MULTIDRUG RESISTANT ENTEROBACTERIACEAE FROM BLOOD STREAM INFECTIONS AT A TERTIARY CARE HOSPITAL OF EASTERN NEPAL

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ABSTRACT

Objective: To find out the Multidrug resistant (MDR) pattern among Enterobacteriaceae isolates obtained from blood stream infection (BSI) with special reference to extended-spectrum-beta-lactamase (ESBL), AmpC, K1, carbapenemase and metallo-β-lactamase (MBL). **Methods:** A cross-sectional study was conducted in the Department of Microbiology, BPKIHS from 1st September 2014 to 31st August 2015. Isolates were screened for ESBL, AmpC, K1, carbapenemase and MBL production by ten disk method. Confirmation for ESBL was done phenotypically by using combined disk method recommended by CLSI, AmpC sterile disk method for AmpC and K1 by combined disk method. Metallo-beta-lactamase (MBL) production was detected by imipenem-ethylene-diamine-tetra acetic acid (IPM-EDTA) double disk synergy test. **Results:** A total of 11,264 blood samples were collected from the patients suspected of BSI. Of these isolates, 192 (1.70%) were Enterobacteriaceae. Among them, 94 (49%) were ESBL, 51 (26.5%) were carbapenemase and 10 (5%) were AmpC producers. Of 51 carbapenemase producers, 22 (11.5%) were MBL producers. None of the isolates were found to produce K1 β-lactamase. A total of 64 (33.4%) isolates were MDR.**Conclusions:** MDR Enterobacteriaceae is found to be prevalent in our set up as an important cause of BSI.

Keywords: Enterobacteriaceae,   Extended-spectrum-beta-lactamase,   Metallo-beta-lactamase, Multidrug resistant, Ten disk method

Introduction

BSI is one of the major causes of morbidity and mortality worldwide. On one hand advancement in the patient care services such as widespread use of indwelling devices, complicated surgery and prolonged hospitalization have emerged as important risk factors for occurrence of BSI. On the other hand involvement of the resistant microorganisms as etiological agents of BSI has posed significant challenge to the clinicians and clinical microbiologists alike. Accurate diagnosis of BSI, timely isolation and identification of the causative agents and determination of their antimicrobial susceptibility are crucial, as effective management depends on the selection and timely administration of the most appropriate antimicrobial agent[1].

Over the recent years, the problem of multidrug resistant Enterobacteriaceae has accelerated dramatically and occupies the third most leading cause of BSI in several settings[2]. The escalating burden of multidrug resistance in Enterobacteriaceae is largely due to production of beta lactamase and its subsets, which are enzymes that bind, deactivate the different types of beta lactam antibiotics and confer broad resistance to them[3].

Despite the attempts of antibacterial stewardship and rigorous endeavor against MDR bacteria, its incidence is not decreasing[4]. This is a worrying public health issue as the infections caused by such enzymes producing bacteria are associated with higher morbidity and mortality. It is a threat to developing countries like Nepal who has greater economic burden as these enzymes can be carried on bacterial chromosomes or may be plasmid mediated and has potential to move between bacterial populations. A comprehensive and cost effective approach is essential for the early identification of the resistant strains and rational institution of therapy.

This study was, therefore, undertaken with an aim to determine ESBL, AmpC, K1, carbapenemase and MBL producing Enterobacteriaceae isolated from BSI at BPKIHS with special reference to the detection of Multidrug resistant Enterobacteriaceae by ten disk approach.

Materials and Methods

A cross-sectional study was conducted in the Department of Microbiology, BPKIHS from 1st September 2014 to 31st August 2015. A total of 11,264 blood specimens were submitted to Department of Microbiology for culture. Only Enterobacteriaceae isolates were included in this study, isolates other than Enterobacteriaceae falls under exclusion criteria. Approximately 3 ml blood was collected in Brain Heart Infusion Broth. Cultures were processed in BD 9050 system (Becton and Dickinson, New York, USA). These broths were aerobically incubated at 35°C for 5 days and observed for the growth of microorganisms. Any sign of growth was followed by sub-culture on MacConkey's agar and Blood agar plates (Hi Media, Mumbai, India). Identification, characterization and sensitivity of the isolates were analyzed as per standard microbiological procedures[5]. Microbiological data were acquired

through the routine microbiological diagnostic and surveillance of multidrug resistant bacteria.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test of all the isolates was performed on Mueller Hinton agar (MHA) (Hi Media, Mumbai, India) by the standard disk diffusion technique of Kirby-Bauer method and interpreted as per CLSI recommendations[6].

