ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence and Antibiogram of Extended Spectrum B-Lactamase Producing E-Coli, Klebsiella and Pseudomonas Species From Clinical Isolates In a Tertiary Care Hospital.

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ABSTRACT

Extended spectrum B-lactamases (ESBL) producing bacteria are emerging pathogens. They have descended by genetic mutation from native B-lactamases found in gram negative bacteria, especially infectious strains of Escherichia coli and Klebsiella species. The present work was carried out to determine the frequency of clinically important isolates like Escherichia coli, klebsiella and pseudomonas spp recovered from various clinical specimens in our set up, to compare the resistance pattern of ESBL producers with that of non ESBL producers, to compare the efficacy among three different cephalosporin's in screening ESBLs and also to compare the efficacy of three different phenotypic confirmatory tests in detecting ESBLs. A total of 300 isolates comprising Escherichia coli, klebsiella pneumonia and pseudomonas aeruginosa species were included in the study, during the period between November 2008 and October 2009. Apart from routine antibiogram, all these 300 isolates were screened for ESBL enzymes by using 3 indicator Cephalosporins- Cefpodoxime (30 ug), Cefotaxime (30 ug) and ceftazidime (30 ug) as per NCCLS guidelines. The ESBL screen positive isolates were further subjected to three phenotypic confirmatory test i.e. Double disc diffusion synergy test, inhibitor potentiated disc diffusion test and E-test using klebsiella pneumonia ATCC 700603 and Escherichia coli ATCC 25922 as positive and negative controls respectively. Out of 20000 samples screened, 300 (15%) isolates belong to Escherichia coli, klebsiella pneumoniae and pseudomonas aeruginosa species. Out of 300 isolates, majority of them were E.coli 196 (65.33). Klebsiella pneumonia isolates, were 64 (21.33%) and pseudomonas aeruginosa 40 (13.34%). Out of 300 isolates, ESBL screen test positive were 150 (50%). Out of 300 isolates,. The resistance rates of ESBL screen positive isolates to most antibiotics were significantly ($p < 0.05$) higher than those of ESBL-negative isolates except for Ampicillin where the resistance rate was not significantly ($p < 0.05$) higher than those of ESBL-negative isolates. However, all ESBL screen positive and negative isolates were susceptible to Imipenem. Among screening tests, Cefpodoxime (94.67%) was more sensitive compared to Ceftazidime (88%) and Cefotaxime (84%). Inhibitor-potentiated disc diffusion test (44%) and E-test (44%) were more sensitive compared to Double disc diffusion synergy test (16%) in confirming the screen positives. The high levels of ESBL producers mainly among gram negative isolates is alarming and warrants special attention, both by the clinicians and the microbiologists. While the clinician has to re-evaluate the antibiotic policies, the lab must be capable of readily identifying these isolates, so that proper therapy can be instituted to avoid misuse or overuse of antibiotics. It is conclude that production of ESBLs by clinically important isolates is emerging as a wide spread problem in our setup. Routine detection of these isolates, appropriate infection control and antibiotic management strategies are needed to the spread of this emerging form of resistance.

Keywords: ESBL, E test, Inhibitory Potentiated disc diffusion method, Screening test.

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ISSN: 0975-8585

INTRODUCTION

The first β-lactamase was identified in *escherichia coli* prior to the release of penicillin for use in medical practice [1]. The age of penicillin saw the rapid emergence of resistance in *staphylococcus aureus* due to a plasmid-encoded penicillinase. This β-lactamase quickly spread to most clinical isolates of *staphylococcus aureus* as well as other species of *staphylococci.*

Over the last 20 years, many new β-lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new b-lactamases emerged that caused resistance to that class of drug. Presumably, the selective pressure of the use and over use of new antibiotics in the treatment of patients has selected for new variants of β-lactamase .one of these new classes was the oxyimino-cephalosporins, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s[1] not suprisingly, resistance to these expanded-spectrum β-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer lactams, SHV-2, was found in a single strain of *kiebsiella* ozaenae because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended spectrum β-lactamases (ESBLs) hydrolyze the expanded spectrum cephalosporins (e.g. Cefotaxime and ceftazidime) and monobactams, do not hydrolyze the cephamycins (cefoxitin), and are inhibited by the β-lactamase inhibitors (eg. Clavulanic acid) [2]

ESBLs have been found in a wide range of gram negative rods. However, the vast majority of strains expressing these enzymes belong to the family *enterobacteriaceae. Kiebsiella pneumoniae* seems to remain the major ESBL producer. Another important organism is *escherichia coli.* Non *enterobacteriaceae* are relatively rare with *pseudomonas aeruginosa* being the most important organism [3] with the spread of ESBLs in hospitals all over the world, it is necessary to know their prevalence in a hospital so as to formulate an empirical therapy in high risk units where infection due to resistant organisms is much higher [4,5] because of the evolving and continuing antibiotic resistance phenomenon.

