Prevalence of ESBL Production in Gram Negative Isolates in a Tertiary Care Hospital in Gulbarga

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Research Article

Abstract: Extended Spectrum Beta lactamases are enzymes that mediate resistance to third generation Cephalosporins rendering treatment with these antibiotics ineffective. Bacterial strains that produce these enzymes pose various problems in the management of infectious diseases. With this objective in mind, a total of 272 gram negative, clinical isolates were initially screened for ESBL production, and then confirmed by CLSI confirmatory method. Of the 231 screen positive (85%) isolates, 151 (56%) were confirmed as ESBL positive. Among these, ESBL production was 72% in *Klebsiella*, 56% in *Ecoli*, 24% in *Citrobacter*, 29% in *Proteus*, 5% in *Enterobacter*, 15% in *Pseudomonas* and 33% in *Acinetobacter*. Considering the statistically significant production of ESBL in these isolates, routine screening of these enzymes is advised in all tertiary care hospitals.

Keywords: Extended Spectrum Beta Lactamases(ESBL), 3rd generation Cephalosporins.

Introduction

With the discovery of Penicillin, and related drugs, even the bacteria started evolving mechanisms to evade these antibiotics so that they could survive under optimal conditions in the host. ESBLs represent an impressive example of ability of gram negative bacteria to develop resistance in the face of the introduction of new antimicrobial agents.(1) The ESBL enzymes are plasmid - mediated enzymes capable of hydrolyzing and inactivating a wide variety of b lactams, including third generation Cephalosporins, Penicillins and Aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-I. Widespread use of third generation Cephalosporins and Aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs.[2] These enzymes have no detectable activity against Cephamycins and Imipenem. Because, of their greatly extended substrate range these enzymes were called extended spectrum beta-lactamases. [3] In addition, ESBL-producing organisms frequently show cross-resistance to many other classes of antibiotics; including Aminoglyco sides and Fluoroquinolones thus treatment of these infections is often a therapeutic challenge.(4)Therefore it is advisable for all clinical laboratories to detect ESBL producing strains in their laboratories.

Materials and Methods

The study was carried out in the Department of Microbiology, in Khaja Banda Nawaz Institute of Medical Sciences in Gulbarga. A total of 272 Gram negative clinical isolates in the year 2011 were initially identified by biochemical reactions(5)They were then screened for ESBL production by Kirby Bauer Disc Diffusion method using Ceftazidime(30ug) and Cefotaxime(30ug).A zone size of ≤ 27mm for Ceftazidime and ≤ 22mm for Cefotaxime was considered as ESBL screen positive. These strains were then subjected to confirmation.(6)

Confirmatory disk diffusion test for ESBL detection(6)

A 0.5 Macfarland turbidity standard of the test strains was swabbed on MH Agar plates. Ceftazidime (30 mcg) was used alone as well as in combination with Clavulanic acid(10 mcg). Similarly, Cefotaxime (30 mcg) was used alone as well as in combination with Clavulanic acid(10 mcg).These discs were placed on MH agar at a distance of 20mm and incubated at 37 °C for 24 h. An increase in the zone diameter for Ceftazidime-Clavulanic acid by ≥ 5mm and Cefotaxime-Clavulanic acid by ≥ 5mm was considered positive for ESBL production(CLSI, 2010). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 strains served as negative and positive controls respectively Statistical analysis was performed by the Chi-square test and a *p* value of less than 0.05 was considered as statistically significant..

Results

Out of 272 gram negative isolates, 249 belonged to *Enterobacterieaceae* family, while 23 were non fermenters. Among these 231 (85%)strains were screened to be ESBL producing ,of which 151(56%) were confirmed to be ESBL producing as CLSI guidelines. The distribution of strains among these ESBL positive isolates was as follows.

Table 1: Distribution of ESBL positive strains among ESBL positive isolates.