Detection of ESBL

ESBL screening test

ESBL screening was done as per CLSI recommendations. Isolate showing zone of inhibition (ZOI) of ≤ 27 mm for cefotaxime (CTX) (30 µg) and ≤ 22 mm for ceftazidime (CAZ) (30 µg) was taken for ESBL confirmation[6].

ESBL confirmatory test

The antimicrobial disks (HI Media, Mumbai, India) used were CTX (30 µg), CAZ (30 µg), cefotaxime-clavulanic acid (CEC) (30 µg/10 µg) and ceftazidime-clavulanic acid (CAC) (30 µg/10 µg). Each disk was kept at least 20 mm apart (center to center). Isolates resistant to CTX and CAZ but sensitive to CEC and CAC with enhanced ZOI ≥ 5 mm was confirmed as ESBL producers[6].

Detection of AmpC

Amp C screening test

Antimicrobial disks used to screen AmpC β-lactamase were cefoxitin (CX) (30 µg) and cefepime (CPM) (30 µg). Isolates resistant to CX (ZOI ≤ 18 mm) but sensitive to CPM (ZOI ≥ 18 mm) indicates AmpC producers[7].

Amp C confirmatory tests

Amp C sterile disk test

Confirmation was done by performing AmpC sterile disk test. MHA plate was inoculated with *Escherichia coli* ATCC 25922. CX disk was placed on MHA plate. Adjacent to it, a sterile disk was placed. On the sterile disk, isolated strain was inoculated several times. After incubation, flattened or intended zone produced around the CX disk was confirmed as AmpC β-lactamase production[8].

Modified Hodge Test

Escherichia coli ATCC 25922 was inoculated on MHA plate. CX disk was placed at the center of plate with the help of sterile forcep. Isolated strain was inoculated perpendicular to the CX disk and was incubated overnight aerobically at 35°C. Presence of clover leaf appearance at the streaking line of the isolated strain was interpreted as AmpC producers[9].

Detection of K1

The antimicrobials used were aztreonam (AT) (30 µg), ceftriaxone (CTR) (30 µg), CTX (30 µg) and CAZ (30 µg). A strain was considered K1 β-lactamase producer if it was resistant to AT (ZOI≤ 27 mm) and CTR (ZOI ≤ 25 mm) and sensitive to CTX (ZOI ≥ 26 mm) and CAZ (ZOI ≥ 21 mm)[10,11].

Detection of Carbapenemase

The isolates showing resistant to ertapenem (ETP) (10 µg) (ZOI ≤ 22 mm) and sensitive to imipenem (IPM) (10 µg) (ZOI ≥ 23 mm) were considered as carbapenemase producers. Besides this, rest of the antimicrobials should be resistant for the isolates to be carbapenemase producers[12].

Metallo β-lactamase (MBL)

All carbapenemase producers were tested for EDTA-IPM double disk synergy test and were confirmed by MHT.

Double disk synergy test

Double disk synergy test was done by using anhydrous ethylene diamine tetra acetic acid (EDTA) disk of concentration 1.5 mg/disk (0.5 mol.) and imipenem (IPM) (10 µg). EDTA-IPM disks were placed at a distance of 10 mm apart (edge to edge). Enhanced ZOI showing synergistic effect between the two disks was considered as indication of MBL productions[13,14].

Modified Hodge Test (MHT)

ATCC *Escherichia coli* was inoculated on MHA plate. IPM disk was placed at the center of MHA plate with the help of sterile forcep. Isolated strain was inoculated perpendicular to the IPM disk. Following incubation, appearance of a clover leaf at the streaking line of the isolated strain was confirmed as MBL producers[15].

