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Phenotypic Detection of Extended Spectrum B-Lactamase Producing Gram Negative Bacteria Isolated From Clinical Specimens

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ABSTRACT

Background: The worldwide emergence of extended spectrum β-lactamase (ESBL) producing gram negative bacteria (GNB) has become a major problem observed over the last decade. ESBLs are considered as a threat since they are coded by plasmid and can be easily transmitted between species. The rate of hospital acquired infections caused by ESBL-producing GNB in Asia Pacific has increased and several studies have identified their increasing prevalence in the region. The aim of this study is to review the prevalence of ESBL-producing GNB in Nepal.

Methods: This descriptive cross sectional study was conducted at Grande International Hospital, Tokha, Kathmandu, Nepal. The study period was from July 2016 to February 2017. The study population included the patients attending the hospital. The processing of the samples sent to Microbiology laboratory for Culture and sensitivity, identification of the isolates and Antibiotic susceptibility testing was performed using standard microbiological procedures. Screening for Multi-Drug resistance (MDR) and Extended Spectrum Beta- Lactamase (ESBL) was done for all *Escherichia coli* and *Klebsiella pneumoniae* isolates using combined Disk (CD) test..

Results: Out of 258 *Escherichia coli* and *Klebsiella pneumoniae* isolates, 42 isolates of *E.coli* and 21 isolates of *K. pneumoniae* were confirmed as ESBL producer.

Conclusion: The regular surveillance of hospital-associated infections including monitoring of antimicrobial susceptibility pattern is needed for the formulation of a definite antimicrobialpolicy.

Keywords: Antibiotic susceptibility testing; combined disk assay; ESBL; Gram negative bacteria; MDR

INTRODUCTION

Multi Drug resistant bacteria are emerging as a threat to the favorable outcome of common infections in community and hospital settings. MDR bacterial infections are spreading worldwide where Extended Spectrum β-Lactamases (ESBLs) are the major MDR related bacterial enzymes in addition to metallo β-lactamases, carbapenemases and AmpC β- lactamases (Chakraborty et al. 2011). ESBLs are considered as a threat since they are coded by plasmid and can be easily transmitted between species.

Antimicrobial resistance among Enterobacteriaceae has increased dramatically in recent year against commonly used antimicrobial agents such as the tetracycline, β-lactams, fluoroquinolones,

aminoglycosides and co-trimoxazole (Bonelli et al 2014). ESBLs havebeen found in wide range of gram negative rods. *Escherichia coli* and *Klebsiella pneumoniae* are important ESBL producing organisms in the Enterobacteriaceae family. ESBL producers are usually selected in hospitals, although outbreaks have been reported in nursing home facilities (Bradford et al 1995; Huppertz et al 2005). The distribution of ESBL isolates in the hospitals is in the wards where patients have a higher risk for infections such as ICU, surgicalwards, neonatal wards, chronic care facilities etc. (Pena et al 1997).
This article aims to give an overview of the current situation regarding ESBLs and management of infections caused by ESBL producing organism. The outcome of this study would be helpful to manage the problem by suggesting appropriate chemotherapeutic agent.

Methods

This descriptive cross sectional study was conducted at Grande International Hospital, Tokha, Kathmandu, Nepal. The study period was from July 2016 to February 2017. The study population included the patients attending the hospital whose samples were sent to Microbiological laboratory for culture and sensitivity. The samples include urine, pus, body fluids, sputum, endotracheal tube (ET) tip, wound swab, cervical swab. Informed consent wastaken from the patients for their participation in the study during the sample collection time.
Culture of different clinical specimens was performed using standard microbiological procedures (Forbeset al. 2007). Isolated colonies from the pure culture were identified by performing Gram staining and the biochemical tests. Susceptibility tests of the different clinical isolates towards various antibiotics were performed by modified Kirby-Bauer disk diffusion method for the commonly isolated pathogens using Mueller Hinton Agar (MHA). The isolates resistant to at least one antibiotic in at least three different antimicrobial categories were considered as MDR.
The MDR isolates were screened for possible ESBL production using ceftazidime (30µg), cefotaxime (30µg) (CLSI 2014). The screen positive isolates, i.e. showing ceftazidime<22 mm, cefotaxime<27 mm zone of inhibition, were subjected to Combined Disk (CD) test using cefotaxime (30µg) and cefotaxime (30µg) plus clavulanate (10µg) for confirmation of ESBL production. An increase in zone of diameter of ≥5mm in the presence of clavulanate was concluded as confirmed ESBL producer.
The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) software.

Results

A total of 1563 different clinical samples were processed. Among them *E. coli* and *K. pneumoniae* were isolated from 184 (71.31%) and 74 (28.69%) samples respectively. Majority of the isolates were obtained from urine (70.93%) followed by sputum (13.95%).

