ESBL and their identification in peripheral laboratories of Nepal

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ABSTRACT

Extended spectrum beta-lactamases (ESBLs) are the enzymes mostly produced by Enterobacteriaceae which are able to hydrolyze extended spectrum cephalosporins. ESBLsare not able to hydrolyze cephamycins and are inhibited by clavulanic acid. Infections caused by ESBL producing organismshave been increasing worldwide including Nepal.The transmission of ESBL infections is rapid because the resistance pattern is plasmid mediated. In order to limit its transmission, early identification andtreatment with appropriate antibiotic is critical. There are variousmethods to detect the ESBL producers in laboratory. Nevertheless, many laboratories in developing countries such as Nepal are unaware of the relevant Clinical and Laboratory Standard Institute (CLSI) guidelines for detecting ESBL producers. One of the reliable, cost effective and simple tests for phenotypic confirmation of ESBL organismsis double disk diffusion test which can easily be adopted in peripheral laboratories of Nepal. Identification and treatment of ESBL organisms are essential, however, are equally challenging because of the lack of routine detection practice and the limited treatment options. Current treatment options for ESBL infections are carbapenem group of drugs such as etrapenem, imipenem and meropenem which are often unaffordable and are unavailable particularly in the peripheral region of Nepal. The main objectives of this review were to provide an overview of ESBL, its prevalence, detection methods and the treatment options.

Keywords: Double disk diffusion test; Enterobacteriaceae; Extended Spectrum β- lactamases; Prevalence.

INTRODUCTION

Bacterial resistance to antibiotic has increased in recent years. Resistance to β-lactams in Gram-negative bacteria has been reported to be associated with Extended Spectrum beta-Lactamases. ESBLs confer resistance to penicillins, cephalosporins and monobactams but not to cephamycins and are inhibited by clavulanic acid.[1]Beta-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam inhibit ESBL produced by microorganisms.

ESBLs are mainly produced by Enterobacteriaceae and *Pseudomonas aeruginosa*.[1] Infections caused by these organisms are mostly treated by cephalosporins. Extensive use of cephalosporins might lead to increase in ESBL producer worldwide[2] that further challenges the treatment of infections caused by ESBL organisms. Identification of the ESBL organisms can help to treat the infections with appropriate antibiotics. Many peripheral laboratories find difficult to identify ESBL producers despite the availability of CLSI recommended simple and cost effective method.

BETA-LACTAMS AND BETA-LACTAMASES

All β-lactam antimicrobial agents share the common central four member β-lactam ring. Additional substituent groups added to the β-lactam ring determines whether the agent is classified as a penicillin, cephem, carbapenem, or monobactam. Beta-lactamases open the β-lactam ring, inactivating the antibiotics. There are over 340 different types of β-lactamases. These are mainly ESBLs, AmpC and carbapenemases. Beta-lactamases which had the ability to confer resistance to the extended spectrum cephalosporins were named as Extended Spectrum β-Lactamases (ESBLs). AmpC type β-lactamases are produced by organisms such as *Enterobacter cloacae*which are not inhibited by clavulanic acid but are inhibited by boronic acid. Carbapenemases are another group of β-lactamases which have the ability to hydrolyze carbapenem group of drugs along with penicillins, cephalosporins and monobactams. Important organisms that produce carbapenemases are *Pseudomonas aeruginosa* and *Acinetobacter* spp.[2]

CLASSIFICATION

Bush Jacoby Medeiros classified β-lactamases on the basis of functional similarity with regards to substrate and inhibitor profile. The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognized (A-D). ESBL comes under Group 2be and Class A by functional and molecular classification respectively.

TYPES

There are various types of ESBL such as SHV, TEM, CTX-M and Toho β-lactamases, OXA, PER, VEB-1, BES-1 and others. More than 700 ESBL have been

described, with more than 100 in the TEM class, over 40 in the SHV family, over 30 in the CTX-M class, and 15 in the OXA family.[3]

TEM-1 is the most commonly encountered β-lactamase in Gram negative bacteria. TEM-1 is able to hydrolyze ampicillin at a higher rate. TEM-2 has the same hydrolytic profile as TEM-1, but differs from TEM-1 by having a more active native promoter and by a difference in isoelectric point.