Definition of MDR

Multidrug resistance (MDR) is the resistance offered by bacteria to three or more different antibiotic classes[16].

Data and statistical analysis

The data generated during the study period were analyzed by using SPSS version 16.0 and interpreted according to frequency distribution and percentage.

Ethical consideration

Ethical approval was obtained from Institutional Review Committee (IRC), BPKIHS, Dharan, Nepal (code no IRC/424/014).

Consent to participate/consent to publish

Informed consent form was not included as this study deals with the bacterial isolates that belong to Enterobacteriaceae family only.

Data availability

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Results

During the study period, a total of 11,264 blood samples were collected from the patients subjected to Blood Stream Infections. Of these isolates, 192 (1.70%) were Enterobacteriaceae comprising of *Escherichia coli* 95 (49.48%), *Klebsiella pneumoniae* 45 (23.44%), *Enterobacter aerogenes* 27 (14.06%), *Citrobacter freundii* 6 (3.13%), *Citrobacter koseri* 6 (3.13%), *Proteus vulgaris* 3 (1.56%), *Salmonella* Typhi 3 (1.56%), *Enterobacter cloacae* 2 (1.04%), *Proteus mirabilis* 2 (1.04%), *Klebsiella oxytoca* 1 (0.52%), *Morganella morganii* 1 (0.52%) and *Salmonella* Paratyphi A 1 (0.52%). (Figure 1)
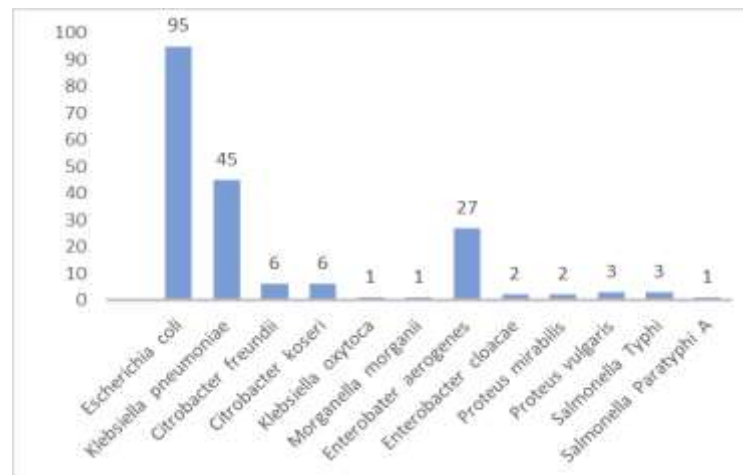
Figure 1: Frequency of Enterobacteriaceae isolates (n=192)

113 (59%) isolates were obtained from male patients and 79 (41%) from female patients. Majority of the isolates were from age group <10 years (71; 37%), followed by group 50-89 years (24%) and 20-29 years (13%).

Frequency of β-lactamases producers

Results of different types of β-lactamases produced by Enterobacteriaceae are given in figure 2. ESBL was the most common β-lactamase 94 (49%), followed by carbapenemase 51 (26.5%), MBL 22 (11.5%) and AmpC 10 (5%). In none of the isolates K1 β-lactamase has been detected.
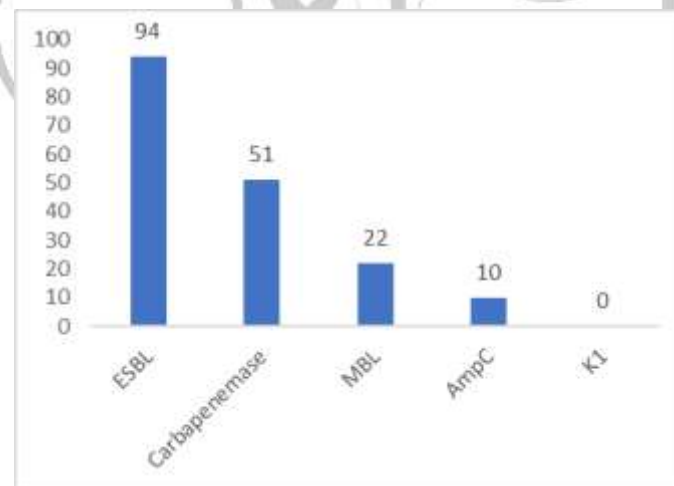

