CHANGING TRENDS IN FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING GRAM NEGATIVE BACILLI IN INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To determine the frequency of isolation of extended spectrum beta lactamase (ESBL) producing Gram negative bacteria from intensive care units (ICUs) of a tertiary care hospital.

Study Design: Retrospective descriptive study.

Place and Duration of Study: The study was carried out at the department of microbiology Army Medical College Rawalpindi from Dec 2003 to Nov 2007.

Materials and Methods: This study was carried out from Dec 2003 to Nov 2007. A total of 590 consecutive Gram-negative bacilli were recovered during the four year study period from various samples including urine, blood, pus, sputum, high vaginal swabs (HVS), ascitic fluid, central venous lines (CVP), chest tubes, catheter tips, NBL (nasobronchial lavage), CSF, tissue, endotracheal tube (ETT) tip and pleural fluid in ICUs. Extended spectrum β-lactamase detection in these isolates was carried out by Kirby- Bauer double disc synergy method.

Results: The frequency of ESBL producing organisms was 84 (66%) (December 03 to November 04), 80(54%) (December 04 to November 05), 80(57%) (December 05 to November 06) and 82 (47%) (December 06 to November 07) ($p<0.0001$)

Conclusion: Our study shows a decrease in the frequency of ESBL producing organisms. However there is an increase in the resistant organisms having same resistance pattern, but not detected as ESBL producers, therefore we need to improve the methods for ESBL detection.

Keywords: Extended spectrum beta lactamases, Frequency, Intensive care units.

INTRODUCTION

The β-lactam antibiotics are among the most frequently prescribed antimicrobial agents worldwide. The emergence of resistance to these agents in the past two decades has resulted in a major clinical crisis [1]. A common mechanism of bacterial resistance to β -lactam antibiotics is the production of β-lactamase enzymes that break down the β-lactam ring of penicillin-like drugs. Extended spectrum β-lactamases (ESBLs) are enzymes inhibited by clavulanic acid and confer resistance to narrow- and expanded-spectrum cephalosporins, but do not affect cephamycin and carbapenem compounds [2]. The increasing use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of ESBL producing microorganisms [3]. Total number of ESBLs now characterized exceeds 2004. Extended spectrum β-lactamases were first isolated in Western Europe in 1983, most commonly in Klebsiella spp., followed by Escherichia coli [4, 5]. These plasmid mediated enzymes, TEM, SHV, CTX-M, OXA, PER and VEB-1 are detected throughout the world. Due to its frequent isolation CTX-M is now considered the most common ESBL type worldwide [4]. Most common ESBL detected in Pakistan is also CTX-M type [6]. These enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but are inactive against cephamycins and imipenem. Extended spectrum β-lactamases have serine at their active site and attack the amide bond in the β-lactam ring of antibiotics causing their hydrolysis. However, due to the variable affinity of these enzymes for different substrates and the inoculum's effect, identification of ESBL producers is a major challenge for the clinical microbiology laboratory. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family [5]. Prevalence of ESBLs vary throughout the world, in France 25 to 35% Klebsiella pneumoniae were ESBL producers whereas in Brazil, Columbia and

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Venezuela it ranges from 30 to 60%. In Turkey it is 58% and in China 34% Escherichia coli and 38% Klebsiella pneumoniae were ESBL producers.2Some studies in our neighboring countries show a prevalence of 6.6 to 68% in India [7] and 40% in Bangladesh [8]. In Pakistan a prevalence of 47% among nosocomial pathogens has been reported from Rawalpindi. The objective of this study was to determine the frequency of ESBL producing Gram-negative bacilli recovered from clinical specimens in ICUs and to see whether the methods of detection for ESBL producers are sufficient in our setup.

MATERIAL AND METHODS

This study was carried out at the department of Microbiology Army Medical College Rawalpindi from December 2003 to November 2007. A total of 590 consecutive non duplicate Gram-negative bacilli recovered from clinical specimens received from surgical and medical ICUs Military Hospital, Rawalpindi were included in the study. Details of patient disease and antibiotics used were not available. The organisms were isolated from various samples including urine, blood, pus, sputum, high vaginal swabs (HVS), ascitic fluid, central venous lines (CVP), chest tubes, catheter tips, nasobronchial lavage (NBL), CSF, tissue, endotracheal tube (ETT) tip and pleural fluid received from patients admitted in Military Hospital, Rawalpindi. The isolates were dealt with when they arrived. The samples received were initially inoculated on blood agar and McConkey agar besides chocolate agar (in case of sputum) where appropriate. Urine samples were cultured on cystiene lactose electrolyte deficient (CLED) agar. The samples were incubated at 37oC under aerobic conditions for 24 hours. The organisms were primarily identified by colony morphology, microscopy of Gram`s stain and routine biochemical tests9. Confirmation to the species level was done by API 20 E & API 20 NE (Biomerieux) where required. Discs of aztreonam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) and cefotaxime (30 µg) were placed 15 mm (edge to edge) from an augmentin (amoxycillin-clavulanate; 20/10 µg) disc. Inoculated plates were incubated overnight at 37oC. Enhancement of the zone of inhibition between the clavulanate disc and any one of the β-lactam discs indicated the presence of an ESBL [7]. Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were used as control strains. E. coli ATCC 25922 was used as the negative control and an in house ESBL producer was used as the positive control.

Data had been analyzed using SPSS version 15. Frequency and percentage were used to describe the data. Chi-squre test was used to check the significance change in different types of isolates year wise p-value <0.05 was considered as significance.