Name of the organism	Total no. of Isolates	ESBL Positive	ESBL Negative
E Coli	126	71(56%)	55(44%)
Klebsiella	95	68(72%)	27(28%)
Pseudomonas	20	3(15%)	17(85%)
Citrobacter	17	4(24%)	13(76%)
Proteus	7	2(29%)	5(71%)
Enterobacter	4	2(50%)	2(50%)
Acinetobacter	3	1(33%)	2(67%)
Total	272	151(56%)	121(44%)

When the Chi square test was applied to these strains ,the difference between ESBL positive and ESBL negative strains was found to be statistically significant at p value 0.05.Also Ceftazidime was found to be a better indicator of ESBL production than Cefotaxime.

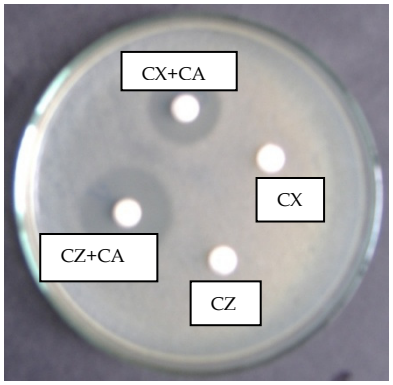

Figure 1: Both Cefotaxime (CX)and Clavulanic acid (CA);Ceftazidime(CZ) and Clavulanic acid(CA) have ≤ 5mm increase in zone diameter as compared to Cefotaxime(CX) and Ceftazidime (CZ) alone.

Discussion

ESBLS producing strains represent a major hurdle in treatment of infectious diseases. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of Ceftazidime and other third generation Cephalosporin use and instrumentation or catheterisation.[2]The prevalence of ESBL production in India varies from28-84%(7). We had 231(85%) of strains which were screened as ESBL producing strains .Of these,151(56%were confirmed as ESBL producing as proposed by CLSI guidelines. This showed that 80(29%)strains that were detected as positive by the screening tests could be derepressed mutants as shown by Rodriques *et al* (8) Derepressed mutants represent strains that were screen positive but there was no increase in zone with an inhibitor. This futher emphasizes the confirmation of ESBLproduction by ClSI method. Kulkarni *et al* had reported 78.8%ESBL positive by screening but were actually confirmed. .in29.16 %.(9)Similarly ,Rao *et al* had reported61.7% ESBL producers among 98.5% screen positive isolates.(10).

The overall prevalence of ESBL positive strains in our study was found to be56% which corresponded to that of Gaurav *et al*(57-61%)(11). Mathur *et al* (12)andSinghal *et al* (13),and Rodrques *et al*(8) had reported the prevalence to be68%,64%,and 53% respectively. The prevalence of ESBL positive strains is generally noted more in *Klebsiella* species than in *E coli* but there have been studies where *Ecoli* have reported more positivity than *Klebsiella*. We had reported maximum isolation of ESBL positive strains in *Klebsiella* species(72%) followed by *E coli*(56%)

Table 2: The comparative study by different authors and isolation rate of different ESBL positive organisms is as follows.

Name of the organism	Percentage of ESBL positive isolates as reported by various authors					
E Coli	73.5%	62.9%	40.7%	19.6%	81%	56%
Klebsiella	58.1%	62.2%	15.9%	41.6%	74%	72%
Pseudomonas	37.1%		24%	11.1%		15%
Citrobacter	50%		10%	33.3%		24%
Proteus	50%	70.5%	25.7%	37.5%		29%
Enterobacter				20%		50%
Acinetobacter	57.1%					33%
	Gaurav *et al* (11)	Rao *et al*(10)	Kulkarni *et al*(9)	Balan *et al*(14)	KharKhar *et al* (15)	Our study

Although *Acinetobacter* had shown positivity of only 33% which was less than that of Gaurav *et al* (11),they are a major cause of metallobetalactamases production ,so they must be screened intensively and reported. Among Cefotaxime and Ceftazidime,Ceftazidime was reported to be more sensitive indicator than Cefotaxime both alone as well as in combination with Clavulanic acid. To conclude, higher prescription of 3rd generation Cephalosporins in the treatment of infections has resulted in the emergence of ESBL positive strains in our hospital. As the ESBL production was stastically significant, routine screening is a must in all tertiary care hospitals. However confirmation must not be ignored also. Judicious use of antibiotics, regular formulation of

antibiotic policies, antibiotic cycling, implementing of infection control measures and continued monitoring of the susceptibility pattern should be carried out in all hospitals.

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