Figure 2: β-lactamase producers (n=192)

Among IPD, ESBL producers were the most common, accounting for 17% (33/192), followed by carbapenemase 12.5% (24/192), MBL 7% (14/192) and AmpC 2% (4/192). Similarly among OPD, ESBL producers 32% (61/192) were the leading one, followed by AmpC 3% (6/192), carbapenemase 14% (27/192) and MBL 14% (27/192). Comparing β-lactamases producers among both IPD and OPD, ESBL, AmpC and

carbapenemase producers were more in OPD but MBL producers were more in IPD.

β-lactamase producers among Enterobacteriaceae

Among 192 Enterobacteriaceae isolates, the most common β-lactamase producers were ESBL, accounting for 94 (49%), followed by AmpC 10 (5%), carbapenemase 51 (26.5%) and MBL 22 (11.5%). Among 95 *Escherichia coli* isolates, 60 (92%) were ESBL producers, 2 AmpC producers, 23 carbapenemase producers and 3 MBL producers. Among the isolates, only one *Klebsiella oxytoca* was isolated which was ESBL producers. The isolates that were non β-lactamase producers were *Morganella morganii* (1) and *P. mirabilis* (2). After ESBL, carbapenemase (51) was the second most β-lactamase producers which included *Escherichia coli* (23), the most predominant one followed by *K. pneumoniae* (18). Among MBL producers, *K. pneumoniae* (13) was the leading one followed by *E. coli* (3), *E. aerogenes* (3), *C. koseri* (2) and *C. freundii* (1). Among AmpC producers, two from each *E. coli, K. pneumoniae, E. aerogenes* and *C. freundii* were isolated. Similarly, different types of β-lactamases producers were elucidated in Table 1.

Table 1: β -lactamases producers among Enterobacteriaceae

Enterobacteriaceae (n=192)	ESBL (n=94)	AmpC (n=10)	Carbapenemase (n=51)	MBL (n=22)
E. coli	60	2	23	3
K. pneumoniae	19	2	18	13
E. aerogenes	8	2	5	3
C. freundii	2	2	1	1
P. vulgaris	2	0	1	0
K. oxytoca	1	0	0	0
C. koseri	1	1	3	2
E. cloacae	1	0	0	0
S. Typhi	0	1	0	0
S. Paratyphi A	0	0	0	0
M. morganii	0	0	0	0

Frequency of AmpC producers

Equal number of *K. pneumoniae, E. aerogenes, C. freundii* and *E. coli* were found to be AmpC producers, and one each of *S.* Typhi and *C. koseri.* Of the total AmpC producers (10), five were positive for AmpC confirmatory test. (Table 2)

Table 2: AmpC producers (n=10)

| Isolates (n=10)	AmpC screening test(n=10)	AmpC confirmatory test (n=5)	Department
K. pneumoniae (n=1)	Positive	Positive	IPD
K. pneumoniae (n=1)	Positive	Negative	OPD
E. aerogenes (n=2)	Positive	Positive	OPD
C. freundii (n=1)	Positive	Positive	IPD
C. freundii (n=1)	Positive	Negative	OPD
E. coli (n=1)	Positive	Positive	OPD
E. coli (n=1)	Positive	Negative	OPD
S. Typhi (n=1)	Positive	Negative	IPD
C. koseri (n=1)	Positive	Positive	IPD