RESULTS

A total of 590 GNRs were included in the study. Year wise distribution of different types of isolates were given in table 1. A significant change in frequency types of isolates was observed year wise (P=0.0001). Among the ESBL producing GNRs 52.15% were Klebsiella pneumoniae, 38.65% were Escherichia coli, 4.60% were Enterobacter cloacae, 3.68% Citrobacter freundii and 0.92% Proteus mirabilis. (Table. 2) During four years Klebsiella pneumoniae isolated were 292. Among them ESBLs were 170(58.22%), non-ESBLs 92(31.51%) and sensitive 30(10.27%). Escherichia coli isolated were 234. Among them ESBLs were 126(53.85%), non-ESBLs 58(24.79%) and sensitive 50(21.37%). Enterobacter cloacae isolated were 32. Among them ESBLs were 15(46.88%), non-ESBLs12 (37.5%) and sensitive 5 (15.62%). Citrobacter freundii isolated were 24. Among them ESBLs were12 (50%), non-ESBLs 8(33.33%) and sensitive 4(16.67%).Proteus mirabilis isolated were 8. Among them ESBLs were 3(37.5%), non-ESBLs 4(50%) and sensitive1 (12.5%). (Table. 3) Among different specimens ESBLs were isolated as pus 22.08%,urine 16.26%,NBL 15.34%, CVP tip 9.51%, catheter tip 8.90%, sputum 6.44%, blood 6.44%, ascetic fluid 5.83%, ETT tip 3.37%, tissue 1.53%, HVS

1.53%, CSF 0.92%, pleural fluid 0.92% and chest tube 0.92%.

Table-1: Frequency of ESBL producers, resistant but non ESBL producers and sensitive organisms among total GNRs during four years 2004-2007 (n = 590)

Year	2004	2005	2006	2007	Total
Total GNRs	128	148	140	174	590
ESBL producers	84(66%)	80(54%)	80(57%)	82(47%)	326(55.25%)
Resistant but non-ESBL producers	18(14%)	42(28%)	48(34%)	66(38%)	174(29.50%)
Sensitive	26(20%)	26(18%)	12(9%)	26(15%)	90(15.25%)

P = 0.000135

Table-2: Frequency of ESBLs among Enterobactericiae (n = 326)

Klebsiella pneumoniae	170(52.15%)
Escherichia coli	126(38.65%)
Enterobacter cloacae	15(4.60%)
Citrobacter freundii	12(3.68%)
Proteus mirabilis	3(0.92%)

Table-3: Frequency of ESBLs, resistant but non-ESBLs and sensitive organisms among respective genus during four years 2004-2007 (n = 590)

Organisms	Total count	ESBLs	Resistant but Non-ESBLs	Sensitive
Klebsiella pneumoniae	292	170(58.22%)	92(31.51%)	30(10.27%)
Escherichia coli	234	126(53.85%)	58(24.79%)	50(21.37%)
Enterobacter cloacae	32	15(46.88%)	12(37.5%)	5(15.62%)
Citrobacter freundii	24	12(50%)	8(33.33%)	4(16.67%)
Proteus mirabilis	8	3(37.5%)	4(50%)	1(12.5%)

DISCUSSION

Extended-spectrum β-lactamases have been increasingly described worldwide since their description in the early 1980s and have raised to prominence among Enterobacteriaceae isolates all over the world. The prevalence of ESBL producing bacteria is variable worldwide; it is 28% in Bulgaria, 16% in Cyprus and Romania, 12% in Portugal, 50% in Russia and 40% in Poland [2]. Sensitive organisms (sensitive to extended spectrum cephalosporins) they are almost decreasing but there is significant increase in resistant but non ESBLs in our study. Frequency of ESBL producing organisms is changing from 66% to 47% in our study which is closest to frequency in India 68%7, in Nepal 60% [10] and in other studies from Pakistan 50%. [11] Most common ESBL producer in our study is Klebsiella pneumoniae (52.15%) followed by Escherichia coli (38.65%) which correlates with other local study done by Shah et al [12]. Among individual organisms isolation of ESBLs is in the same pattern as in total enterobateriaciae. We observed a significant change for ESBLs and non-ESBLs during four year study. In this study there is decrease in the detection of ESBL producing organisms. The reason may be that the methods we are using for ESBL detection are not sufficient. Variable affinity of these enzymes for different substrates and inoculum's effect may be the other factors interfering with ESBL detection [5]. We used the commonly employed double disc synergy method for ESBL detection, which has limitations for ESBL detection as 79% positivity was reported by Thomson et al [7] The non ESBL producing but resistant organisms in our study may be due to hyper production of beta lactamases. These do not allow the beta lactamase inhibitors to show the zone of enhancement as required for ESBL detection. Another reason is AmpC β-lactamase production, which can be detected by using AmpC disk test or Modified three dimensional test. Also multiple β-lactamases within one organism (e.g., multiple ESBLs or ESBL-AmpC combinations) can make

phenotypic identification of the β-lactamases difficult [10, 13, 14].

CONCLUSION

The frequency of ESBL producing organisms has decreased during four years in our study. However, organisms showing same resistance pattern as ESBLs are on the rise. This shows that there may be new ESBLs which are even resistant to beta lactamase inhibitors and are not detected as ESBLs by standard double disk synergy method. Hence there is a need to improve the methods used for ESBL detection.

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