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Research Article

Determination of Incidence of Extended Spectrum Beta Lactamases (ESBL) Producing Gram Negative Bacilli at a Tertiary Care Hospital Serving a Rural Population

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Abstract: Extended spectrum beta lactamase (ESBL)-producing organisms pose unique challenge to clinical microbiologists, clinicians, infection control professionals and scientists. ESBLs are enzymes produced by some bacteria or germs that can make them resistant to certain antibiotics. ESBLs are enzymes capable of hydrolyzing penicillin, broad-spectrum cephalosporins and monobactams that are generally derived from TEM and SHV- type enzymes. ESBLs are often located on plasmid that are transferable from strain to strain and between bacterial species.The present study was undertaken to determine the incidence, detect ESBL producers and study the antibiotic resistance pattern of gram negative bacilli (GNB) in clinical isolates of different specimens in a tertiary care hospital of rural West Bengal so that more effective treatment protocols can be planned. In this hospital based prospective study, 855 samples collected over a two month period were analyzed phenotypically according to CLSI specifications for GNB isolates which produced ESBL. 126 isolates proved to be GNB out of which 19 were ESBL producers by CLSI confirmatory tests. The majority, were Escherichia coli followed by Klebsiella spp, Pseudomonas spp and others. Approximately 13% of the isolates were ESBL producers which is a lower figure than the other hospitals of this country and some other parts of the world. Intervention with proper antibiotic use and awareness will help in preventing higher resistance in the hospital isolates..
Keywords: ESBL, incidence, Antibiotic, GNB, Double Disk Approximation Test.

INTRODUCTION

Beta-lactam agents such as penicillins, cephalosporins, monobactams and carbapenems are molecules of choice to treat a variety of infections. Their introduction into therapy was rapidly followed by the reports of resistance [1]. ESBLs are enzymes that compromise the efficacy of all beta-lactams, exceptcephamycin and carbapenems, by hydrolysis of the beta-lactam ring. They are encoded by plasmids generally derived from TEM or SHV, but the predominance of CTX-M has increased considerably since 1995 in most parts of the world[2, 3].

ESBLs are often encoded by genes located on large plasmids, which also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulfonamide, tetracycline, and chloramphenicol. Furthermore, recent studies have demonstrated the co-transfer of *qnr*, encoding reduced susceptibility to the quinolones, with ESBLs on a plasmid. The acquisition and accumulation of resistance determinants have given rise to multidrug resistant ESBL producers, further limiting therapeutic options and subsequent dissemination of these populations because of their co-selection by various antimicrobials[4,5].

The aim of our study was to determine of ESBL-producing GNB from different isolates ina tertiary care hospital of rural West Bengal.

MATERIAL AND METHODS

For isolation and identification, all the collected samples were inoculated on sterile MacConkey's agar and Blood agar plates and the plates were incubated at 37°C for 18 to 24 hours. Based on growth in MacConkey's agar isolates were identified as lactose fermenting and non- lactose fermenting GNB. The gram stained smear was then examined under oil immersion light microscope to see the presence of the organisms. Biochemical tests such as iodole test, urease production, citrate utilization, triple sugar iron tests, and motility tests were carried out for identification of different

genus of GNB as per standard microbiological guidelines[6].

Antimicrobial susceptibility testing the isolates were tested by the modified Kirby-Bauer disk diffusion method on Muller Hinton agar (Hi-Media) and interpreted according to CLSIguideline. The antibiotics included in the study were Amikacin 30µg; Amoxy-clavulanic acid 30 µg; ; Cefotaxime 10 µg; Cefoxitin 30 µg;Ceprofoxacin 30 µg ;Imipenem 10 µg; Nalidixic acid 30µg(Hi-Media)[7].

According to CLSI screening method the isolates were tested for their susceptibility to the third generation cephalosporins (3GCs) e.g. ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg). If a zone diameter of < 22 mm for ceftazidime, < 27 mm for cefotaxime and < 25 mm for ceftriaxone were recorded, the strain was considered to be "suspicious for ESBL production". Only those isolates which were resistant to one of the 3 GCs were selected for the study and were processed for confirmation of ESBL production[8].

