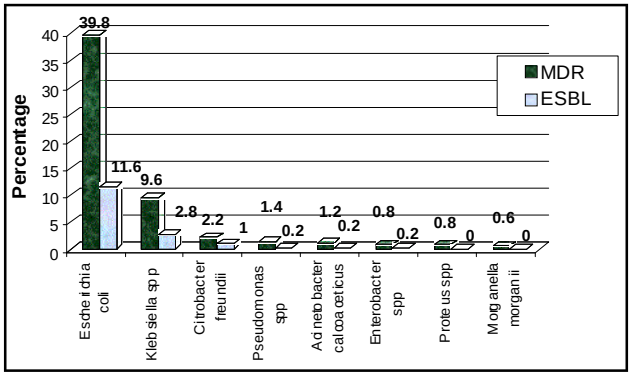

Fig. 5: MDR versus ESBL Strain

For testing ESBL producing strain, Combination disk method was found to be superior to Double disk synergy test *(Table 8).*

Table 8: Comparison of methods for the phenotypic confirmation of ESBL strains

No of suspected ESBL cases	Double Disk Synergy Test (AMC / CAZ)	Combination Disk Method (CAZ / CAZ+CA)	True ESBL
112	75	80	80

Nitrofurantoin (68.63%) was found to be effective to MDR *Escherichia coli* followed by Ceftazidime *(Table 9).*

Shows that Ceftazidime (64.58%) was found to be effective to MDR *Klebsiella* spp followed by Gentamicin *(Table 10).*

Discussion

The genes that code for production of ESBL are often linked to other resistance genes[15] causing extended spectrum of drug resistance[1]. In this study too, all ESBL strains were found only in MDR cases.

Table 9: Antibiotic Sensitivity Profile of MDR *E.coli*

Antibiotics	No. of isolates on which each antibiotic was used	*E.coli* (N=199) S	R	I
Ampicillin	199	12 (6.03%)	185 (92.96%)	2 (1.01%)
Ciprofloxacin	198	55 (27.78%)	143 (72.22%)	-
Cotrimoxazole	164	32 (19.51%)	130 (79.27%)	2 (1.22%)
Cephalexin	175	40 (22.86%)	126 (72.00%)	9 (5.14%)
Norfloxacin	186	53(28.49%)	131 (70.43%)	2 (1.08%)
Nitrofurantoin	51	35 (68.63%)	16 (31.37%)	-
Ceftazidime	199	132 (66.33%)	63 (31.66%)	4 (2.01%)
Gentamicin	151	79 (52.32%)	67 (44.37%)	5 (3.31%)

Table 10: Antibiotic Sensitivity Profile of MDR *Klebsiella* spp.

Antibiotics	No. of isolates onwhich each antibiotic was used	*Klebsiella* spp (N=48) S	R	I
Ampicillin	48	0 (0.00%)	48 (100.00%)	-
Ciprofloxacin	46	12 (26.09%)	34 (73.91%)	-
Cotrimoxazole	36	6 (16.67%)	30 (83.33%)	-
Cephalexin	45	13 (28.89%)	32 (71.11%)	-
Norfloxacin	44	12 (27.27%)	32 (72.73%)	-
Nitrofurantoin	22	6 (27.27%)	16 (72.73%)	-
Ceftazidime	48	31 (64.58%)	17 (35.42%)	-
Gentamicin	39	15 (38.46%)	21 (53.85%)	3 (7.69%)

MDR and ESBL strains from LRTI isolates

In this study, with regards to LRTI, 30.47% sputum samples showed significant growth; out of which 47.57% were MDR which included *Klebsiella* spp (16.50%), *Pseudomonas* spp (15.53%) and others were *E. coli*, *Acinetobacter calcoaceticus*, *S. aureus* and *Citrobacter* spp. Besides, 24.27% isolates were found to be ESBL-producing with *Klebsiella* spp being the predominant one (10.68%). This result is similar to that by Long J et al who showed that 20.1% of the isolated strains in patients with LRTI were ESBL-producing with *Klebsiella* spp being the chart topper[26].