Frequency of MBL producers
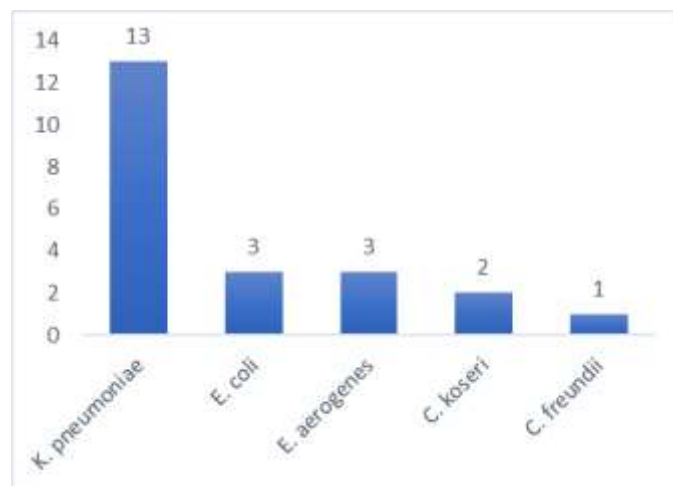
Figure3: MBL producers (n=22)

Among the isolates, 22 (11.5%) were found to be MBL producers, which was elucidated in *Figure 4*. Among these, *K. pneumoniae* (13, 59.1%) was found to be most MBL producers followed by *E. coli* (3, 13.6%), *E. aerogenes* (3, 13.6%), and *C. koseri* (2, 9.1%). Among the isolates, only one *C. freundii* was isolated which was MBL producers. (Figure 3)

Antibiotic sensitivity pattern of Enterobacteriaceae

Most of the isolates were resistant to third generation cephalosporins, cefotaxime (141; 73.40%), ceftazidime (139; 72.40%) and ceftriaxone (105; 54.70%). Similar sensitivity pattern for cefepime (113;

58.80%) was observed compared to third generation cephalosporins. Most of the isolates of *E. aerogenes* (17; 63%) and *C. freundii* (4; 67%) were sensitive to cefepime. All three isolates of *S.* Typhi were sensitive to cefepime. Regarding monobactam, 77 (40%) isolates were found sensitive and the number of resistant isolates were equal to the sensitive isolates. Among carbapenems, 22 (11.5%) isolates were resistant to imipenem and 51 (26.5%) isolates were found resistant to ertapenem. Most of the isolates (164; 85.4%) were sensitive to imipenem. Almost 99% of the isolates were found sensitive to tigecycline. (Table 3)

Table 3: Antibiotic sensitivity pattern of the Enterobacteriaceae

| Antibiotic used	Sensitive (%)	Resistance (%)	Intermediate (%)
Cefotaxime	25.50	73.40	1.10
Ceftazidime	23.96	72.40	3.64
Ceftriaxone	42.70	54.70	2.60
Cefotaxime-clavulanic acid	71.90	28.10	0.00
Ceftazidime-clavulanic acid	68.30	31.70	0.00
Cefoxitin	41.70	41.70	16.6
Cefepime	39.60	58.9	1.50
Aztreonam	40.10	56.25	3.65
Ertapenem	66.63	26.56	7.81
Imipenem	85.40	11.50	3.10
Colistin	96.40	3.10	0.50
Tigecycline	98.96	0.00	1.04

MDR Enterobacteriaceae
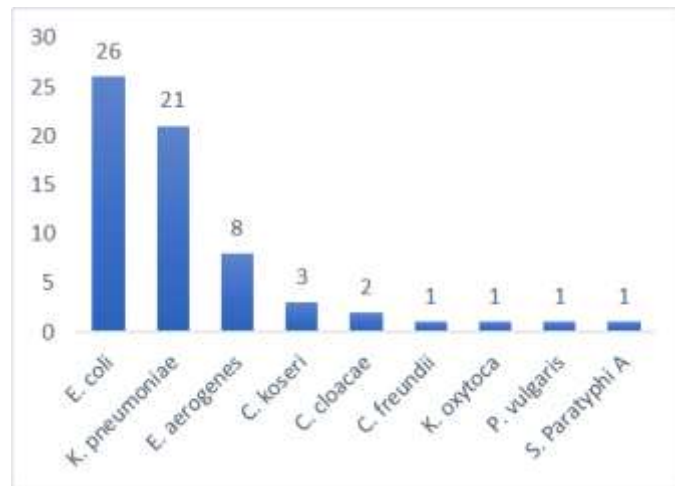
Figure 4: MDR Enterobacteriaceae (n=64)

