FREQUENCY OF EXTENDED SPECTRUM BETA–LACTAMASES (ESBL) PRODUCING NOSOCOMIAL ISOLATES IN A TERTIARY CARE HOSPITAL IN RAWALPINDI

Arif Maqsood Ali, *Brig Shahid Ahmed Abbasi, Mubasher Ahmed

Combined Military Hospital Okara, *Army Medical College Rawalpindi

ABSTRACT

Objectives of Study: To find out the frequency of extended spectrum beta–lactamases (ESBL) producing organisms among Gram negative rods from clinical specimens.

Design: This was a descriptive study.

Setting: The study was carried out in the Microbiology Department of Army Medical College, Rawalpindi from 1 Jan 03 to 31 Dec 03 on clinical samples received from admitted patients in Military hospital, Rawalpindi.

Materials and Methods: It was carried out on clinical specimens of urine, blood, pus, catheter tips, fluids including CSF, sputum, chest tube, HVS and i/v canula/CVP line obtained from admitted patients in Military Hospital, Rawalpindi. The organisms were identified by standard techniques. Confirmation to the species level was done by API 20 E & API NE where required. Sensitivity testing was carried out by Modified Kirby Bauer disc diffusion method on Mueller Hinton agar incubated at 35o C in ambient air for 24 hrs. ESBL producing strains were identified by double disc diffusion method test according to Jarlier et al. Clavulanate was applied as the inhibitor of beta lactamases (AMO/CLAV disc). The results were tabulated as frequencies.

Results: Forty three percent of clinical isolates yielded ESBL producing gram negative rods.

Enterobacter cloacae (76%), Klebsiella oxytoca (68%) Acinetobacter baumanni, (63%) and Aeromonas hydrophila (50%) were the most frequent ESBL producing bacteria.

Conclusion: Production of ESBL among Gram negative rods is frequent in Military Hospital, Rawalpindi. Infection control measures are required to control their spread.

Keywords: ESBL, infection control, antibiotics resistance

INTRODUCTION

Esistance to antimicrobial agents, which was recognized more than 50 years ago, continues to be a major cause of increased morbidity, mortality and health care cost. Overuse of antibiotics is considered the major contributing factor. However, poor implementation of infection control measures, prolonged hospitalization, admission to intensive care units and the use of invasive procedures are other contributing factors. Antibiotic resistance has become a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is unknown [1].

Drug resistance is more frequently encountered in hospital-acquired pathogens. However, the incidence of antibiotic-resistant pathogens in community-acquired infections has also been on the rise in recent years [2].

More worrisome is the emergence of multidrug resistance shown by certain strains of gram-negative bacteria such as Pseudomonas sp, Klebsiella sp., Enterobacter sp., Acinetobacter sp., Salmonella species and gram-positive organisms such as Staphylococcus sp., Enterococcus sp. and Streptococcus species. In recent years there has been a steady increase in frequency of methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus species and extended spectrum beta-lactamase producting Klebsiella pneumoniae and Escherichia coli [3].

ESBL-producers are detectable using standard disk interpretive criteria or by the

Correspondence: Maj Arif Maqsood Ali, Pathologist, Combined Military Hospital Okara
Email: drarifmaqsood@hotmail.com

Received 28 Nov 2007; Accepted 27 May 2008

currently available automated methods according to the CLSI, previously NCCLS guidelines [4].

OBJECTIVE OF THE STUDY

This descriptive study was carried out in the Microbiology Department of Army Medical College, Rawalpindi from 1 Jan to 31 Dec 2003 on clinical samples received from admitted patients in Military hospital, Rawalpindi.