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MATERIALS AND METHODS

The present study carried out in the department of microbiology, navodaya medical college, Raichur in recent past 2 years.
The patients of navodaya medical college hospital and research centre, raichur, formed subject for the study.

Sample size

All the clinical samples, that came to the microbiology laboratory during the study period b 300 strains of gram negative organisms comprising Escherichia coli, klebsiella Spp and pseudomonas Spp we isolated from the various clinical samples such as urine, sputum, exudates/pus, blood, and CUF from patients attending navodaya medical college hospital and research Centre.

Methods of collection of samples [7]

Specimens were collected as follows.

Urine: about 3-5ml of midstream urine was collected by clean catch method in a sterile container

Sputum: patients were instructed to take a simple mouthwash and then deep coughed sputum 5ml was collected in a sterile wide mouthed container.

Blood: venepuncture site was prepared with tincture iodine and 70% alcohol. Blood (5-10ml) was drawn with sterile syringe and transferred into the bottle containing 50ml brain heart infusion broth under aseptic precautions.

Exudates: after cleaning the surface of the lesion with sterile swab soaked in saline, two swabs taken from the depth of the wound or lesion. Care was taken not to touch the adjacent skin margins.

Pus:pus was collected with swab and transported tolab in a sterile screw cap container
Csf: 3-5ml of csf was collected aseptically by lumbar punctures in a fresh sterile screw cap Container.

Laboratory procedures [8-11]

Specimens were brought to the laboratory within two hours of collection and further processing done.

Gram stain: smears were made from the samples except blood, heat fixed and stained by gram stain. Smears were examined for pus cells and presence of pus cells and presence of gram negative rods.

Culture: sputum and exudates were inoculated onto mac-conkey agar and blood agar. The media were incubated aerobically overnight at 37^0 c and observed for growth on the next day.

Urine samples were inoculated with standard loop determination of significant on mac-conkey and ClED agar.

brain heart infusion broth of blood culture after 48 hours of incubation was inoculated on to mac-conkey and blood agar.

the colonies of different gram negative bacilli belonging to Escherichia spp, klebsiella spp and pseudomonas spp were studied as follows.

E. coli

Nutrient agar: medium sized thick, grayish white, moist, non mucoid, smooth, opaque or partially translucent colonies.

Blood agar: colonies are sometimes hemolytic on blood agar:

Mac conkey agar: the colonies are typically large mucoid and pink due to lactose fermentation.

Pseudomonas spp:

Nutrient agar: large, opaque, irregular colonies with bluish green diffused pigment and an earthy smell.

Blood agar: colonies of many strains are hemolytic on blood agar.

Mac-colonies agar: flat, irregular, pale colonies indicating non lactose fomenters, and identified by biotyping.

Antibiotic sesitivity testing was as per the NCCLS criteria.

Pseudomonas aerginosa ATCC 27853 and Escherichia coli ATCC 25922 were used as quality control strains.

In the pesent study susceptibility was tested against ampicillin (10mcg), amoxclav (20/10 ug), cotrimoxazole (1.25/23.75 mcg), amikacin (30 ug),imipenem (10 ug), gatifloxacin (5 ug), tobramycin (10 ug), these disks were obtained from himedia laboratoriespvtltd

Screening tests for esbl

All organisms belonging to escherichiacoli, klebsiella pneumonia and pseudomonas aeruginosa were screened for ESBL production by using three different third generation indicator cephalosporins ceftazidime (30 ug), cefotaxime (30 ug) and cefpodoxime (30 ug).

A zone a diameter of <22 mm for certazidime, <27 mm for cefotaxime and <17 mm for cefpodoxime was recorded as resistant. The strain which showed resistance to at least one of these cephalosporins was considered positive on ESBL screening test.

Such strains which showed positive result on screening test were further tested using the following phenotypic confirmation methods for ESBL production.

Phenotypic confirmation ESBL methods for

Double disk diffusion synergy test (DDST) [13-14]

All isolates belonging to Escherichia coli, klebsiella pneumonia and pseudomonas aeruginosa were tested for ESBL production by DDST. Ceftazidime (30 ug), cefotaxime (30 ug), cefpodxime (30 ug), and co- amoxclav (amoxicillin 20 ug + clavulanic cid 10 ug) (himedia laboratories ltd. Mumbai) were used for esbl detection.

klebsiella pneumonia ATCC 700603 and Escherichia coli ATCC 25922 were used as positive and negative controls respectively [7-13].

