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Review Article

EXTENDED SPECTRUM BETA-LACTAMASES AND BACTERIAL RESISTANCE

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SUMMARY: In modern medical practice, newer antimicrobial drugs have been used extensively resulting in the emergence and rapid dissemination of resistant bacterial strains. Extended spectrum beta-lactamases (ESBLs) are enzymes that originate by mutations in genes for common plasmid mediated beta-lactamases such as TEM-1, TEM-2 and SHV-1 and are transmitted among bacterial species. Klebsiella sp. and E. coli are the two most frequently ESBLs producing bacteria worldwide with different degree of resistance in different countries. Resistance to third generation cephalosporins and susceptibility to beta-lactamase inhibitor compounds such as clavulanic acid, sulbactam and tazobactam are considered in favour of ESBL. These bacteria are considered resistant to all extended-spectrum penicillins, cephalosporins and monobactams but beta-lactamase-stable beta-lactam (e.g., imipenem) are active in vitro and also appear to be clinically effective. The genes that code for production of ESBLs are often linked to other resistance genes causing extended spectrum of drug resistance. Although different laboratory techniques to detect ESBL are available but there is no International consensus regarding its standardization. But general guidelines are advised to follow by the laboratories to detect ESBL on their own settings.

INTRODUCTION

Bacterial resistance to the beta-lactam drugs and the mechanisms leading to this resistance are gaining importance as a field of interest of medical researchers throughout the world. The term Extended-spectrum beta-lactamase (ESBL) refers to beta lactamase enzymes produced mainly by Klebsiella sp and E. coli that confer resistance to beta-lactam antibiotics[1].

Soon after the first detection of multiple transferable drug resistant strains in Japan its importance in microbial genetics have launched wider searches for it in different bacteria. Moreover, quick emergence of resistance to the third generation cephalosporins by gram-negative bacilli in 1980s has also widened the area of research in a new dimension[2]. Bacteria acquire resistance to beta-lactam drugs by several mechanisms, which include spontaneous mutation in chromosomal DNA or by acquiring transferable plasmid that mediates beta-lactamases[3]. ESBLs are newly described enzymes that arise by mutations in genes for common plasmid mediated beta-lactamases such as TEM-1, TEM-2 and SHV-1 and much of the dramatic increase in bacterial resistance to beta-lactam antibiotics has been associated with it[4]. Difficulty in the detection of ESBL production using routine antimicrobial susceptibility testing methods has also been documented by researchers[5].

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ESBL producing isolates are considered to be resistant to all extended spectrum of penicillins, cephalosporins (e.g. ceftazidime, cefotaxime and ceftriaxone) and monobactams (e.g. aztreonam) even if they appear to be susceptible to these agents in vitro. But drugs like cephamycins (e.g. cefoxitin and cefotetan) or carbapenems (e.g. meropenem or imipenem) show good efficacy in clinical applicaitons[6].

GENETIC BASIS OF ESBL

A common mechanism of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase enzymes that break down the structural beta-lactam ring of penicillin and its synthetic derivatives. Although the genetic control of beta-lactamase production resides either on plasmids or on the chromosome, the expression is either inducible or constitutive. Also beta-lactamases are encoded by genes located on transposons[7]. It is assumed that most probably beta-lactamases were selected in environments where beta-lactam producing fungi compete with bacteria for survival. The genes that code for production of ESBLs are often linked to other resistance genes thus, ESBL-producing isolates are sometimes multiple drugs resistant (e.g. resistant to aminoglycosides and trimethoprim-sulfamethoxazole)[8].

Until now more than 40 of these genes encoding extended spectrum enzymes (TEM-2, TEM-3, SHV-2 etc.) have been discovered and their enhanced stability and extended spectrum in the presence of beta-lactam antibiotics resulted from point mutations[9,10]. Further analysis at molecular level revealed that SHV-2 and TEM-7 differ from their progenitors by a single amino acid substitution[11].