TEM and SHV are transferred by both plasmid and chromosome.SHV refers to sulfhydryl-variable. The SHV type ESBL are more frequently found in clinical isolates than any other type of ESBL. SHV type ESBL has been detected in a wide range of Enterobacteriaceae. Outbreaks of SHV producing *Pseudomonas aeruginosa* and *Actinobacter*spp have been reported in different studies.[4, 5]

CTX-M enzymes were named for their greater activity against cefotaxime than other oxyimino-β-lactam drugs.[6] Recently CTX-M type ESBL has emerged and has become increasingly common over TEM and SHV type of ESBL.[7]

OXA type β-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin. These enzymes are mainly found in *Pseudomonas aeruginosa*[8].

PER type of ESBL shares around 25% to 27% homology with known TEM and SHV type of ESBL. Toho-1 and Toho-2 are β-lactamases related structurally to CTX-M type β-lactamases. Each ESBL has unique hydrolytic activity on oxyimino β-lactams.

DISCOVERY

The first report of plasmid encoded β-lactamase, capable of hydrolyzing the extended spectrum cephalosporin was published in 1983 from Germany.[8] ESBL was then detected in France among *Klebsiella* spp. China reported first *K. pneumoniae* harboring SHV-2 type ESBL in 1988.[9] Consequently, the spread of ESBL became global.

PREVALENCE

Early ESBL infections were commonly reported in hospitalized patients. However, in recent years, ESBL producing organisms are commonly isolated from community acquired infections. In the last few years, CTX-M type enzymes appear to be the most widespread variants of the ESBL types. High prevalence of CTX-M in both the community and the hospital environment is suggestive of a dynamic flow of genes coding for these enzymes.

The prevalence of ESBL producers varies greatly from place to place. A national surveillance reported increase in ESBL in European countries.[10, 11]*E. coli* isolated from urine was the most common organism producing ESBL in Dhaka Medical College, Bangladesh.[12]Many studies from India reported increase in prevalence rate of ESBL organisms.[13-15] Similarly, China reported increase in ESBL *E. coli* of CTX-M type.[16] There are very few authentic studies on ESBL organisms in Nepal. Most of them are from the central part of the country. Prevalence rate of ESBL organisms in Nepal ranges from 14.26%-72% as reportedbyvarious studies over the past decade.[17-27]

MECHANISM OF DRUG RESISTANCE

Beta-lactamase production is the important mechanism of drug resistance among the Gram negative bacteria. Acquired resistance to β-lactam antibiotics is mainly mediated by ESBL. Increasing prevalence of ESBL producers among Enterobacteriaceae might be because of dissemination of resistant traits, proliferation of epidemic strains, and the transfer of the resistant gene carrying plasmids.[28]Plasmids carry the resistant gene to other commonly used antibiotics such as quinolones and aminoglycosides as well which is transmitted to

Table 1. Epidemiology of ESBL organisms in Nepal

Year	Organism	Place	Author	Prevalence
2015	*E.coli*, *Klebsiella* spp	Pokhara	Raut S[19]	22.4%
2015	*E.coli*	Chitwan	Ansari S[20]	24%
2014	*E.coli*	Kathmandu	Pokhrel RH[22]	25.8%
2013	*E.coli*	Kathmandu	Thakur S[17]	31.57%
2013	*E. coli, K. pneumoniae*	Kathmandu	Chander A[23]	14.26%
2013	Gram negative bacilli	Kathmandu	Khanal S[24]	25%
2012	*Salmonella* spp	Kathmandu	Gautam K[25]	72%
2012	Gram negative bacilli	Kathmandu	Dhakal S[26]	42.37%
2011	Gram negative bacilli	Dharan	Shrestha S[27]	54%
2011	*E.coli, K. pneumoniae*	Kathmandu	Paudyal S[18]	62.7%

other organisms together. In addition, exposure to antibiotics particularly to ceftazidime and aztreonam has been associated with an increased prevalence of ESBL producing organisms.[29]

METHODS OF ESBL DETECTION:

Many laboratories have reported challenges in detecting ESBL mediated resistance mainly because of the cost cutting practices. In addition, many peripheral laboratories are unaware of the relevant CLSI guidelines for ESBL detection. Since the early years when ESBLs were detected, a number of testing methods have been suggested.