Among 192 isolates of Enterobacteriaceae, 64 (33.3%) isolates were found to be multidrug resistant. *Escherichia coli* (26) was found to be the leading one among the MDR isolates, followed by *Klebsiella pneumoniae* (21), *Enterobacter aerogenes* (8), *Citrobacter koseri* (3), *Citrobacter cloacae* (2) and one each of *Citrobacter freundii, Klebsiella oxytoca, Proteus vulgaris,* and *Salmonella* Paratythi A. (Figure 4)

Enterobacteriaceae resistance to all beta-lactam drugs

Among 192 Enterobacteriaceae, 22 isolates were resistant to all β-lactam drugs - extended spectrum cephalosporins, cephamycins, monobactum and carbapenems. (Figure 5)
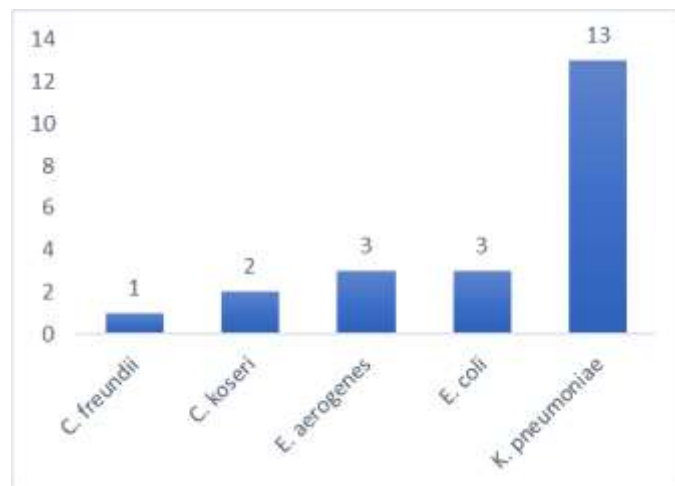

Figure 5: β -lactam drugs resistant Enterobacteriaceae (n=22)

A total of 22 isolates were found to be resistant to all β-lactam drugs. Almost all were isolated from inpatients (21; 95.5%) and only one from outpatient. All these

were MBL producers (n=22), among which 13 (59%) were *K. pneumoniae*, 3 (13.6%) each were *E. coli* and *E. aerogenes*, 2 (9%) were *C. koseri* and 1 (4.5%) was *C. freundii*. Distribution among different departments of origins were as follows; 8 (36%) from ER, 4 (18%) from ICU, 3 (13.6%) from DICU, 2 (9%) each from nursery and NICU and 1 (4.5%) from medicine outpatient department.

Distribution of Enterobacteriaceae among different wards

Escherichia coli (30.2%) was found to be more common among the isolates in ER, followed by *Klebsiella pneumoniae* (8.3%) and *E. aerogenes* (7.3%). In ICU, *K. pneumoniae* was the leading one, followed by *E. coli* and *E. aerogenes*. In PICU and DICU, only one isolate, *K pneumoniae*, in each ward was isolated. Two isolates were from NICU, *K. pneumoniae* and *C. koseri*.

Discussion

This study is conducted with an aim to evaluate multidrug resistance pattern in Enterobacteriaceae isolated from BSIs with special reference to ten disk method, and to guide the clinicians with the most appropriate antibiotics against those pathogens. MDR pattern is most commonly seen in Gram-negative bacteria compared to Gram-positive bacteria (GPB). Particularly resistance in GNB is of great importance as there is a dearth of novel antibiotics directed against these organisms[16]. Among the antimicrobials used, β-lactams are the most commonly used therapeutic class of antimicrobials for treatment of bacterial infections because of their broad antibacterial spectrum and excellent safety profile[17].