LABORATORY DETECTION OF ESBL PRODUCTION

International consensus guidelines on the detection of ESBLs have yet to be developed. In particular, there is no agreement on which isolates should be tested for these enzymes, what indicators should direct further testing, what methods should be used, and how the findings should be reported. Klebsiella pneumoniae and Escherichia coli are most frequently associated with ESBL production[12] while Enterobacter aerogenes, E. cloacae, Serratia marcescens, Morganella morganii, K. oxytoca, Citrobacter freundii, and C. koserii appear to less frequent bacteria[13]. However, the detection methods used for K. pneumoniae and E. coli have not shown to be valid for other ESBL-producing bacteria.[14]

Disk approximation method is one of the currently available methods for the detection of the ESBLs[15]. In this method, amoxicillin-clavulanic acid disk is placed in the center of an inoculated plate with the test bacteria containing ceftriaxone, aztreonam, cefotaxime and ceftazidime disks that are placed 20 to 30 mm away from the amoxicillin-clavulanic acid disk. Enhancement of the zone of inhibition between either of the cephalosporins disks and clavulanate containing disk indicates the presence of an ESBLs. This is known as double disk diffusion method for detection of ESBLs production. Double disk tests can lack sensitivity because of various problems like optimal disk spacing, the inability of clavulanate to inhibit all ESBLs and the limitations of the test in detecting ESBLs in strains that also produce chromosomal cephalosporinases[16].

Another method for detecting ESBLs known as three-dimensional test, is a modification of the disk diffusion test with the advantage of simultaneous determination of antibiotic susceptibility and beta-lactamase substrate profile information. Thompson et al.[17] have compared between these two methods for ESBL detection and found sensitivity of 93% and 79% in the modified and disk diffusion test respectively. The vitek (bioMereiux Vitek, Hazelwood, Mo.) susceptibility cards for ESBL test is the recent addition. Vitek cards are interpreted by the Vitek AutoMicrobic System by using appropriate software. Sanders et al. (1996) compared the vitek ESBLs test care with double disk test and found almost same results[18].

Detection and validation of ESBL-producing enteric bacilli by E-test is also being performed

by different centres with some encouraging results[19].

Despite the advent of newer technologies, ESBLs detection remains difficult because they have different levels of activity against various cephalosporins. Thus, the choice of antimicrobial agents to be tested remains critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime minimum inhibitory concentrations (MICs) of 256 g/ml, but have poor activity on cefotaxime, producing MICs of only 4 g/ml. Therefore, if an ESBL is detected, all penicillins, cephalosporins and aztreonam should be reported as resistant[20].

The National Committee for Clinical Laboratory Standards (NCCLS) has developed broth microdilution and disk diffusion screening tests using selected antimicrobial agents[4]. The sensitivity of screening for ESBLs in enteric bacteria can vary depending on types of antimicrobial agents tested. The use of more than one of the five antimicrobial drugs suggested for screening will improve the sensitivity of detection. Cefodoxime and ceftazidime show the highest sensitivity for ESBL detection.

NCCLS also recommends performing phenotypic confirmation of potential ESBL-producing isolates of K. pneumoniae, K. oxytoca, or E. coli by testing both cefotaxime and ceftazidime alone and in combination with clavulanic acid[21]. K. pneumoniae ATCC 700603 (positive-control) and E. coli ATCC 25922 (negative-control) should be used for quality control of ESBL tests. Some organisms with ESBLs contain other lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. These lactamases include AmpCs and inhibitor-resistant TEMs. Moreover, detection of organisms with multiple-lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing[22]. Currently, these methods are not available in most of the clinical laboratories.

ESBL PRODUCING ORGANISMS IN DIFFERENT COUNTRIES

ESBL have been reported from many parts of the world since 1983. The distribution of ESBL in E. coli is 5% and 23.3% in Korea and Indonesia respectively which is higher when compared to North America[23] or Europe, but similar to that of South America[24]. 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 sp. were reported from Korea[25].

In the United States, the frequency of resistance to ceftazidime has increased from 1.5% (1987 to 1999) to 3.6% (1990 to 1991) as reported by the National Nosocomial Infections Surveillance system[13]. A surveillance trial involving 102 medical centers in the United States detected 10.3% and 23.8% ceftazidime resistant E. coli and K. pneumoniae respectively. Antimicrobial susceptibility pattern of Acinetobacter sp was reported by Nalinee et al. (1998) from Thailand[26] and found that more than 50% of the isolates were resistant to tetracycline and co-trimoxazole, 30-50% resistant to amikacin, cefotaxime, ceftazidime and ciprofloxacin, 10-21% of the isolates resistant to sparfloxacin, cefepime and piperacillin-tazobactam only 2.5% were resistant to ampicillin-sulbactam and none was resistant to imipenem. In France, Jarlier et al. (1988) studied the transmissible resistance in E. coli K12 recipient and reported that the drug resistance particularly by ESBL producing plasmids are transferable[27].