DOUBLE DISK DIFFUSION TEST (DDDT)

This test employs a β-lactamase inhibitor, usually clavulanate, in combination with an oxyimino cephalosporin such as ceftazidime or cefotaxime. Clavulanate inhibits ESBL, which is evidenced by increased zone size of inhibition with oxyimino-cephalosporin+clavulanate disk as compared to the zone size of inhibition with an oxyimino-cephalosporin disk alone.[30]

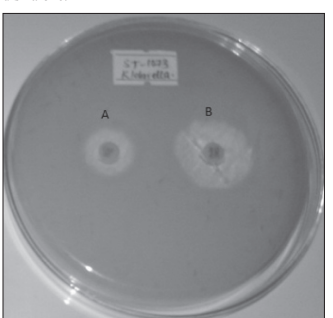

Fig. 1.Double Disk Diffusion Test; Disk A: Ceftazidime (30μg) alone, and Disk B: Ceftazidime+Clavulanic acid (30μg+10μg)

DOUBLE DISK SYNERGY TEST

This test is performed by placing disk containing amoxicillin+clavulanate and disk containing one of the oxyimino β-lactam antibiotics, 30 mm apart in the lawn culture of the test isolate. Enhancement of the zone of inhibition of the oxyimino β-lactam disk caused by the synergy of the clavulanate in the amoxicillin+clavulanate disk is a positive result.

SCREENING BY DILUTION ANTIMICROBIAL SUSCEPTIBILITY TESTS

Test organism is grown in a broth containing 1μg/ml of one of the five expanded spectrum β-lactam antibiotics (aztreonam, cefotaxime, ceftazidime, cefpodoxime and ceftriaxone). Turbidity in the broth is to be reported as suspicious for the presence of an ESBL. Screening test is followed by a phenotypic confirmatory test that consists of determining MIC of either ceftazidime or cefotaxime with and without the presence of clavulanate (4μg/ml). A decrease in the MIC of ceftazidime or cefotaxime >3 twofold dilutions in the presence of clavulanate is indicative of the presence of an ESBL.[31]

E-TEST FOR ESBL

E-test ESBL strips are two sided strips that contain a concentration gradient of ceftazidime on one end and ceftazidime plus clavulanate on the other end. A positive test for an ESBL is a >3 two-fold dilution reduction in the MIC of ceftazidime in the presence of clavulanate as compared to the MIC of ceftazidime alone.[32] This method is convenient and easy to use, however, it is expensive.

BD PHOENIX AUTOMATED MICROBIOLOGY SYSTEM

This testuses growth of organisms in the presence of cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime, with or without clavulanic acid, to detect ESBL production. Semi-automated systems are widely used for species identification and susceptibility testing in clinical laboratories. This test consumes less time and is cost effective.[33]

VITEK ESBL CARDS

This test utilizes either ceftazidime or cefotaxime and the combination with clavulanic acid (4μg/ml). A predetermined reduction in growth in wells containing clavulanic acid compared to those containing cephalosporin alone indicates the presence of an ESBL.[34]

MOLECULAR DETECTION METHODS:

Early detection of genes responsible for β-lactamase production was performed using DNA probes. However, the easiest and most common molecular method used to detect the presence of a β-lactamase gene is PCR with oligonucleotide primers that are specific for a β-lactamase gene.[1]

DDDT is the efficient and the cost effective methods for use in many clinical laboratories. This method is highly sensitive and specific for phenotypic confirmation of ESBL organisms even when compared to the genotypic methods.[8] Currently, CLSI recommends ESBL phenotypic confirmatory test only for *E. coli, Klebsiella* sppand

Proteus mirabilis.There are no CLSI recommendations for detection and reporting of ESBL in other members of Enterobacteriaceae.[30]

CLINICAL SIGNIFICANCE OF ESBL PRODUCTION

ESBLs are most often detected in *K. pneumoniae* and *E. coli.* The majority of ESBL producing organisms have been reported from hospitalized patients admitted to intensive care units (ICU).[35] Frequency of colonization and infection with ESBL *K. pneumoniae* is very high in ICU patients.[36]ESBL productions may predict therapeutic failure with extended spectrum cephalosporin drugs. Isolation of ESBL organisms from a clinical sample is an indication for using combination drugs like cephalosporins plus clavulanic acid or carbapenems.