Table 1: Overall growth and distribution of *E. coli* and *K.pneumoniae* within the clinicalsamples

Sample *E.coli*(%)	Total (%)	Bacterial Isolates	*K.pneumoniae (%)*
Urine	183 (70.93)	153 (83.15)	30 (40.54)
Pus	8 (3.10)	5 (2.71)	3 (4.05)
Sputum	36 (13.95)	8 (4.34)	28 (37.83)

Catheter/Foley's tip	2 (0.77)	2 (1.08)	0 (0)
Blood	14 (5.46)	8 (4.34)	6 (8.10)
Wound swab	13(5.03)	6(3.26)	7(9.45)
ET/suction tips	2 (0.77)	2 (1.08)	0 (0)
Total	**258(16.50)**	**184 (71.31)**	**74 (28.69)**

Resistance pattern of the isolates

The antibiotics sensitivity pattern of *E.coli* and*K.pneumoniae* showed high rate of resistance to cephalosporins used: Ceftriaxone, Cefotaxime and Ceftazidime. Other drugs resistance to *E.coli* and *K.pneumoniae* were Cotrimoxazole, Ofloxacin and Ciprofloxacin. Gentamicin, Amikacin, Nitrofurantoin and Imipenem showed the lowest rate of resistance

Table 2: Resistance pattern of the isolates

Antibiotics Used	**Resistance pattern of the isolates**	
*E. coli(*N=184)		*K.pneumoniae*(N=74)
Cotrimoxazole	100 (54.34)	35 (47.29)
Ampicillin	107 (58.15)	50 (67.56)
Nitrofurantoin	17 (9.23)	18 (24.32)
Amikacin	23 (12.5)	20(27.02)
Ciprofloxacin	91 (49.45)	28 (37.83)
Ofloxacin	92 (50)	28 (37.83)
Ceftriaxone	93 (50.54)	42 (56.75)

Cefotaxime	82 (44.56)	41 (55.40)
Ceftazidime	84 (45.65)	38 (51.35)
Gentamicin	25 (13.58)	27 (36.48)
Imipenem	5 (2.71)	13 (17.56)

Out of 258 isolates, 148(57.36%) were MDR. Among 184 *E. coli* and 74 *K. pneumoniae*,MDR was seen in 111(60.32%) and 37 (50%) isolates respectively. (Table 3)

Table 3. Pattern of MDR for the isolates

Bacterial Isolates	MDR	Non-MDR
E. coli	111 (60.32)	73 (39.68)
K. pneumonia	37 (50)	37 (50)
Total	148 (57.36)	110 (42.64)

Out of 258 isolates, 98 isolates of *E. coli* and 43 isolates of *K. pneumoniae* were suspected as ESBL producer on primary screening test. Among them, 42 isolates of *E. coli* and 21 isolates of *K. pneumoniae* were confirmed as ESBL producer. Of the total isolates 24.41% wereESBL producer. (Table 4)

Table 4. ESBL producer among the isolates

Organisms	**Number of isolates**		**ESBL producer**
Screening %	**Confirmed %**		
E. coli	184	98 (53.26)	42 (22.82)
K.pneumoniae	74	43 (58.10)	21 (28.37)
Total	258	141(54.65)	63 (24.41)

ESBL producing isolates showed highly resistance towards all the cephalosporins; cefotaxime (100%), ceftazidime (98.41%) and ceftriaxone (96.82%). Resistance was also seen in case of ampicillin(84.12%), cotrimoxazole(82%), ciprofloxacin(73.01%), ofloxacin(73.01%), gentamicin(39.68%), amikacin(36.5%), nitrofurantoin(30.15%) and imipenem(7.93%). (Table 5)

Table 5. Antibiotic resistivity pattern of the ESBL positive isolates

Antibiotics Used	**Resistance pattern of the isolates**		**Total(%)** (N=63)
	***E.coli*(N=42)**	***K.pneumoniae*(N=21)**	

Cotrimoxazole	33(78)	19(90)	52(82)
Ampicillin	35(83)	18(85)	53(84.12)
Nitrofurantoin	10(23.80)	9(42.8)	19(30.15)
Amikacin	13(30.95)	10(47.61)	23(36.50)
Ciprofloxacin	32(76.19)	14(66.66)	46(73.01)
Ofloxacin	32(76.19)	14(66.66)	46(73.01)
Ceftriaxone	40(95)	21(100)	61(96.82)
Cefotaxime	42(100)	21(100)	63(100)
Ceftazidime	42(100)	20(95.23)	62(98.41)
Gentamicin	12(28.57)	13(61.90)	25(39.68)
Imipenem	1(2.38)	419.04)	5(7.93)

Discussion

Escherichia coli followed by *Klebsiella pneumoniae* is the most common gram-negative pathogen associated with a wide spectrum of infections, such as urinary tract infection (UTI), pneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis and pyogenicliver abscess (Vading M et al 2018). During the last decades ESBL producing *E.coli* and *K.pneumoniae* have dramatically increased worldwide and mostly associated with dissemination of ESBLs and other horizontally transmissible resistance genes.