The samples received were initially inoculated on blood agar and Mac Conkey agar beside chocolate agar (in case of CSF & sputum). Urine samples were inoculated on Cystiene lactose electrolyte deficient (CLED) agar. The samples were incubated at 37^{O} C under aerobic conditions. The organisms were identified by standard techniques including sugar sets. Confirmation to the species level was done by API 20 E & API NE along with the sensitivity testing on the following day Modified Kirby Bauer disc diffusion method on Mueller Hinton Agar. ESBL production was detected by by double disc diffusion method of Jarlier et al [4]. A susceptibility disk containing amoxicillin-clavulanate was placed as the inhibitor of beta lactamase in the center of the plate, and cefotaxime, ceftazidime, ceftriaxone and aztreonam disks were placed 30 mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-β-lactam caused by the synergy of the clavulanate in the amoxicillin-clavulanate disk indicates β-lactamase production. The quality control strains used for disk diffusion testing were *E. coli* ATCC 25922 and *K.* pneumoniae ATCC 700603. A total of 2032 Gram negative nosocomial isolates from various samples including urine, blood, pus, catheter tips, fluids including CSF, sputum, chest tube, HVS and i/v canula/CVP lines were included in the study.

Inclusion Criteria

All the specimens received for culture & susceptibility were processed. All Gram negative rods were included in the study.

Exclusion Criteria

Duplicate specimens yielding ESBL producing Gram negative rods from same patient were excluded from the study.

Data Analysis

Data had been analyzed using SPSS version 10.0. Frequencies alongwith percentages were used to describe the data.

RESULTS

Total 2125 gram negative rods were included in the study. Forty three percent (920/2125) gram negative rods yielded ESBL. Frequency of different samples yielding ESBLs was given in table 1. Proteus vulgarism and providencia sp were 100% ESBL producers followed by Entero bacter (76%), Klebsiella oxytoca (68%). Acinetobacter baumanni (63%) and Aeromonas hydrophila (50%) (Table-2). ESBL producing Escherichia coli and Klebsiella pneumoniae were most frequently isolated in catheter tips, urine, pus and blood (Table-2)

DISCUSSION

Gram-negative bacteria are important causes of nosocomial infections. These include Escherichia coli, Citrobacter sp., Klebsiella sp., Serratia sp, Proteus sp, Morganella sp, Providencia and Enterobacter species, Pseudomonas aeruginosa, Acinetobacter species, Stenotrophomonas maltophilia and Burkholderia cepacia. The majority of the infections caused by these organisms occur in hospitalized patients in the intensive care units. Risk factors include underlying illnesses such as malignancy, immunosuppressive disorders, burns, prematurity in pediatric patients, intravascular and/or central nervous system devices, mechanical ventilation and

indwelling urinary catheters. The spectrum of nosocomial infections comprises blood stream, urinary tract, respiratory tract, gastrointestinal tract, central nervous system, skin and wound infections [5].

Over the past several decades the treatment of most Gram-negative bacteria has been problematic due to their intrinsic and acquired ability to develop antimicrobial resistance. However, the most important mechanism of antimicrobial resistance among Gram-negative organisms is the production of beta-lactamases [6]. In 1983, a new group of enzymes designated extended-spectrum beta-lactamases (ESBL) was detected among K. pneumoniae and Serratia marcescens [7].

These plasmid-mediated groups of enzymes are the end products of point

Table-1: Distribution of Specimens Cultured, Esbls & Gram Negative Rods.

Type of Specimen	No. of Specimens	No. of ESBLs isolated	No. of Gram Negative Rods isolated	% of ESBL
Urine	6365	245	712	34%
Blood	9791	162	359	46%
Pus	1698	269	605	45%
Catheter tip	325	66	90	74%
Fluids (pertonial, Asctic)	1361	84	113	74%
CSF	977	9	11	82%
Sputum	1635	60	177	34%
HVS	1545	14	51	27%
Cvp/iv Canula	25	4	6	67%
Chest tube	24	7	8	87%
Total	23746	920	2125	43%

Table-2: Number of ESBLs, Gram Negative Rods Isolated Among Different Specimens and their Frequency

