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Original article

Prevalence of Extended Spectrum Beta Lactamases from the clinical isolates of Enterobacteriacae family

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Abstract

Introduction-The incidence of Extended spectrum beta lactamases (ESBL) producing strains among the clinical isolates has been increasing over the years creating major therapeutic difficulties resulting in the limitation of treatment options.

Methodology- A total of 50 serial isolates of Gram negative bacteria were obtained from clinical samples such as pus, urine, sputum, blood, ear discharge & pleural fluid. These Gram negative isolates of Enterobacteriacae family were isolated, identified and its antibiotic susceptibility pattern was done & interpretated as per NCCLS guidelines.

Result- A total of 50 Gram negative bacteria were tested for ESBL production out of 50 isolates ,35 (70%) were positive for ESBL production by Double disk approximation test. A total of 20 isolates (40%) were positive by NCCLS conformatory test. Maximum ESBL production was detected in Klebsiella pneumoniae isolates followed by E-coli by both the test. Majority of ESBL producing Gram negative isolates were from Surgical wards 25 (50 %) followed by Medical wards 15 (30%). All the ESBL producers were Multidrug resistant.

Empirical use of broad spectrum antimicrobial agents & clinical and bacteriological surveillance of patients admitted to critical areas will minimize the emergence and the spread of ESBL producing organisms.

Key words- ESBL, multidrug resistance, Enterobacteriacae.

Introduction-

Beta lactam antibiotics are the most frequently prescribed antimicrobial agents for the treatment of bacterial infections. Production of the beta lactamases is the most common mechanism of the bacterial resistance for these antibiotics. These enzymes are numerous, and they mutate continuously in response to the heavy pressure of antibiotic use, leading to the development of Extended spectrum beta-lactamases(ESBLS) (1). These enzymes are found in the variety of Enterobacteriaceae species. These enzymes are plasmid mediated , capable of hrydrolyzing and inactivating a wide variety of beta-lactam antibiotics, including third generation cephalosporins, monobactams, penicillins and aztrreonam (2). They have no detectable activity against chephamycins. Abruent use of third generation cephalosporins and aztreonam is the major cause of mutations in these enzymes, that led to the emergence of ESBLS(2). These ESBLS are inhibited by

clavunalic acid, Sulbactam and tazobactum and are put into functional classification in group 2be(3). Exended spectrum drug resistance is caused by the genes that code for production of ESBLS and other resistance genes (4) .These ESBLS have become widespread serious problem and several aspects of them are worrying. These enzymes are increasing expressed by many strains of pathogenic bacteria with potential for dissemination. They compromise the activity of wide spectrum antibiotics creating major therapeutic difficulties with a significant impact on outcome of the patient (5) . ESBL producing Gram negative bacteria are increasing being associated with hospital infections(6).Thus continued emergence of ESBLS present diagnostic challenges to the clinical microbiology laboratories, who should be aware of the need for their detection (5) by accurately identifying the enzymes in clinical isolates. In the peesent study ,, we report the prevalance of ESBL producing Gram negative bacteria from the hospitalized patients.

Materials & Methods-

The study was conducted in the microbiology department of Dr.D.Y.Patil Medical College Pimpri, Pune during Jan 2006 to Jan 2008 . 50 serial isolates of Gram negative bacteria from Enterobacteriaceae family from various clinical samples were studied.

The primary aim of the study was to identify the prevalence of ESBL producing Enterobacteriaceae from the various clinical samples.

Gram negative bacteria from the Enterobacteriaceae family were obtained from clinical samples such as pus, urine, sputum, blood, ear-discharge, pleural fluid.

These samples were processed by plating on Blood agar and Macconkeys agar. All the isolates were identified using Standard Biochemical tests (7) Antimicrobial susceptibility of the isolates was performed by Disc diffusion technique Kirby Bauer Method according to NCCLS guidelines. (8) and the results were interpretated as per its NCCLS guidelines (8) Gram negative isolates with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to third generation Cephalosporins were selected for ESBL production. (8, 9)

ESBL detection was done by Double disk approximation test (10) and NCCLS confirmatory test (11)

Tests for ESBL production:-

a) Double disk approximation test -

Mueller Hinton Agar Plates were inoculated with direct colony suspension equivalent to 0.5 MC Farland Standard (12) to form a lawn culture.