DISCUSSION

ESBLs are enzymes found in a variety of Enterobacteriaceae and are frequently resistant to many classes of antibiotics, resulting in treatment failures. Major problems with these resistant strains are difficulty in detecting the presence of ESBLs, limited treatment options and deleterious impact on clinical outcomes. The NCCLS has recently described screening and confirmatory tests for detection of ESBLs. In addition, several phenotypic characteristics can be used to assess the mechanism of resistance without additional testing[28]. In the mid 1980's, it became evident that a new type of beta-lactamase was being produced by Klebsiella sp. and in some cases by E. coli that could

hydrolyze the extended spectrum cephalosporin[29]. Moreover, beta-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam do not inhibit these extended spectrum beta-lactamases sufficiently. Recently, it has been evident that the cephamycins (cefoxitin, cefotetan, moxalactam) have diminished activity against the ESBL-producing bacteria. More troubling is the observation that resistance to non-beta-lactamases is associated with ESBLs. Other members of Enterobacteriaceae, such as Salmonella sp., Proteus mirabilis, and isolates of Pseudomonas aeruginosa also produce ESBLs[30]. Unfortunately, at this time, standardized methods for screening of ESBL and phenotypic confirmatory testing of these isolates have not been determined and/or recommended. Nonetheless susceptibility testing using the revised criteria still may fail to detect low or relatively low expression of ESBL production. Additional testing to detect such production on a routine basis is not considered clinically necessary or cost-effective. Selective testing for ESBL production should be considered for gram-negative enteric bacilli isolated from normally sterile body sites and where the infection may have been nosocomially acquired.

CONCLUSION

Development of bacterial resistance against various antimicrobials is a long continued problem in Clinical Medicine. Although there is increasing observations and detection of Klebsiella and E. coli as well as other Enterobacteriaceae that express ESBLs but the available laboratory identification techniques are still in an evolving state. Moreover, many ESBLs producing E. coli and Klebsiella strains do not appear resistant to newer cephalosporins or aztreonam in routine in-vitro susceptibility tests.

The identification of resistant phenotypes is particularly important in developing countries where there is no good control of antibiotic abuse and medical centres that do not maintain adequate epidemiological surveillance. Failure to recognize ESBL producing strains may not only result in inappropriate beta-lactam therapy and consequent treatment failure but also have infection control implications and threaten the future usefulness of many beta-lactam agents.

A series of work by many investigators have tried to establish the general guidelines for suspected and probable ESBLs producing strains of Klebsiella sp. and E. coli. Laboratories can effectively follow these guidelines in their own situations to help monitor the emergence of ESBLs producing bacteria in order to face the challenging issue of antibiotic resistance.

REFERENCES

1. Sanders CC, Sanders WE Jr. Beta-lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin Infect Dis 1992;15:824-83.
2. Moosden F. The evolution of resistance to cephalosporins. Clin Infect Dis 1997;24:487-93.
3. Philippon A, Arlet G, Lagrange PH. Origin and impact of plasmid mediated extended spectrum beta-lactamases. Eur J Clin Microbiol Infect Dis 1994;13:17-29.
4. Wayne PA. National Committee for Clinical Laboratory Standard. Journal of Clinical Microbiology, 2001;39:591-95.
5. Livermore DM. Beta-lactamases in laboratory and clinical relevance. Clin Microbiol Rev 1995;8:557-84.
6. Meyer KS, Urban C, Eagan JA et al. Nosocomial outbreak of Klebsiella infection resistant to late-generation cephalosporins. Ann Intern Med 1993;119:353-8.
7. Sirot D, De Champs C, Chanal C, et al. Translocation of antibiotic resistance determinants including an extended spectrum beta-lactamase between conjugative plasmids of Klebsiella pneumoniae and Escherichia coli. Antimicrob Agents Chemother 1991;35:1576-81.
8. Jacoby GA. Genetics of extended spectrum beta-lactamases. Eur J Clin Microbiol Infect Dis 1994;10:867-78.
9. Poyart C, Mugnier P, Quesne G et al. A novel extended-spectrum TEM-type beta-lactamases (TEM-52) associated with decreased susceptibility to moxalactam in Klebsiella pneumoniae. Antimicrob Agents Chemother 1998;42:108-13.
10. Hall A, Knowles Jr. Directed selective pressure on a beta-lactamases to analyse molecular changes involved in development of enzyme function Nature 1976;264:803-4.
11. Dubois SK, Marriott MS, Amyes SGB. TEM and SHV-derived extended spectrum beta-lactamases: relationship between selection, structure and function. J Antimicrob Chemother. 1995;35:7-32.