Organism	Urine	Blood	Pus	Catheter tip	Fluids (pertonial, Asctic)	CSF	Sputum	Hvs	Cvp/iv canula	Chest Tube	Total
E. coli	140/512	60/96	63/192	25/32	19/22	2/2	18/30	9/37	3/4	3/4	342/931 (37%)
K.pneumoniae	57/94	82/185	61/143	17/25	8/13	1/1	12/30	4/10	1/2	1/1	244/504 (48%)
K.oxytoca	-	-	3/3	3/3	1/1	-	6/12	-	-	-	13/19 (68%)
Citrobacter	3/10	3/15	3/3	6/6	0	0	0	0	0	0	15/34 (44%)
Enterobacter	23/40	14/17	31/35	3/3	9/9	1/1	3/6	0	0	1/1	85/112 (76%)
Acinetobacter baumanii	9/9	2/19	30/42	6/6	18/28	2/2	3/6	0/1	0	2/2	72/115 (63%)
Acinetobacter lwofii	3/3	1/14	3/6	0/1	0	0	0	0	0	0	7/24 (29%)
Proteus mirabilis	6/12	0	4/27	0/2	9/10	2/2	0	0	0	0	21/53 (40%)
Proteus vulgaris	0	0	6/6	3/3	0	0	0	0	0	0	9/9 (100%)
Aeromonas hydrophila	0	0	3/6	0	0	0	0	0	0	0	3/6 (50%)
Providencia	0	0	1/1	0	0	0	0	0	0	0	1/1 (100%)
Pseudomonas	4/32	0/9	61/139	3/8	20/30	1/3	18/93	1/3	0	0	108/317 (34%)

mutations at the active site of TEM, SHV and OXA enzymes [8].The ESBLs hydrolyze penicillins, extended spectrum cephalosporins with an oxyimino side chain including ceftazidime, ceftriaxone and cefotaxime, and oxyimino-monobactam antibiotics such as aztreonam. The frequency of ESBL-producing Gram-negative bacteria has increased in recent years [9, 10]. In some US hospitals, the rate of ESBL positive K. pneumoniae is as high as 40% and in some French hospitals the rate approaches 50% [11].An Indian study showed the prevalence of ESBL phenotypes in E. coli and K. pneumoniae to be >61% and >55% respectively [12]. In a recent international multicenter study Paterson et al found 18.7% of 455 K. pneumoniae isolates to be ESBL-producers [13].

In our study, 48% of K Pneumoniae and 37 % of E. coli were ESBL producing.

ESBLs are commonly expressed in K. pneumoniae but have also been detected in other Enterobacteriaceae and P. aeruginosa. In

an Italian multicenter study, ESBL were found in 28.1% of 96 isolates of Proteus stuartii, 20.5% of 151 isolates of Enterobacter aerogenes and 16.3% of 85 isolates of Proteus mirabilis but was also seen in Citrobacter, Klebsiella, Proteus, Enterobacter species, E. coli, Morganella morganii, and Serratia marcescens [14].

In a study carried out in All India Institute of Medical Sciences, New Delhi 68% isolates were found to be ESBL producers. Among the bacterial species, ESBL production was most common in Klebsiella spp. (80%) [15]. In a study carried out in a tertiary care institute in India 63.7% (214/336) of E. coli followed by K. pneumoniae 14% (47/336) and 11.3% (38/336) of Citrobacter spp. were ESBL producers [16]. In our study chest tubes (87%), CSF (82%) catheter tips (74%) and fluids (74%) yielded the highest frequency of ESBLs, although these specimens were less than other specimens studied. In our previous study ESBL producers detected were Enterobacter cloacae (79 %) followed by K. oxytoca (66.66%), Proteus mirabilis (61%) and K. Pneumoniae (57 %) [17].

The frequency of ESBLs in nosocomial infections reported from Islamabad and Armed Forces Institute of Pathology, Rawalpindi were 48% and 35% with Escherichia coli and Klebsiella spp. being the most frequent respectively [18]. The frequency of 43 % ESBL producing isolates in present study is similar to earlier reported frequencies in our setup but shows slight increase [17].

A number of measures have been adopted to control nosocomial outbreaks caused by ESBL producing bacteria. Since increased cephalosporin usage is associated with increase in infections caused by ESBL producing organisms and control of cephalosporin usage is associated with a decrease in infections, several centers have targeted cephalosporin usage as part of their control program [19, 20]. ESBL producing organisms are spread between patients in a manner similar to that of other nosocomial organisms, namely through the contaminated hands and equipment of healthcare workers [21]. A substantial number of ICU staff had hand colonization with nosocomial strains of Klebsiella species during an outbreak [22].

