Int J Pharm Bio Sci 2014 July ; 5(3): (B) 465 - 472

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

STUDY ON OCCURRENCE OF ESBL AND AMP C BETA LACTAMASE AMONG GRAM NEGATIVE CLINICAL ISOLATES FROM GOVERNMENT HOSPITAL, MANGALORE

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ABSTRACT

Resistant bacteria have emerged and established themselves worldwide as a threat to the favourable outcome of common infections in community and hospital settings. Beta lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. ESBL and AmpC beta lactamases are typically associated with multiple antibiotic resistances, leaving few therapeutic options. In this study, a total of 116 patient samples were evaluated for occurrence of ESBL and AmpC by Combination disk test and Modified three dimensional test respectively. 58.1% of isolates were ESBL producers, 23.3% were AmpC producers and 15.5% were both ESBL and AmpC producers.

KEYWORDS: DRUGRESISTANCE,ESBL,CEPHALOSPORIN,AMPC,KLEBSIELLA,E.COLI

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INTRODUCTION

Drug resistance can be described as a state of insensitivity or of decreased sensitivity to drugs that ordinarily cause growth inhibition or cell death.[1,2].ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid. This property differentiates the ESBLs from the AmpC-type beta-lactamases which have third-generation cephalosporins as their substrates, but which are not inhibited by clavulanic acid. [2]

MATERIALS AND METHODS

Sample collection: Different clinical samples collected from patients admitted in the various wards at the Government Wenlock Hospital, Mangalore included pus, urine, blood, respiratory specimen, catheter tips to body fluids etc.

Identification: Various clinical isolates, including bacteria of family *Enterobacteriaceae* Acinetobacter spp *&* Pseudomonas spp were identified by routine biochemical reactions.

Antimicrobial susceptibility testing

It was performed by Modified Kirby Bauer's disk diffusion method as per CLSI recommendations.

Antibiotics used [5]:

For urinary isolates: - Amikacin (10 µg), Cephotaxime (30 µg), Cotrimoxazole (25 µg)

For exudate and blood samples: - Amikacin (30 µg), Ampicillin (10 µg), Cephotaxime (30 µg), Ciprofloxacin (5 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg). The antibiotic disks were obtained from Span, HiMedia & BD diagnostics.

Screening tests

A) FOR ESBL's

According to the CLSI guidelines, isolates showing the inhibition zone size of

≤ 27mm with Cephotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production [4].

B) FOR INDUCIBLE AmpC BETA LACTAMASE BY DISC ANTAGONISM TEST

It was done by placing Cefoxitin disc (30 µg) at a distance of 20mm from Ceftazidime (30 µg) on the surface of Mueller-Hinton agar plates. Beta lactamase inducibility was recognized by blunting of the Ceftazidime zone adjacent to Cefoxitin disc [6].

Confirmatory tests

A) MODIFIED THREE DIMENSIONAL TEST for AmpC enzyme production

A modification of Manchanda & Singhs method was done in which overnight broth culture of isolates in Mueller Hinton broth was used. A lawn culture of E. coli ATCC 25922 was prepared on Mueller Hinton agar plates and Cefoxitin discs (30 µg) placed. Linear slits (3cm) were cut using a sterile surgical blade 3mm away from the Cefoxitin disc. Small circular wells were made on the slits at 5mm distance, inside the outer edge of the slit by stabbing with a sterile Pasteur pipette on the agar surface. The wells were loaded with the overnight broth culture in 10µl increments until the well is filled to the top. Approximately 30-40 µl of the Mueller Hinton broth culture was loaded in the wells. The plates were kept upright for 5-10 minutes until the solution dried, and were then incubated at 37°C overnight [8]. Quality control was achieved using a known AmpC positive isolate of *Providentia alcalifaciens* . Three kinds of results were recorded. The isolates showing clear distortion of zone of inhibition of cefoxitin was taken as AmpC producers. The isolates with no distortion as AmpC non producers and the isolates with minimum distortion were taken as indeterminate strains [6].

B) PHENOTYPIC CONFIRMATORY TEST WITH COMBINATION DISK FOR ESBL

In this, a third- generation cephalosporin antibiotic disk alone (Ceftazidime 30µg) and in combination with clavulanic acid (Ceftazidime + Clavulanic acid 30/10µg) was

used. Both the disks were placed at least 25mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate. The plates were incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured [(4)]. Interpretation: When there is an increase of ≥ 5mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disk alone, it cofirms ESBL production [(4)].