12. Katsanis GP, Spargo J, Ferraro MJ, Sutton L, Jacoby GA. Detection of Klebsiella pneumoniae and Escherichia coli strains producing extended-spectrum beta-lactamases. J Clin Microbiol 1994;32:691-696.
13. Burwen DR, Banerjee SN, Gaynes RP. The National Nosocomial Infections Surveillance System J Infect Dis 1994;170:1622-25.
14. Katsanis GP, Spargo J et al. Detection of Klebsiella pneumoniae and Escherichia coli strains producing extended-spectrum beta-lactamases. J Clin Microbiol 1994;32:691-96.
15. Jacoby GA, Han P. Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli. J c\Clin Microbiol 1996;34:908-911.
16. Rahal JJ, Urban C, Horn D, et al. Class restriction of cephalosporins use to control total cephalosporins resistance in nosocomical Klebsiella. JAMA. 1998;280:1253-1257.
17. Thompson KS and Sanders CC. Detection of extended spectrum beta-lactamases in members of the family Enterobacteriaceae: Comparison of the double-disk and three-dimensional tests 1992;36:1877-82.
18. Sanders CC, Barry AL et al. Detection of extended spectrum beta-lactamases in members of the family Enterobacteriaceae with the vitek ESBL test. J Clin Microbiol 1996;34:2997-3001.
19. Cormican MG, Marshall SA, Jones RN. Detection of Extended-Spectrum beta-lactamases (ESBL)- producing strains by the Etest ESBL Screen. J Clin Microbiol 1996;34:1880-1884.
20. Jones RN, Pfaller MA et al. Antimicrobial activity and spectrum investigation of eight broad spectrum beta-lactamases drugs: A 1997 surveillance trial in 102 medical centres in the United States. Clin Infect Dis 1998;35:215-28.
21. Ambler RP, Coulson AFW et al. A standard numbering scheme for the class A beta-lactamases. Biochem J 1991;276:269-72.
22. Livermore DM. Beta-lactamases in laboratory and Clinical Resistance. Clin Microbiol Rev 1995;8:557-584.
23. Medeiros A. A. Nosocomial outbreaks of multiresistant bacteria: extended spectrum beta-lactamases have arrived in North America. Ann. Intern. Med 1993;119:428-30.
24. Moland ES, Thompson KS. Extended-spectrum beta-lactamases of Enterobacteriaceae. J Antimicro Chemother 1994;33:666-668.
25. Wiener J, Quinn JP, Bradford PA et al. Multiple antibiotic-resistant Klebsiella and Escherichia coli in nursing homes. JAMA. 1999;281:517-523.
26. Nalinee Aswapokee, Surapee Tiengrim et al. Antimicrobial resistant pattern of Acinetobacter sp. J Infect Dis 1998;15:43-48.
27. Jarlier V, Nicolas MH, Fournier G and Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactum agents in enterbacteriaceae: hospital prevalence and susceptibility pattern. Rev Infect Dis 1988;10:867-878.
28. Emery CL, Weymouth LA. Detection and Clinical Significance of Extended-Spectrum beta-lactamases in a Tertiary-Care Medical Centre. J Clin Microbiol 1997;35:2061-2067.
29. Bush K. Characterization of beta-lactamases. Antimicrob Agents Chemother 1989;33:259-63.
30. Watanabe NF, Katsu K, Moriyama M, Kitoh K. Transferbale imipenem resistance in Pseudomonas aeruginosa Antimicrob. Agents. Chemother 1991;35:147-51.