MDR and ESBL strains from UTI isolates

Out of total 1766 urine samples, 28.31% showed significant bacteriuria. Out of total isolates, 60.40% were MDR which included *E. coli* (39.80%), *Klebsiella* spp (9.60%) and also *Citrobacter* spp, *S. aureus, Enterococcus faecalis*, *Pseudomonas* spp, *A. calcoaceticus, Proteus* spp and *Morganella morganii*. Likewise, 16.00% isolates were found to be ESBL-producing with *E. coli* being the predominant one (11.60%). Much lower prevalence (2.70%) of ESBL-producers was seen in *E. coli* isolates from urine samples in a multicentric study by Smaoui H et al[27]. On the contrary to 15.80% in our study, much higher prevalence (58%) of ESBL-producers in urinary isolates of Gram negative bacilli was observed in India by Mathur et al[22,28]. Ampicillin had the lowest activity against MDR *E. coli* isolates with resistance rate of 92.96%. Nitrofurantoin showed low-level resistance to multidrug-resistant urinary isolates as compared to other drugs which is consistent with Iqbal M et al[29]. In the case of MDR *Klebsiella* spp, ceftazidime was found to be the most sensitive which showed sensitivity rate of 64.58% and this result is similar to that of surveillance test done in Brooklyn, New York where 62.00% of *Klebsiella* spp were found susceptible to ceftazidime[30].

Worldwide pattern of ESBL

The prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution[31]. These differences may be due to geographical variations, local antibiotic prescribing habits,[32] etc. Although the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of world, 10-40% of strains of *E. coli* and *K. pneumoniae* express ESBLs[12]. In the United State, occurrence of ESBL production in *Enterobacteriaceae* ranges from 0 to 25%, depending on the institution, with the national average being around 3%[31,33]. In Korea and Indonesia, the distribution of ESBL in *E. coli* is 5% and 23.3% respectively which is higher when compared to North America[1,34] or Europe, but similar to that of South America[1]. Moreover, the prevalence rate of ESBL in *E. coli* is much lower when compared to that of *Klebsiella* isolates and the highest ESBL rates in *Klebsiella* spp were reported from Korea[1,35]. In the Netherlands, a survey of 11 hospital laboratories showed that <1% of *E. coli* and *K. pneumoniae* strains possessed an ESBL[31,36]. In Japan, the percentage of beta lactam resistance due to ESBL production in *E. coli* and *K. pneumoniae* remains very low. In a recent survey of 196 institutions across the country, <0.1% of *E. coli* and 0.3% of *K. pneumoniae* strains possessed an ESBL. Elsewhere in Asia, the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies like 8.5% in Taiwan[31,37] and 12% in Hong Kong[31]. Similarly, at the University Hospital of the West Indies, a tertiary care hospital in Jamaica, 18.2% of the total *K. pneumoniae* were found to be ESBL producers while there was no ESBL producing *E. coli*[38]. A study done in a general hospital in Saudi Arabia showed that 6% of all isolates were MDR and 4.8% were positive for ESBL[39].

Phenotypic test results of ESBL

The accurate detection and reporting of ESBL-production in clinical isolates are crucial[40]. For this purpose, when DDST and Combination Disk method were employed in our study for the phenotypic confirmation of ESBL producers, the latter was found to be more sensitive which was in agreement to the result shown by Yan J-J et al[37]. Besides, this method is recommended by NCCLS. Similar result was found by Babypadmini S et al in South India among *E. coli* and *K. pneumoniae* isolated from urine samples. Their study revealed that DDST failed to detect ESBLs in a total of 5 out of 166 isolates when compared with combination disk method. [22]

Conclusion

The percentage of MDR and ESBL-producing strains causing LRTI was found to be 47.57% and 24.27% respectively.

The percentage of MDR and ESBL-producing strains causing UTI was found to be 60.40% and 15.80% respectively.

The Combination Disk method was found to be better in terms of sensitivity than the DDST method for the phenotypic confirmation of ESBL-producing strains.

Most common organism causing LRTI was found to be *H. influenzae* followed by *Pseudomonas* spp, *Klebsiella* spp, *E. coli*, *S. aureus*, *Streptococcus pneumoniae*, *Acinetobacter calcoaceticus*, *Citrobacter freundii*, *Enterobacter* spp.

Most common organism causing UTI was found to be *E. coli* followed by *Klebsiella* spp, *S. aureus*, *Enterococcus faecalis*, *Citrobacter* spp, *Pseudomonas* spp, *Acinetobacter calcoaceticus*, *Proteus* spp, *Staphylococcus saprophyticus*, *Morganella morganii*, *Enterobacter* spp., Providencia spp.