In our study, the frequency of BSIs by Enterobacteriaceae was found to be 1.7% (192/11,264) out of total blood culture positive 17.3% (1948/11,264). A similar study conducted by Abdallah *et al* in 2015 in Egyptian patients and in Nepal by Joshy M Easow *et al* in 2010 where the number of Enterobacteriaceae isolated found to be 94 and 96 respectively, which was less as compared to our study[17, 18].

All together 192 Enterobacteriaceae isolates were included in this study. Among them, most of the isolates (115; 60%) were obtained from outpatient departments, while 77 (40%) were obtained from inpatient departments. The number of isolates were more from outpatient departments compared to inpatient, male to female ratio was found to be 1.4:1 which was found similar to the finding of study conducted in the western region of Nepal by Joshy M Easow *et al* in 2010[18]. The etiological agents of BSIs caused by Enterobacteriaceae are listed in table 1. Majority of the isolates were *Escherichia coli* 49.5% as compared to the other isolates. Similar results have been documented by Abdallah *et al*[17]. In a study conducted by Joshy M Easow *et al* 2010, *Klebsiella pneumoniae* 13.5% was found to be the predominant isolates causing BSIs, a finding different as compared to our study[18]. Isolation of *Escherichia coli* (66), *Enterobacter aerogenes* (14), *Enterobacter cloacae* (2), *Salmonella* Typhi (2) and *Proteus vulgaris* (2) were more from OPD as compared to inpatient departments.

In Enterobacteriaceae, β-lactamase production remains the most important mediator of β-lactam resistance[17]. The rates of antimicrobial drug resistance are

increasing among Enterobacteriaceae particularly of multidrug pattern which limits the activity of antimicrobial agents[19].

In the present study, 49% ESBL were isolated. Among them, 64% were *Escherichia coli* followed by *K. pneumoniae* 20%. In a study conducted by Shrestha *et al* at BPKIHS in the year 2007, prevalence of ESBL among the clinical isolates of pyogenic infections were reported as isolates are- *E. coli, K. pneumoniae, P. mirabilis, Enterobacter* species and *Citrobacter* species 53%,14.8%,12.9%,5.5% and 5.5% respectively[20]. Another study conducted at BPKIHS by Abhilasha Sharma in the year 2012, found the prevalence of ESBL as *E. coli* 73%, *K. pneumoniae* 60.5%, *Enterobacter* species 46% and *Citrobacter* species 25%. These data suggest that at our institution *E. coli* remains as the most frequent ESBL producers followed by *K. pneumoniae*, *Enterobacter* species and *Citrobacter* species. Many studies have been conducted in Nepal regarding prevalence of ESBLs. In a study conducted by Raut *et al.* 2015 at Manipal Teaching Hospital, Pokhara, the prevalence of ESBL in *E. coli* was found to be 81.6% and in *K. pneumoniae* (4.1%) which is similar to our study[21]. A comparable results were reported by Poudyal *et al.* 2011, i.e. the predominance of ESBL *E. coli* 80% as compared to *K. pneumoniae* 5.8%[22]. Emergence of ESBL may be due to widespread use of third-generation cephalosporins and aztreonam which is believed to be the major cause of mutations in TEM and SHV enzymes[23].

Data regarding AmpC producing strains in Nepal is limited and very little information is available. In the present study, 10 (5%) isolates were AmpC positive. Out of these, 5 isolates were positive by confirmatory test (Table 2). The rate of AmpC production was less compared to other study done in Nepal suggesting lesser spectrum of resistance among our isolates[24].

In the present study, K1 β-lactamase was not found in any of the isolates. This enzyme was first detected in *Klebsiella pneumoniae*. It is also detected in *Klebsiella oxytoca*[25, 26]. In the present study, 46 (24%) *Klebsiella* species were isolated. Among these, 40 were positive for β-lactamases- ESBL 20, AmpC 2, carbapenemase 18. Among 18 carbapenemase producers 13 were MBL producers, but all were negative for K1 β-lactamase. Carbapenemase producers in this study was found to be 51 (26.5%). It was further tested by performing sensitivity test with tigecycline and colistin. Among the isolates, 190 (99%) were sensitive to tigecycline and 185 (96%) were found sensitive to colistin. Two *E. coli* were found to have intermediate susceptibility to tigecycline. Talking about activity of colistin, six isolates were resistant to colistin, one had intermediate susceptibility. In this study, Tigecycline was very active and appears to be an excellent option compared to colistin for treatment of infections caused by these multidrug-resistant Enterobacteriaceae[27, 28].