MODES OF SPREAD OF ESBL PRODUCING ORGANISMS

Various common environmental sources have been identified which can contribute to the spread of ESBL organisms within hospitals. Some of them are ultrasonography coupling gel, bronchoscopes, blood pressure cuffs, and glass thermometers.[8] Cockroaches have been implicated as possible vectors of ESBL infection. In one recent study, ESBL producing *K. pneumoniae* isolated from cockroaches was indistinguishable from those infecting patients.[37] Transient carriage on the hands of health care workers has been found as a means of transfer of multidrug resistant organisms from patient to patient.[38]

RISK FACTORS

Previously hospitalized patients and patients who received cephalosporins during the previous months have an increased risk of bacteremia by ESBL *E. coli.* Mortality rate has been found to be higher in patients with ESBL infections.[39] Various studies have found factors associated with ESBL infections such as increased length of stay in the ward or ICU, severity of illness, respiratory or urinary tract infections, malignancy, indwelling urinary catheter, dialysis, age > 65 years, functional dependence, emergency abdominal surgery, and prior administration of antimicrobials.[40,41] ESBL Gram negative bacilli were commonly isolated from cancer patients particularly, who were exposed to antibiotics and were on indwelling urinary catheter.[42]

DRUG OF CHOICE

Carbapenems are considered to be the drugs of choice for the treatment of severe infections caused by ESBL organisms.[8] Etrapenem has been suggested for the treatment of community acquired infections.[43] Studies have reported temocillin to have good *in vitro* activity against ESBL producing *E. coli*.[44]Tigecycline is another alternative for treatment of such infections.[45] Drugs like fosfomycin, nitrofurantoin and mecillinam are important oral treatment options for ambulatory urinary tract infections caused by ESBL *E. coli*.[46] However, these*in vitro* promises will require support from more clinical studies. Currently, carbapenems are the only hope for treating ESBL infections effectively. Unfortunately, carbapenem resistant organisms have been evolved. Colistin is preferred to treat MBL-producing pathogens which are resistant to carbapenems.[47]

DRUG RESISTANCE AND THEIR IMPACT ON SOCIETY

Drug resistance has huge impact on the society both clinically and economically. In order to improve the situation, firstly, the practice of antibiotic prescriptions for viral infections such as common cold needs to be closely scrutinized and discouraged. Secondly, use of appropriate antibiotic to treat the infections on the basis of antibiotic susceptibility tests needs to be prioritized**.** Prescribing inappropriate antibiotics has been found to be an important factor that can lead to increase in treatment expenditure, increase incidence of preventable adverse effects, consequent treatment failure, prolonged stay in the hospital and subsequent antibiotic resistance.[48]

CONCLUSION

The prevalence of ESBL organisms (prevalence from various studies ranged from 14 to 72%) are at rising trend in Nepal, however, much of these organisms are undetected primarily because they are not routinely tested. ESBL organisms can be identified easily by DDDT in any peripheral laboratory of Nepal equipped with minimum resources and facilities. DDDT ought to be introduced immediately to every laboratory nationwide in order to detect and control ESBL infections. In addition, routine identification of ESBL organisms in laboratories can help to assess theactual burden of ESBL organisms in the country. For the treatment of these ESBL infections, currently, carbapenems are the recommended drug of choice. Rational use of antibiotics (based on the laboratory detection and sensitivity) is imperative to prevent the increasing emergence of drug resistant bacteria including ESBL producing organisms.

ACKNOWLEDGEMENT

We would like to thank the e-library of Lumbini Medical College for the generous support in literature search.

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