This is the first report of ESBL-producing strains from Nepal and it holds significance in that infection with ESBL-producing bacteria can result in avoidable failure of treatment and increased cost in patients who have received inappropriate antibiotic treatment.

Recommendation

Even if ceftazidime and/or cefotaxime appears to be sensitive *in vitro*, but the zone of inhibition is ≤ 22 mm and ≤ 27mm respectively, the organism should be suspected of producing ESBL. If the organism is confirmed to do so, it should be reported to be resistant to all penicillins, cephalosporins and aztreonam.

If the patient is not responding to the third generation cephalosporin antimicrobials, the clinician should think for the ESBL-producing organism and request for its test.

The detection of resistant clones is particularly important in developing countries like Nepal where there is no prudent use of antibiotic and health centers that do not conduct adequate epidemiological surveillance which may eventually increase national problem. Therefore, a careful laboratory practice is required to trace MDR as well as ESBL-producing strains in order to prevent and control such clones in the community.

The study should be continued and the strains should be subjected for genetic study to acquire their detail genetic make-up to characterize mechanism of drug resistance.

References

1. Ahmed I, Salam A. Extended spectrum beta-lactamases and bacterial resistance. *Pak J Med Sci* 2002;**18:**151-5.

2. Araj GF, Samaha-Kfoury JN. Recent developments in ?-lactamases and extended spectrum b lactamases. *BMJ* 2003;**327:**1209-13.

3. http://www.pulsus.com/Infdis/10_06/gard_ed.

4. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983;**11:**315-7.

5. Thompson KS. Controversies about extended spectrum and Amp-C beta lactamases. *Emerg Infect Dis* 2001; **7:**333-6.

6. Jacoby GA, Medeiros AA. More extended-spectrum ?-lactamases. *Antimicrob Agents Chemother* 1991; **35:** 1697-704.

7. Philippon A, Labia R, Jacoby GA. Extended-spectrum ?-lactamases. *Antimicrob Agents Chemother* 1989; **33:**1131-6.

8. Shah AA, Hasan F, Ahmed S, Hameed A. Extended-spectrum beta-lactamases (ESBLs): characterization, epidemiology and detection. *Crit Rev Microbiol* 2004; **30:**25-32.

9. Rupp ME, Fey PD. Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*: considerations for diagnosis, prevention and drug treatment. *Drug* 2003;**63:**353-65.

10. Gold HS, Moellering RC. Antimicrobial-drug resistance drug therapy. *N Engl J Med* 1996;**335:**1445-53.

11. Virk A, Steckelberg JM.. Clinical aspects of antimicrobial resistance. *Mayo Clin Proc*, 2000;**75:** 200-14.

12. Ahmed AA, Osman H, Mansour AM, et al. Antimicrobial agent resistant in bacterial isolates from patients with diarrhea and urinary tract infection in Sudan. *Am J Trop Med Hyg* 2000;**63:**259-63.

13. Tuladhar NR, Banjade N, Pokhrel BM, et al. Comparative study of multi-drug resistant bacterial strains of respiratory pathogens among in and out patients of Tribhuvan University Teaching Hospital, Kathmandu, Nepal. *J NAMLS* 2004;**6:**19-24.

14. Tenover FC, Schwaber MJ, Raney PM, et al. Utility of NCCLS guidelines for identifying extended-spectrum ?-lactamases in non-*Escherichia coli* and non-*Klebsiella* spp. of *Enterobacteriaceae*. *J Clin Microbiol* 2004;**42:**294-8.

15. Sotto A, Lavigne JP, Chanal C, Mahamat A, Michaux-Charachon S. Molecular epidemiology of *Enterobacteriaceae* isolates producing extended-spectrum ?-lactamases in a French hospital. *J Clin Microbiol* 2004;**42:**3805-8.

16. Dhakal BK, Pokhrel BM, Ahnn J. Microscopic detection of urinary tract infection in Nepalese patients. *The Journal of Microbiology* 2002;**40:** 267-73.

17. Baron EJ, Finegold SM. Microorganisms encountered in the urinary tract. Bailey and Scott's Diagnostic Microbiology. 8th ed. St. Louis Missouri Baltimore: CV Mosby -Yearbook Inc, 1990; 253-61.