In screening of MBL, 22 (11.5%) isolates were found to be imipenem resistant, whereas 51 (26.5%) were resistant to ertapenem. The MBL producing *Klebsiella*

species in the present study was found to be higher in number than that shown by Shrestha *et al.*[29]. In a study carried out by Vinod Kumar *et al.*, 20% resistance to imipenem and 17% rate of MBL production was reported. Similarly, Kamble *et al.*, reported 20% MBL production[30].

In most centers, detection of β-lactamase production is not routinely done which results in the spreading of β-lactamase producing strains in hospitals. It is also due to irrational use of antimicrobials leads to increased percentage of β-lactamase production. Therefore, irrational use of antibiotics should be done as little as possible and specific therapeutic antibiotics should be used for short period as suggested by Singh *et al.*[30].

Antimicrobial susceptibility profile of Enterobacteriaceae showed a high degree of resistance to the antimicrobials. Resistance for cefotaxime and ceftazidime were highest (72–74%) as compared to the resistance pattern for other antimicrobials. In BSIs, third-generation cephalosporins have been used extensively as a first-line antibiotic, as a result of which they are rendered useless. Our isolates showed least resistance for imipenem and ertapenem, 11.5%, and 26.5% respectively. The rate of resistance to the various drugs was in concordance with other studies[23, 30, 31]. Present study showed good activity of tigecycline (99%) and colistin (96%) against the isolates. Only two isolates were found to have intermediate susceptibility to tigecycline. Similar results were documented by Sader *et al*[32] and Chen *et al.* in 2011[33]. The clinical efficacy of tigecycline in BSI has not yet been established. In vitro, evaluation of its efficacy in ESBL and MBL producing isolates in septicemia have been reported by Roy *et al.* in two different studies[34, 35].

In the present era, the emergence of MDR organisms and their spread in the community is of great concern. Infections by MDR organisms lead to prolonged hospitalization, increased mortality, morbidity and cost of treatment[36]. As per the definition, MDR in Enterobacteriaceae is defined as "the resistant offered by bacteria to three or more than three antimicrobials of different classes"[37]. Isolates exhibiting co-resistance to at least any three of the following drugs were considered as MDR and these drugs were: extended spectrum cephalosporins (cefixime/ceftriaxone/ceftazidime/cefepime), cephamycins and monobactam or resistant to any two of the above drugs and any one of the carbapenems. In our study, 64 (33.3%) isolates were found to be MDR. Various authors have reported high percentage of MDR in their study[23, 31, 38]. Present study has documented the increasing antimicrobial resistance among isolates from BSI which is a matter of concern for clinicians and microbiologists. This reflects the need for early detection and prevention of further spread of resistance to other bacteria.

Conclusion

In conclusion, our study has revealed the presence of ESBL, AmpC, carbapenemase and MBL producers in our setting. Occurrence of multidrug resistant Enterobacteriaceae as important etiological agent of BSI is a serious matter of concern. Therefore, it is

imperative to take timely steps for the prevention and control of spread of these resistant pathogens. Coordinated efforts from various departments, education of hospital staffs regarding problem of drug resistance, prudent use of antimicrobials and early detection of resistant isolates are required for the achievement of this task. Clinical microbiology laboratory has vital role to play by early and accurate detection of resistant Enterobacteriaceae isolates by ten disk method so that timely action can be taken to curb their spread.

Competing interest

We declare that we have no conflict of interest.

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Author's contributions

Conceptualization: AY, NRB, BK. Investigation: AY. Methodology: AY, NRB, BK. Resources: AY, NRB, BK. Supervision: NRB, BK. Writing-original draft: AY. Writing-review and editing: NRB, BK. All authors read and approved the final manuscript.

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