The suspension for inculcation was prepared from 4-5 isolated colonies and turbidity was compared with 0.5 McFarland standards. Sterile cotton swab soaked in this suspension was used to make lawn culture on mueller-hinton agar plates. Co-amoxclav (20 ug amoxicillin + 10 ug clavulanic acid) and ceftazodime (30 ug) were placed at distance of 20mm from center to center. Plates were incubated at 37^0c overnight. Enhancement of zone of inhibition of the ceftazidime towards the co-amoxclav disc was considered positive result. This occurs because the clavulanic acid present in co-amoxclav disc inactivates the ESBL produced by the test organism.

Inhibitor potentiated diffusion test (IPT) [15-16]

potassium clavulanate powder was procured from fluke company with certificate of analysis from sigma aldrich.the test inoculums was streaked onto muller hinton agar plates,one acid and one without clavulinicacid. ceftazidime(30µg), cefotaxime(30µg) & cefpodoxime (30µg) disks were placed on both of these plates.adifferance of more than or equal to 10mm was taken as positive foresbl.

E test [7, 17, 18].

E-strips for ESBL detection were procured from an biometric solna, Sweden. The e test ESBL strip carries two gradients; on the hand, ceftazidime (0.5 to 32 ug/ml) and on the opposite end, ceftazidime (0.125 to 8 ug/ml) plus clavulanic acid (4 ug/ml). The test was performed by following the manufacturer's instructions. Briefly, after overnight growth on brain heart infusion agar, the organisms were suspended in saline to turbidity equal to that of a 0.5 McFarland agar plates by swabbing them with a cotton swab. After drying, the e test strips were placed on the plates and the plates were incubated overnight in air at 37^0c. The MIC's on other ends of the strip were interpreted as the point of intersection of the inhibition exlipse with the e-test strip edge. According to the manufacture, a ratio of ceftazidime mic/ ceftazidime-clavulanate mic equal to or greater than 8 indicates the presence of ESBL enzymes.

RESULTS

Table-1: sample-wise percentage distribution of positive isolates from total samples Screened

Clinical specimens	Total no. Screened	Total no. Of isolates belonging to E .Coli, klebsiella & pseudo moans spp	percentage
Sputum	233	32	13.74
Urine	1301	200	15.37
Exudates/pus	445	65	14.61
HVS	7	1	14.29
Stool	14	2	14.29
Total	2000	300	15

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Above table shows that out of 2000 samples screened, 300 (15%) isolates baking toE.coli, klebsiella pneumonia and pseudomonas aeruginosa species. Out of 233, 445,7 and 14 specimens of sputum, urine, exudates/pus, HVS, and stool samples screened, 32 (13.74%), 200 (15.37%), 65 (14.67%), 1 (14.29%), and 2 (14.29) were study isolates belonging to Escherichia coli, klebsiella pneumonia and pseudomonas species from respective samples

Table –2: isolates from different specimens

clinical specimens	total isolates	Percentage
Sputum	32	10.67
Urine	**200**	**66.67**
Exudates/pus	65	21.67
Hvs	1	0.33
Stool	2	0.67
Total	300	100

It can be observed from the above table that our of the 300 isolates (E.coli klebsiella pneumoniae and pseudomonas aeruginosa species),32 (10.67%), 200 (66.67%), 65 (21.67%), 1 (0.33%) and 2 (0.67% were from sputum, urine, exudates/pus, HVS, and stool respectively.

Figure-3: organism-wise distribution of total isolates

organism	total isolates	percentage
E. Coli	**196**	**65.33**
Klebsiella pneumonia	64	21.33
Pseudomonas aeruginosa	40	13.34
Total	300	100

From above table, it can be observed that out of 300 isolates, majority of them wee E.coli 196 (65.33%). Klebsiella pneumonia isolates, were 64 (21.33) and pseudomonas aeruginosa 40 (13.3)

Table-4: specimens-wise distribution of total isolates

Clinical specimens	E. Coli	Klebsiella pneumonia	pseudomonas aeruginosa
Sputum	7	16	8
Urine	**163**	**35**	2
Exudates/pus	23	12	**29**
Hvs	1	0	0
Stool	2	0	0
Total	196	64	40

From the above table it can be observed tha out of 196 e.coli isolates, 163 were from urine, 23 from exudates/pus, 7 from sputum, 2 from stoll and 1 from high vaginal swab. out of 64 klebsiella pneumonia isolates, 35 were from urine, 16 from sputum, 12 from sputum and 12 fromexudates/pus. Out of 40 isolates of pseudomonas aeruginosa, 29 were from exudata/pus, 8 from sputum and 2 from urine.