18. Collee JG, Duguid JP, Fraser AG, Marmion BP, eds. Mackie and McCartney Practical Medical Microbiology. 13th ed. Edinburg: Churchill Livingstone, 1989.

19. Cheesbrough M. Medical Manual for Tropical Countries Vol-II: Microbiology. 1st ELBS ed. Cambridge: University Press, 1984.

20. Pokhrel BM, Shrestha B, Sharma AP. A prospective study of adult lower respiratory tract infections at TUTH in Kathmandu. *J Inst Med* 1997;**19:**30-6.

21. Pokhrel BM, Rai C, Sharma AP. A prospective study in antibiotic sensitivity profile of the organisms associated with clinical infections among the patients attending TU Teaching Hospital. *J NAMLS* 2001;**3:**13-6.

22. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum ?-lactamases in members of the family *Enterobacteriaceae* at a veterans medical center: seek and you may find. *J Clin Microbiol* 1997; **35:**2593-7.

23. Babypadmini S, Appalaraju B. Extended spectrum-beta lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. *Indian J Med Microbiol* 2004;**22:**172-4.

24. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility test, 7th edition. Approved standards, NCCLS document M2-A7, 20; No 1; Wayne PA 2000.

25. Jacoby GA. Genetics of extended spectrum beta-lactamases. *Eur J Clin Microbiol Infect Dis* 1994; **10:**867-78.

26. Long J, Lv SC. Drug resistance analysis of Gram negative bacilli producing extended spectrum beta lactamases in lower respiratory tract infection. *Di Yi Jun Yi Da Xue Xue Bao* 2004;**22:**172-4.

27. Smaoui H, Mahjoubi F, Boutiba I, et al. Antibiotic

resistance among *E. coli* isolates from urinary tract infections (1999-2000): a multicentric study. *Tunis Med* 2003; **81:**390-4.

28. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing Gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; **115:**153-7.

29. Iqbal M, Patel IK, Shah SH, et al. Susceptibility patterns of *Escherichia coli*: prevalence of multidrug-resistant isolates and extended spectrum beta-lactamase phenotype. J Pak Med Assoc 2002; **52:**407-11.

30. Saurina G, Quale JM, Manikal VK, Oydna E, Landman D. Antimicrobial resistance in Enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage pattern. *J Antimicrob Chemother* 2000;**45:** 895-8.

31. Bradford PA. Extended-spectrum ?-lactamases in the 21st century: characterization, epidemiology and detection of this important threat. *Clin Microbiol Rev* 2001; **14:**933-51.

32. Nag VL, Ayyagari A, Venakatesh V, Ghar M, Yadav V, Prasad KN. Drug resistant Haemophilus influenzae from respiratory tract infection in a tertiary care hospital in North India. *Indian J Chest Dis Allied Sci* 2001; 43.

33. http://www.cdc.gov/ncidod/hip/surveill/nnis.htm

34. Medeiros AA. Nosocomial outbreaks of multiresistant bacteria extended spectrum beta-lactamases have arrived in North America. *Ann Intern Med* 1993; **119:** 428-30.

35. Weiner J, Quinn JP, Bradford PA et al. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA* 1999; **281:** 517-23.

36. Stobberingh EE, Arends J, Hoogkamp-Korstanje JAA, et al. Occurrence of extended-spectrum beta-lactamases in Dutch hospitals. *Infection* 1999; **27:**348-54.

37. Yan J-J, Wu S-M, Tsai S-H, Wu J-J, and Su I-J. Prevalence of SHV-12 among clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum ?-lactamases and identification of a novel AmpC enzyme (CMY-8) in Southern Taiwan. *Antimicrob. Agents Chemother* 2000; **44:**1438-42.

38. Nicholson AM, Gayle P, Roye-Green K. Extended spectrum beta-lactamase producing organisms at the University hospital of the West Indies. *West Indian Med J* 2004; **53:**104-8.

39. Kader AA, Kumar AK. Prevalence of extended spectrum ?-lactamase among multidrug resistant Gram negative bacterial isolates from a general hospital in Saudi Arabia. *Saudi Med J 2004*; **25:**570-4.

40. Ho PL, Chow KH, Yuen KY, Ng WS, Chau PY. Comparison of novel, inhibitor-potentiated disc-diffusion test with other methods for the detection of extended-spectrum ?-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. *J Antimicrob Chemother* 1998; **42:**49-54.