CONCLUSION

Resistance to beta lactam antimicrobial agents among Gram negative rods due to ESBL's is quite frequent in nosocomial isolates. This rise may be due to overuse of penicillins and cephalosporins in hospitalized patients. Infection control measures to implement infection control polices in addition to judicious use of antibiotics are the needs of hour.

REFERENCES

1. Richet HM, Mohammed J, McDonald LC, Jarvis WR. Building communication networks: international network for the study and prevention of emerging antimicrobial resistance. Emerg Infect Dis 2001; 7: 2: 319-22.
2. Hooton TM, Levy SB. Antimicrobial resistance: a plan of action for community practice. Am Fam Physician 2001; 63: 6: 1087-98.
3. Ang JY, Ezike E, Asmar BI. Antibacterial resistance. Indian J Pediatr 2004; 71: 229-39.
4. Jarlier V, Nicolas M, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10: 4: 867-78.
5. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended-spectrum ß-lactamases conferring transferable resistance to newer ß-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10: 867-78.
6. Ang JY, Ezike E, Asmar BI. Antibacterial resistance. Indian J Pediatr [serial online] 2004 [cited 2007 Oct 21]; 71:229-39.
7. Burns JL. Mechanisms of bacterial resistance. Pediatr Clin of North America 1995; 42: 3: 479-507.
8. Mandell. In Mechanisms of Antibiotic Resistance. Principle and Practice of Infectious Diseases, 5th ed. Churchill Livingstone, Inc., 2000.
9. Kaye KS, Fraimow HS, Abrutyn E. Pathogens resistant to antimicrobial agents. Epidemiology, molecular mechanisms, and clinical management. Infect Dis Clin of North America. 2000; 14: 293-319.

10. Thomson KS. Controversies about extended-spectrum and AmpC beta-lactamases. Emerg Infect Dis 2001; 7: 2: 333-6.

11. Mathai D, Rhomberg PR, Biedenbach DJ, Jones RN. The India Antimicrobial Resistance Study Group. Evaluation of the invitro activity of six broad-spectrum B-lactam antimicrobial agents tested against recent clinical isolates from India: a survey of ten medical center laboratories. Diagn Microbiol Infect Dis 2002; 44: 367-77.

12. Patterson JE. Extended spectrum beta-lactamases: a therapeutic dilemma. Pediatr Infect Dis J 2002; 21(10): 957-60.

13. Paterson DL, Ko WC, Gottberg AV, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organism producing extended-spectrum B-lactamases: Implications for the clinical microbiology laboratory. J Clin Microbiol 2001; 39(6): 2206-2212.

14. 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.

15. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol [serial online] 2006 [cited 2007; 12]; 24: 208-211.

16. Ali AM, Rafi S, Qureshi AH. Frequency of Extended Spectrum Beta Lactamase producing Gram Negative Bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi. J Ayub Med Coll Abottabad 2004; 16: 1: 35-7.

17. Shah AA, Hasan F, Ahmed S, Hameed A. Extended-spectrum beta-lactamases in Enterobacteriaceae: related to age and gender. New Microbiol 2002; 25: 3: 363-6.

18. Zaman G, Karamat KA, Abbasi S, Rafi S, Ikram A. Prevalence of Extended-spectrum beta-lactamase (ESBL) producing enterobacteriaceae in nosocomial isolates. Pak Armed Forces Med J 1999: 49: 2: 91-6.

19. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of Klebsiella infection resistant to late-generation cephalosporins. Ann Intern Med 1993; 119: 353-8.

20. Rice LB, Eckstein EC, DeVente J, Shlaes DM. Ceftazidime-resistant Klebsiella pneumoniae isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. Clin Infect Dis 1996; 23: 118-24.

21. Gaillot O, Maruejouls C, Abachin E, Lecuru F, Arlet G, Simonet M, Berche P . Nosocomial outbreak of Klebsiella pneumoniae producing SHV-5 extended spectrum b-lactamase, originating from a contaminated ultrasonography coupling gel. J Clin Microbiol 1998; 36: 1357-60.

22. Casewell M, Phillips I. Hands as a route of transmission for Klebsiella species. Br Med J 1977; 2: 13.