ISSN: 0975-8585

Table-5: Antibiogram pattern of all isolates

Pattern	total isolates (n=300)		percentage	
	sensitive	resistant	sensitive	resistant
A	14	286	4.67	95.33
Ac	231	69	77.00	23.00
Co	166	134	55.33	44.67
Ak	247	53	82.33	**17.67**
I	**300**	**0**	**100.00**	**0.00**
Gf	209	91	69.67	30.33
Tb	231	69	77.00	23.00
Cep	158	142	52.67	47.33
Ca	168	132	56.00	44.00
Ce	174	126	58.00	42.00

The above table shows that majority of isolates were resistant to ampicillin (95.33%), followed by cefpodoxity (47.33%), cotrimoxazole (44.67%), ceftazidime (44.00%), and cefotaxime (42.0%), all the isolates were susceptible to imipenem (100%), while majority were sensitive to amikacin(82.33%), amoxclav (77.0%), tobramycin (77.0%), followed by gatifloxacin (69.67%), and cefotaxime (58.0%).

Table-6: percentage of ESBL screen test positive & negative isolates among total isolates

organisms	Total isolates	screening test positive	screening test negative
E. Coli	196	100	96
Klebsiell pneumoniae	64	32	32
Pseudomonas aeruginosa	40	18	22
Total	300	150	150

The above table shows that out of 300 isolates, ESBL screen positive are 150 (50%). Among total of 196, 64, and 40 isolates of E.Coli. Klebsiella pneumoniae and pseudomonas aeruginosa respectively, 100, 32, and 18 are screen test positive. Among isolates, out of 196, 64, and 40 isolates of E.Coli, klebsiella pneumoniae and pseudomonas aeruginosa respectively. 96, 32, and 22 are screen test negative.

Table-7: sample-wise distribution of ESBL screen +ve isolates among total isolates

clinical specimens	total isolates	screen +ve	percentage
Sputum	32	17	53.12
Urine	200	102	51.00
Exudates/pus	65	30	46.15
Hvs	1	0	0.00
Stool	2	1	50.00
Total	300	150	50.00

The above table shows specimen wise distribution of ESBL screen +ve isolates among total isolates. In sputum, out of 32 isolates 17 (53.12%) are screen +ve. In Utine, out of 200 isolates 102 (51%) are screen +ve. Out of 2 isolates from stool sample, 1 (50%) is screen +ve.

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Out of 65 isolates from exudates/pus, 30 (46.15%) are screen +ve. From hvs only isolate is found and it is negative for ESBL screen.

Table-8: organism-wise distribution of ESBL screen +ve isolates among total isolates

Organisms	total isolates	ESBL screen +ve isolates	Percentage
E. Coli	196	100	51.02
Klebsiella pneumoniae	64	32	50
Pseudomonas aeruginosa	40	18	40
Total	300	150	50.00

It can be observed from the above table that out of 196,64, and 40 isolates of E.coli, klebsiella pneumonia and pseudomonas aeruginosa respectively, 100 (51.02%), 32 (50%) and 18 (45%) are screen test positive.

Table-9: Antibiogram pattern of the ESBL screen positive isolates

pattern	Screen +ve isolates (n=150)		percentage	
	Sensitive	resistant	sensitive	resistant
A	4	146	2.67	97.33
Ac	104	46	69.33	30.67
Co	65	85	43.33	56.67
Ak	111	39	74.00	26.00
I	150	0	100.00	0.00
Gf	83	67	55.33	44.67
Tb	101	49	67.33	32.67

The above table shows antibiogram pattern among ESBL screen +ve isolates. Majority of them are sensitive to imipenem (100%) followe by amikacin (74%), amoxclav (69.33), tobramycin (67.33%), fatifloxacin (55.33%), cotrimoxazole (43.33%) and ampicillin (2.67%). majority of them show resistance to ampicillin (97.33%) followed by cotrimoxazole (56.67%), gatifloxacin (44.67%), tobramycin (32.67%), amoxclav (30.67%), amikacin (26%).

Table-10: comparison of resistance pattern of the ESBL screen positive with that of ESBL Screen negative

pattern	Screen +ve isolates (%) (n=150)	Screen –ve isolates (%) (n=150)	p-value
A	146 (97.33)	140 (93.33)	p >0.05
Ac	46 (30.67)	23 (15.33)	p >0.002
Co	85 (56.67)	49 (32.67)	p >0.0001
Ak	39 (26)	14 (9.33)	p >0.0002
I	0 (0)	0 (0)	
Gf	67 (44.67)	24 (16)	p >0.0001
Tb	49 (32.67)	20 (13.33)	p >0.0001

The above table shows the results of resistance pattern of antibiotic susceptility testing.