A Ceftazidime disk (30 ugm) was placed near the centre of an agar plate. Keeping a centre to centre distance of 15 mm, Amoxycillin/Clavulanic acid (Augmentin 20/10 ugm) disk was placed in line with ceftazidime. The plates were incubated at 37 ^{0} C & examined after overnight incubation. If the strain was ESBL producer, the zone arround the ceftazidime disk was extended on the side nearest the amoxicillin/ clavulanic acid (10).

b) NCCLS confirmnatory test -

While performing the test, ceftazidime (30 ugm) and ceftazidime plus Clavunalic acid (30/10ugm) were placed on inoulated Mueller Hinton agar & incubated at 37 ^{0} C overnight.

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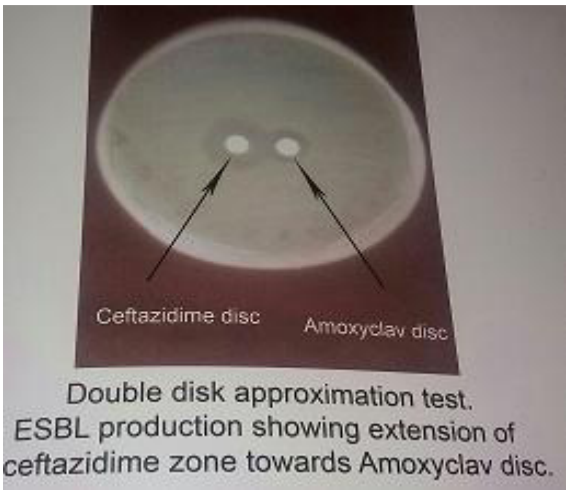
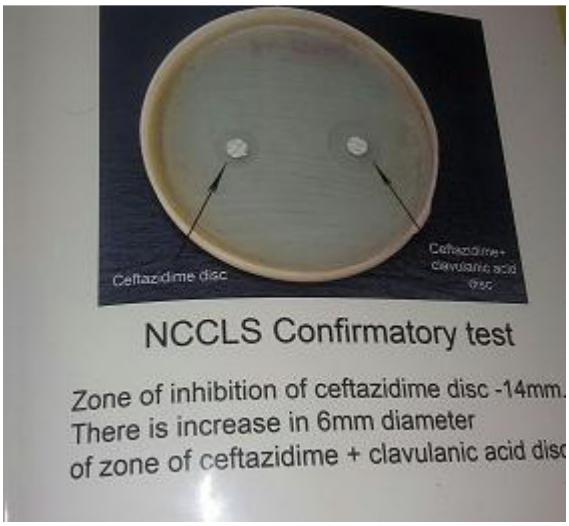
Organism was considered as ESBL producer if there was a ≥ 5 mm increase in zone diameter of ceftazidime/ Clavulanic acid disk and that of ceftazidime alone(11).

E-coli ATCC 25922 (Negative Control) Klebsiella pneumoniae 48188 (Positive Control) were used as control strains in the study.





Results:-

50 Gram negative bacteria from various clinical samples were tested for ESBL production.

Table No 1 :- Distribution of clinical samples from which Gram negative bacteria were isolated.

Clinical Samples	No of samples	(%)Percentage
Urine	26	52
Pus	13	26
Sputum	4	8
Plueral Fluid	3	6
Ear discharge	2	4
Blood	2	4
Total	50	

Table No 2:- Gram negative bacteria isolated from Enterobacteriaceae family.

Name of organism	No of samples	(%)Percentage
Klebsiella Pneumoniae	28	56
E-coli	15	30
Proteus Vulgaris	2	4
Proteus mirabilus	3	6
Citrobacter freundii	2	4
Total	50	

Klebsiella pneumoniae 28 (56%) followed by E-coli 15 (30%)

Table No 3:- Gram negative isolates, positive for ESBL production by Double disk approximation test (Screening test)

Gram negative isolate	No of Gram negative isolates positive for ESBL production	(%)Percentage
Kleb. Pneumoniae (n=28)	23	65.71
E-coli (n=15)	10	28.57
Proteus Vulgans n=(2)	0	0
Proteus mirabilus (n=3)	01	2.85
Citrobacter fruendii(n=2)	01	2.85
Total (50)	35	

35 (70%) of Gram negative bacteria were positive by Double Disk approximation test.

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Table no 4:- Gram negative isolates positive for ESBL production by NCCLS confirmnatory test.

Gram negative isolate	No of Gram negative isolates positive for ESBL production	(%)Percentage
Klebsiella Pneumoniae(n=23)	11	55
E-coli (n=10)	8	40
Proteus Vulgaris (n=1)	1	5
	20	

20 isolates (40%) were positive by NCCLS confirmnatory test.