According to CLSI phenotypic confirmatory method the strains suspected to be ESBL producer by screening method were confirmed for enzyme production by phenotypic confirmatory disk diffusion method. Ceftazidime disk without clavulanic acid and ceftazidime with clavulanic acid combination disk were placed on the same plate. The plates with disks were incubated aerobically overnight (18-24 hours/35°C). The isolates showing an increase in zone size of 5 mm or more around ceftazidime with clavulanic acid as compared to ceftazidime alone were confirmed to be ESBL producer. No enhancement of zone indicates ESBL non-producer isolates[9].

RESULTS AND DISCUSSION

From the 855 samples collected over a period of three months, approximately 30% showed positive growth. This is the usual isolation rate for this microbiology laboratory over the past few years and corresponds to similar rates in other Indian hospitals[10].

Out of the isolates about 50% were GNB. This may be explained by the fact that nearly 70% of the samples were urine where GNB are the major source of infection.

When these GNB were screened for the presence of ESBL, 46.8% showed screening test positivity. In fact, more than 50% the urinary isolates were found to be screening test positive according to the CLSI guidelines. A significant point emerged that pus (from surgical site infections) yielded less percentage of resistant strains that other samples.

When the screened isolates were subjected to confirmatory tests, only 15% of the GNB proved to be confirmed ESBL producers. Here too pus samples which were primarily from admitted and/or operated patient showed a lower rate of ESBL producing isolates. This perhaps reflects that community acquired resistant strains still predominate in this hospital and hospital acquired resistant strains are not so prevalent. Here one may say that proper antibiotic use guided by such data and antibiotic policy preparation may prevent rise of hospital acquired resistant strains.

When analyzing data from the perspective of bacterial species, it was found E. coli had maximum resistant isolates. The higher prevalence of ESBL producers in Asia than in Europe and America was observed in a previous study. Previous studies in Bangladesh revealed 23% to 31% ESBL producers from GNB[11], which is little higher than the present study.

Table 1: Types of sample collection including % measure

Sample	Number (%)
Urine	577(67.49)
Pus	135 (15.79)
Blood	40(4.67)
Others	103(12.05)
Total	855(100)

Table 2: ESBL screening test positive in GNB from samples

Sample	Gram Negative Bacilli	ESBL Screening+ (%)
Urine	80	43 (53.75)
Pus	30	10(33.33)
Others	15	6 (40)
Blood	1	0(0)
Total	126	59(46.82)

Table 3: ESBL confirmatory test positive in GNB from samples

Sample	Gram Negative Bacilli	Confermatory ESBL$^+$
Urine	80	14
Pus	30	2
Others	15	3
Blood	1	0
Total	126	19

Table 4: Percentage of confirmed different organisms that produce ESBL from different samples

Organisms	No. of Isolates	Confirmed ESBL (%)
Escherichia coli	72	11 (15.27%)
Klebsiella sp.	29	3 (10.34%)
Pseudomonas sp.	16	2 (12.5%)
Others	22	3(13.63%)
Total	139	19(13.66%)

CONCLUSION

Although bacterial infection rates are similar to other Indian hospitals, as proved by the isolation of over 30% specimens revealing growth of organisms, the percentage of resistant strains was less compared to Indian (25-80%) as well as figures from some other parts of the world.

Escherichia coli were the predominant organism isolated which produced ESBL, which is different from data of other researchers showing Klebsiella to be the predominant organism having resistance.

The isolates from the community (urine cultures) yielded more percentage of resistant strains compared to the hospital strains of pus maybe indicating that hospital acquired resistant strains were less prevalent.

This data therefore shows different as well as lower rates of resistant isolates in samples analyzed in the study. Thus, to prevent emergence of flora with higher resistance in the patients proper antibiotic policy may be adopted for use in the hospital this may prevent further treatment failures. Also, infection control practices like good hand hygiene and taking antibiotics only when they are really necessary will prevent further development of resistant strains.

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