In general, resistance rates of isolates to I (0%), Ak (26%), ac (30.67%), Tb (32.67%) and (44.67%) were low. Co (56.67%) showed moderate activity on isolates. A (97.33%) is shown to be the ;east active active antibiotic against isolates. Their resistance rates of ESBL screen positive isolates to most antibiotics were significantly $p < 0.05$) higher than those of

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ESBL-negative isolates except for ampicillin where the resistance rate was not was not significantly ($p>0.05$) higher than those of ESBL- negative isolate. However, all ESBL screen positive and negative isolates were susceptible to imepenem.

Table 11- Antibiogram pattern of theESBL screen negativeisolates.

Pattern	screen +ve isolates (n=150)		percentage	
	sensitive	resistant	sensitive	resistant
A	10	140	6.67	93.33
Ac	127	23	84.67	15.33
Co	101	49	67.33	32.67
Ak	136	14	90.67	9.33
I	150	0	100.00	0.00
Gf	126	24	84.00	16.00
Tb	130	20	86.67	13.33

The above table shows antibiogram pattern among ESBL screen –ve isolates.

Majority of them show resistance to ampicillin (93.33%) followed by cotrimoxazole (32.67%), gatifloxacin (16%), amoxclav (15.33%), tobramycin (13.33%), and amikacin (9.33%)&sensitive
to Imipenem(100%)

Table 12:efficacy of screening tests.

Tests	ESBL producer (%) (n=150)	ESBL non-producer (%) (n=150)	P-value
Cep(cefpodoxime)	142 (94.67)	8 (5.33)	
Ca(ceftazidime)	132 (88.0)	18 (12.0)	p<0.01
Ce(ceftoxime)	126 (84.0)	24 (16.0)	

The above table show that there is statistically significance difference ($p<0.01$) among three screening test results. From the table, it was found that Cep (94.67%) gives more efficient results screening test compared to ca (88%) and ce (84%).

Table-13: percentage positivity of ESBL producers among screen positives by different Confirmatory tests

Tests	Total isolates	ESBL producer	percentage
Double disc diffusion synergy test	150	24	16
Inhibitor-potentiated disc diffusion test	150	66	44
E test	150	66	44

The above table shows ESBL producers among screen positives by different confirmatory tests, majority or them were IPD test (44%) and E-test (44%) followed by DDST (24%).

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Percentage positivity of ESBL producers among screen positives by different

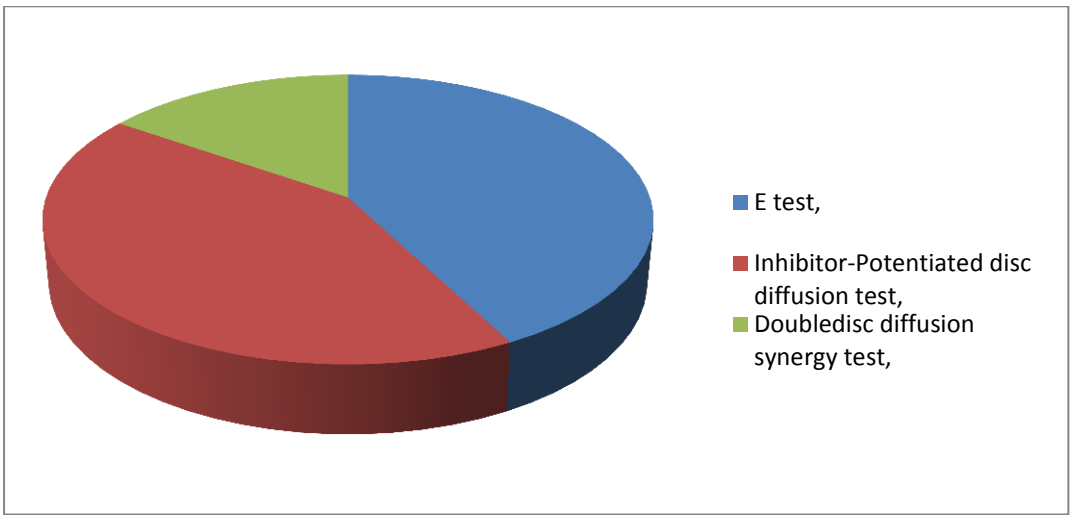


Table-14: comparison of the efficacy of phenotypic confirmatory tests

tests	ESBL Producer(%) (n=150	ESBL Non-producer(%) (n=150)	p<value
Double disc diffusion synergy test	24(16)	126 (84)	p<0.0001
Inhibitor-potentiated disc diffusion test	66 (44)	84 (60)	
E test	66 (44)	84 (56)	