The overall positivity of ESBL production among Gram negative bacteria from Enterobacteriaceae family from 50 serial isolates is 20 (40%).

Table No 5:- Distribution of ESBL producing bacteria in various areas of the hospital.

Ward	No of ESBL producing Organisms isolated	(%)
Surgical	25	50
Medical	15	30
Gynaecology	3	6
Orthopedics	3	6
ENT	2	4
ICU	2	4
Total	50	

Majority of ESBL producing Gram negative isolates were from surgical wards 25 (50%) followed by Medical wards 15 (30%)

Table No 6:- Antibiotic Susceptibility pattern of ESBL positive isolates (urine samples).

Name of Organism	Ca (%)	Ak (%)	NX(%)	Nf(%)	Na(%)
Kleb.Pneumoniae (13)	13 (100)	4(30.76)	8(61.53)	10 (7.69)	11(84.06)
E.Coli (5)	5(100)	2(40)	1 (20)	2(40)	5 (100)
Proteus mirabilus (1)	1(100)	0	1(100)	1(100)	1(100)
Total	19	06	10	13	17

All ESBL producers were multidrug resistant. They showed resistance to at least 3 drugs.

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Table No 7:- Antibiotic Susceptibility pattern of ESBL positive isolates (other samples)

Name of Organism	Ca (%)	Am(%)	G (%)	Ak (%)	Cf (%)
Kleb.Pneumoniae (10)	10 (100)	10 (100)	4 (40)	3 (30)	8 (80)
E.Coli (5)	5 (100)	5 (100)	2 (20)	1(20)	4(80)
Total	15	15	6	4	12

All the ESBL producers were multidrug resistant. They showed resistant to at least 3 drugs.

Discussion:-

ESBL producing strains in the hospitals all over the world have spread so rapidly, that it is important to know the prevalance of ESBL positive strains in the hospital so as to formulate a policy of empirical therapy. (13). This is necessary to avoid the misuse of expanded spectrum cephalosporins, which still remain an important component of antimicrobial therapy. (13)

The prevalance of ESBLs among clinical isolates varies from country to country and from institution to institution. The Incidence varies from 0% to 69%. In U.S., occurrence of ESBL production in Enterobacteriaceae renges from 0 to 25% (3,8). In netherlands percentage of ESBL production in E-coli & K.pneumoniae showed less than 1%(3) Pai et al reported the prevalance of ESBL production in Korea in 1998 was 4.8%(14). In Europe, Babini et.al reported 69% of ESBL production (15)

In India , the prevalance of ESBL production varies from 6.6% to 86.6%. Ananthan et, al from Chennai is 2000 reported 6.6% ESBL production (16) and Dutta et. al from New Delhi showed 12.6% of ESBL production in 2002 (17) . Also ESBL production was reported by Supriya et al 43.8%, C. Rodrigues et al 53% and Ananthkrishanan 58% respectively (18, 19, 20) . In the present study, ESBL production was seen in 70% isolates by Double disk approximation test Detection of ESBL productions by Double disk approximation test ranges from 6.6%. Ananthan et.al(21) to 76.5% Hansotia et.al (22).

40% of the isolates were positive by NCCLS confirmnatory test in the Present study. Shukla et.al(23) reported 30.18% of isolates positive by NCCLS confirmnatory test. Similarly Petro et.al (24) documented 39.3% of ESBL production by NCCLS confirmnatory test.

Most of the ESBL producing isolates were for surgical wards i.e. 50% followed by Medical wards 30% in the present study. This may be due to the fact that all the patients who underwent surgery were given broad spectrum antibiotics pre-operatively. Specific risk factors for colonization with ESBL producing organisms include length of the hospital stay , severity of illness, time in ICU , intubation and mechanical ventilation, urinary or arterial catheterization and previous exposure to antibiotics. Many of patients infected with ESBLs are found in ICUs but they can also occur in surgical wards as in most other areas of the hospital(25) .

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The reason for the isolates which are not comfirmed by NCCLS confirmatory test may be due to masking by the expression of chromosomal Amp-C B-lactams (26).

Thus the ESBL producing organisms pose a major problem for therapeutics. The incidence of ESBL producing isolates has been steadily increasing over the past few years resulting in the limitations of therapeutic options. Therefore control measures such as empirical use of broad spectrum antibiotics, third generation cephalosporins and imipenem, antibiotic cycling and bacteriological surveillance of patients admitted to ICUs and critical areas will minimize the emergence of spread of ESBL producing organisms.(1)

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