Antibiotic susceptibility testing has become a very essential step for the proper treatment of infectious diseases. Antibiotic susceptibility test was performed by using modified Kirby- Bauer disc diffusion test for all the isolates. Ampicillin was found less effective (60.85% resistant) for both the isolates. Since, Ampicillin is the first line drug which is easily hydrolyzed by the bacterial enzymes; it is found to be less effective in the treatment of gramnegative bacterial infections (Thakur et al 2013).

Nitrofurantoin was only tested for urinary isolates in this study because of better pharmacokinetics for treatment of urinary tract infections than other site infections (Munoz- Davila, 2014). Nitrofurantoin (13.56% sensitive) was found most effective drug for the treatment of UTIs. Comparatively, *E. coli* showed less (9.23%) resistance than *K. pneumoniae* (24.32%). This might be because Nitrofurantoin is more active against infectionscaused by *E. coli* than *K. pneumoniae* (Ahmed et al 2014).

The resistance among pathogens is also increased due to horizontal spread of successful clones of resistant bacteria that may occur either due to poor hygiene, transfer of patients from one ward to another or from a hospital to a nursing home, as well as interregional migration and international mobility (Dalhoff 2012).

Incidence of MDR was found 57.36%. E. coli (60.32%) was found predominant MDRproducer than *K. pneumoniae* (50%). In different studies conducted, MDR ranges from 38- 95.2% for *E. coli* and 25-100% for *K. pneumoniae* (Baral et al, 2012; Poudyal et al, 2011; Thakur et al, 2013; Yadav et al, 2015). Risk factors for MDR organism include institutionalization, previous use of any antibiotic, previous hospitalization, ICU stay, age, chronic underlying disease, urinary catheters and gut colonization (Chaudhary and Murthi 2013).

Out of 54.65% isolates that were suspected for ESBL production only 24.41% i.e. 63 were confirmed as ESBL producer. ESBL production was high in *K. pneumoniae*(28.37%) than *E. coli* (22.82%). The prevalence of ESBL producing *E. coli* and *K. pneumoniae* was found as low up to 18.2% and 4.1% respectively in a study conducted by Raut et al (2015) and high up to 80% for *E. coli* (Poudyal et al 2011) and 90.9% for *K. pneumoniae* (Pathak and Pokharel 2015). Global prevalence of ESBL producing organisms presently varies from <1% to 74% (Thokar et al 2010). The Prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution.

Two different combination discs i.e. Ceftazidime and Cefotaxime with their respective clavulanate were used for the confirmation of ESBL. The ceftazidime -clavulantecombination disc identified 59 isolates as confirmed ESBL producer. However, cefotaxime- clavulanate combination disc identified only 58 isolates as confirmed ESBL producers. The confirmation rate was found more in ceftazidime-clavulante than cefotaxime-clavulanate. Phenotypic differentiation of organisms producing different types of ESBLs can be difficult. The difficulty is due to overlapping phenotypes resulting in interference from other β- lactamases produced by the organism (Pitout et al 2004). However, it is adequate to use cefotaxime, which is consistently susceptible to CTX-M and ceftazidime, which is consistently a good substrate for TEM and SHV variants (Paterson et al 2005).

ESBL producing isolates showed higher rate of resistant towards third generation cephalosporins; ceftazidime (98.41%), cefotaxime (100%) and ceftriaxone (96.82%) which was in coherence to the study conducted by Mishra et al(2015) and Chander and Shrestha (2013).

ESBL is located on a plasmid that can be transferred from one organism to another rather easily and can incorporate genetic material coding for resistance to other antimicrobial classes (Alipourfard et al 2010).Therefore, it is common for organisms expressing an ESBL to express co-resistance to other antibiotics, such as aminoglycosides, trimethoprim sulfomethoxazole, and tetracycline, leading to treatment failure (Shashwati et al 2014).

The combination of β-lactam and β-lactmase inhibitors is usually active against organisms that possess single ESBL. However, multiple ESBLs are produced by many organisms which reduce the efficacy of β-lactam/β-lactamases combinations.

The most effective drug for the treatment of ESBL producing isolates was found to be imipenem, nitrofurantoin, amikacin, and gentamicin. ESBL producing strains are often multidrug resistance. In our study, ESBL producing isolates of E. coli and K. pneumoniae were found resistant to different classes of antibiotics. Such resistant isolates pose serious problems to the physicians as therapeutic options are limited. The early detection of beta lactamases producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such strains (Wadekar et al 2013).

Conclusion

The incidence of ESBL producing *E. coli* and *K. pneumoniae* in clinical settings was found in an

alarming situation. Third generation cephalosporins used were highly resistant to ESBL producer. These pathogens also showed reduced susceptibility towards co-trimoxazole and fluoroquinolones (ciprofloxacin and ofloxacin). The high prevalence of ESBL producing bacterial isolates warrants detection in routine laboratory, immediate infection control, antibiotic stewardship programs in hospitals and programmesawaring consumers about the danger of inappropriate use of antibiotics in order to limit the spread of β-lactamase producing organisms.

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