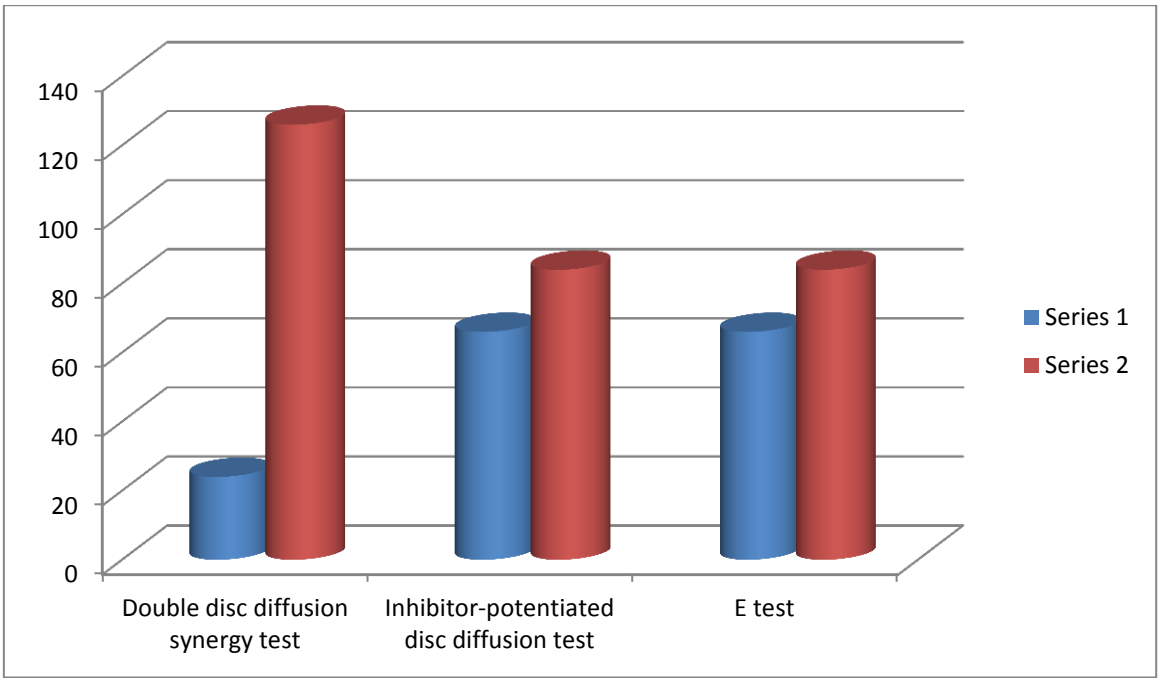
The above table shows that there is statistically significance difference (p<0.0001) among three confirmatory test which shows the results given by three test are vary. From the table, it was found that, inhibitor-potentiated disc diffusion test (44%) and e-test (44%) gives more efficacy result as confirmatory test compared to double disc diffusion synergy test (16%).

Table-14: department-wise distribution of ESBL producers

wards	screen +ve	confirmative +ve	percentage
Medicine	41	13	31.71
Surgery	20	9	45.00
Obg	31	15	48.39
Orthopaedic	8	2	25.00
Ophthalmology	0	0	0.00
Paediatric	5	2	40.00
Ent	7	3	42.86
Medical spl.	1	1	100.0
Urology	35	20	57.15
Casualty	1	0	0
Dermatology	1	1	100.00
Total	150	66	44.00

ISSN: 0975-8585

Comparison of the efficacy of phenotypic confirmatory test



The above table shows that majority of the ESBL producer were isolated from medical SPL (100%) and dermatology (100%) followed by urology (57.15%) Obg (48.39%) surgery (45%) ent (42.86%), pediatric (40%), medicine (31.71%), and orthopedic (25%) none from ophthalmology and casuality.

Table-15: sample-wise ESBL producers

clinical specimens	screen +ve	confirmative +ve	percentage
Sputum	17	5	29.42
Urine	102	46	45.10
Exudates/pus	30	14	46.67
HVS	0	0	0
Stool	1	1	100.00
Total	150	66	44.00

as can be observed from the above table that, ESBL producers were more often isolated from stool (100%), followed by exudates/ pus (46.67%, urine (45.10%), and sputum

Table-16: Organism-wise ESBL producers

organisms	screen +ve	confirmative +ve	Percentage
E. Coli	100	50	50.00
Klebsiella pneumonia	32	11	34.38
Pseudomonas aeruginosa	18	5	27.78
Total	150	66	44.00

From the above table, it can be observed that majority of ESBL producers were e. Coli (50%) followed by klebsiella pneumonia (34.38%) and pseudomonas aeruginosa (27.78%).

DISCUSSION

The present work was carried out in the department of microbiology, in recent past 2 years. In this study, all the clinical samples that came to the department of microbiology during the study period constituted the material for the study.

Prevalence of gram negatives:

Out of 2000 samples screened, 300 (15%) isolates belong to E.Coli, klebsiella pneumonia and pseudomonas aeruginosa species. Out of the 300 isolates, majority of them were from urine 200 (66.67%), followed by exudates/pus 65 (21.67%), sputum 32 (10.67%), stool 2 (0.67%) and HVS 1 (0.33%). Escherichia coli196 (65.33%), which is the most important cause of urinary tract infections was the most frequent bacterial isolate in the present study followed by klebsiella pneumonia 64 (21.33%) and pseudomonas aeruginosa 40 (13.34%). It is on par with many studies in the region.

Chan dtm et al reported 61% E.coli and 16% klebsiella spp [19]

However, in contrast to our study, in a study by acharyavn et al. Klebsiellae were the major pathogens, but the study group comprised of children which probably was the reason for the difference [20].

Table-17: prevalence of gram negative organisms in different studies

author	place	prevalence (%)
Wattal c et al[21]	Delhi	59.00
Babypadmini et al[36]	Chennai	49.00
Supriya et al	Nagpur	48.80
Present study	Raichur	15.00

Prevalence of ESBL producers

The prevalence of ESBL producing organisms varies worldwide extent and the rationale of use β-lactam antibiotics. Prevalence of ESBLs differs in different parts of India based on various risk factors and local reasons. In our study 150 isolates (50%) were found to potential ESBL producers on screening tests out of which 66 isolates (44%) were confirmed as ESBL producers on IPT and e-test. It means in our study 22% of total isolates (66/300)were ESBL producers. The prevalence of ESBL shows a wide range from 6.6% to 91.7% in different parts of India. Prevalence of ESBLs is high i referral centers and ICUS where antibiotic use is profuse. In a study by wattle et al. In Delhi, the prevalence was as high as 91.7% which was probably due to high use of cephalosporin's, high rate of patient transfer from peripheral canters and associated patient risk factors like chronic ill health etc

Prevalence of ESBLs in uropathogens and in different organisms

In our study, ESBL producers were more often isolated from urine (45.10%) next only to exudates/pus (46.67). Sputum (29.42%) showed relatively less ESBL producers. Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in india [06,76,79]. Hence, routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI.

In our study, majority of ESBL producers were Escherichia coli (50%), followed by klebsiella pneumonia (34.38%) and pseudomonas aeruginosa (27.78%).

A large survey of 1610 escherichia and 785 k. Pneumoniae isolates from 31 centers in 10 european countries found that prevalence of ESBL in these organisms ranged from as low as 1.5% in germany to high as 39-47% in russia, poland and turkey [24,25].

In India high prevalence of ESBL producing klebsiella strains has been reported by various groups. Reported frequency of ESBL producing klebsiella spp in India ranged from 6 to 87%[43].

Prevalence of ESBL producing klebsiella spp:

As reported by other investigators were 25.6%, 25.8%. 30.18%,80.0% and 86.6% [28].

Various Indian authors have reported high prevalence of ESBL producing p.aeruginosa, 22.2% to 25.8%, [31-33]. In our study also similar prevalence (27.28%) was found**:** our study showed 47.50% ESBL producers were in females as compared to compared to 40% in males which is similar to observation by baby padmini et al[36]. In our study, majority of ESBL produces were in the in the age group 0-10 year (100%), followed by 31-20 years (59.10%), 61-70 years (52.95%) and 51 Another study by zabeen k zabeen et al reported high prevalence of ESBLs at extremes of age i.e. Less than 5 years and above 60 years of age[41.]

In our study majority of the ESBL producer were isolated from medical SPL ward (100%) and dermatology (100%) followed by urology (57.15%), OBG (48.39%), surgery (45%), ent (42.86%), pediatric (40%), medicine (31.71%), and Orthopedic (25%). None from ophthalmic and casualty. This is in contrast to the observation of purva mathur et al[30] where ESBLs were mainly isolated from ICCU, medical ICU and surgical ICU. This difference may be due to change in type and number of sample**:**

In the present study, majority of the ESBL producers showed resistance to ampicillin (97.33%) followed by resistance to co-trimoxazole (56.67%). Resistance to amikacin was (26%). Similar resistance rates were shown in studies by spanu t et al [34], babypadmini at al [36]. T menon et al and emily sb et al [35]. All ESBL producers were resistant to ampicillin and more than 44% were resistant to co-trimoxazole in a study by jain a et al [42] which is in concordance with our study. Analysis of the antimicrobial resistance patterns in the present study showed that resistance to β-lactam and non β-lactams is more frequent in ESBL

producing strains than in those which did not produce the enzyme which is in concordance with the observation by jain a et al [24].

in our study, all the ESBL strains were sensitive to imipenem (100%). Similar results were observed by tankhiwale ss et al [28], babypadmini et al [36], t menon at al [32] and shoba. Recent studies have demonstrated that 40-45% of ESBL producing isolates are resistant to fluoroquinolones which is in concordance with our study[98]. This is probably because of their prophylactic use in neutropenic and cirrhotic patients and for the treatment of urinary tract infections.

In our study resistance to tobramycin was 32.67 & which is in contrast to 50-60% seen in study by sehgal r et al[39]. This variable resistance to aminoglycosides raises the question of their usefulness in combination regimens to treat ot late onset especially when ESBL producers are suspected.

in our study, resistance to third generation cephalosporins was found to co-exist with resistance to other antibiotics which is concordance like floroquins and aminoglycosides which is concordance with reports by Subha et al [49] and Duttaroy b et al [12]. In the present study, all the ESBL –producers were found resistant to two or more drugs, whereas multi-drug resistance in non-ESBL producers was less comparatively. Our study showed that ESBL production was high among uropathogens and that the ESBL producers we mostly multidrug resistant. This is in concordance with the study by shelvakumar bn et al [100]. Hence routine ESBL testing for uropathogens is essential. Antibioigram patterns would be useful for required therapy.

In general, in the case of routine susceptibity testing, negative predictions are to be taken with caution as a large number of ESBL producers can be missed.

Several studies recommend ceftazidime [1, 42-48]. As the most sensitive screening agent, while others recommend cefotaxime or cefpodoxime [11, 37, 19]. This difference may be caused by various types of ESBL which may be epidemic in different environments. In our study cefpodoxime (94.67%) was more sensitive as a screening test compared to ceftazidime (88%) and cefotaxime (84%) which is comparable to study by Emery Weymouth. The sensitivity of ESBL screening could be improved by adding cefotaxime and cefpodoxime along with ceftazidime. To sum up the results of the screening tests in predicting ESBL production, it is important to mention that for the screening test, negative results are a better guide than positive results. Following all positive results might lead to unnecessary avoidance of conventional β-lactams in a good number of cases.In our study, 44% of the isolates positive on screening were confirmed to be ESBL producers where as in a study by jain a et al [24], In our study inhibitor-potentiated disc diffusion test (44%) and e-test (44%) appear to be acceptable for clinical use, and each was more sensitive in confirming screen positives compared to double disc diffusion synergy test (16%) which was least sensitive.

In our study, disks were placed at a fixed distance of 20 mm, which was probably not optimal for all the strains. The reason why this test is recommended for detection of ESBLs is because it is easy to perform, there is no need to measure zone sizes and it can be easily read based on the presence or absence of synergy.

The use of ipt and e-test may contribute to a wider recognition and more careful monitoring negatives (E. Coli, klebsiella and pseudomonas spp). Furthermore, it is likely that these tests will also prove to be useful for selecting strains for more detailed molecular analysis. The important limitations of all phenotypic based on synergy is their inability to detect inhibitor resistant, OXA and amp c enzymes which are or growing concern [16]. Hyper production of tem and/or SHV β-lactamases if accompanied with ESBL can cause false negative result by phenotypic test. A genotypic confirmation of ESBL gene needs to be done to evaluated the efficacy of phenotypic confirmatory tests to identify ESBL production of bacterial isolates in our hospital.

CONCLUSION

with the spread of ESBL producing strains in hospitals all over the world, it is necessary to know their prevalence in a hospital so as to formulate antibiotic policy of empirical therapy in high risk units where infections due to resistant organisms is much higher. Equally important is the information on an isolate from a patient to avoid misuse of extended spectrum cephalosporin's,which still remain an important component of antimicrobial therapy in high risk wards. Differences in susceptibility patterns of organisms and frequency of infection between hospitals and communities make knowledge of local prevalence and resistance data extremely important. This has direct bearing on choice of empirical therapy. Multidrug resistance in ESBLs is a common problem in hospitals as seen in our study also, which emphasizes the need for judicious use of antimicrobial agents and their continuous in vitro monitoring.

the routine susceptibility test done by clinical laboratories fail to detect ESBL positive strains and can erroneously detect isolates sometimes to be sensitive to any of the test is indispensable for detecting ESBLs. In conclusion, inhibitor potentiated disc – diffusion test is a sensitive and convenient method with a potential for incorporation into routine clinical laboratory service. The e test for ESBL with ceftazidime substrate appears to be a useful method for detecting or validating the presence of ESBL in gram negative bacilli.

It is concluded that ESBL isolates are prevalent in our setting. Routine detecting of these isolates and proper control measures are recommended so that appropriate management can be instituted and spread of these organisms curtailed.

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