RESULTS

A total of one hundred and sixteen consecutive non repeat clinical specimens with one hundred and twenty nine isolates of gram negative bacteria of family *Enterobacteriaceae, Acinetobacter spp & Pseudomonas spp* were studied.The maximum number of samples were obtained from pus/wound swab (38.8%) closely followed by urine (30.2%), blood (14.7%), catheter tip (6.0%), sputum/ endotracheal tip (6.0%) and body fluids (4.3%). On testing with the routine antimicrobial agents, the isolates showed varying degree of antibiotic susceptibility.The isolates were most sensitive to Amikacin (66.7%) and showed a high degree of resistance to Ampicillin (93.2%), Cephotaxime (71.3%) , Cotrimoxazole (65.9%) , Gentamicin (65.2%) and Ciprofloxacin (50.6%). [TABLE 1]

TABLE 1
% SENSITIVITY TO ROUTINELY USED ANTIMICROBIALS

DRUG	SENSITIVE		INTERMEDIATE		RESISTANT	
	Number	%	Number	%	Number	%
AMPICILLIN	3	3.4	3	3.4	83	93.2
CEPHOTAXIME	13	10.1	24	18.6	92	71.3
GENTAMICIN	29	32.6	2	2.2	58	65.2
CIPROFLOXACIN	31	34.8	13	14.6	45	50.6
COTRIMOXAZOLE	22	17.1	22	17.1	85	65.9
AMIKACIN	86	66.7	13	10.1	30	23.2

Higher frequency of ESBL was detected in *E.coli* (40%) , followed by 34.7% in *Klebsiella spp*, 16% in *Pseudomonas spp*, 4% in *Acinetobacter spp*, 2.7% in *Enterobacter spp*, and 1.3% each in *Citrobacter spp* and *Proteus spp*. [TABLE 2.1]

From the confirmed ESBL isolates 29 were obtained from pus (38.6%), 28 from urine (37.3%), 8 from blood (10.7%), 5 from the catheter tip (6.7%), 3 from sputum/ ET tip (4%) and 2 from body fluids (2.7%).[Chart (1-A)]

The confirmed Extended spectrum beta lactamase producers were then traced to different wards of the hospital. The highest number of isolates were obtained from Orthopaedics wards (29.3%) followed by Surgery ward (20%) and Paediatrics ward (16%). [Chart (1-B)]

Table 2.1
Frequency of confirmed ESBL in different isolates.

ISOLATE	NUMBER OF ESBL CONFIRMED	% OF ESBL CONFIRMED
Escherichia coli	**30**	**40**
Klebsiella spp.	**26**	**34.6**
Pseudomonas spp.	**12**	**16**
Acinetobacter spp.	**03**	**4**
Enterobacter spp.	**02**	**2.7**
Citrobacter spp.	**01**	**1.3**
Proteus spp.	**01**	**1.3**

Chart (1-A)
Frequency of confirmed ESBL isolates from different clinical samples.

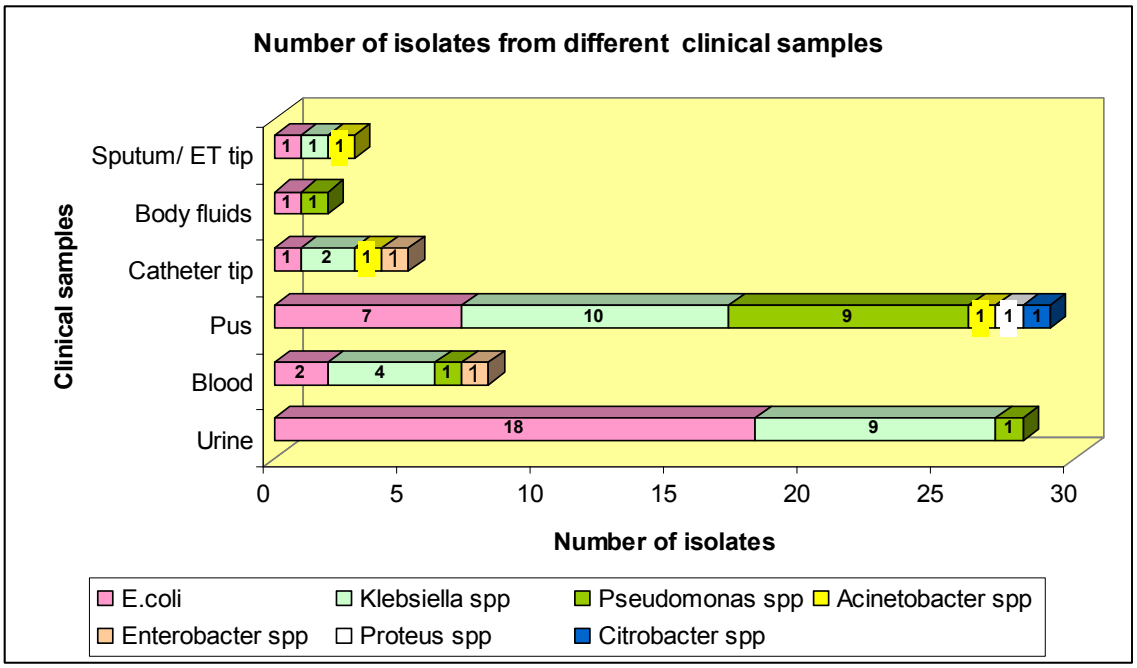
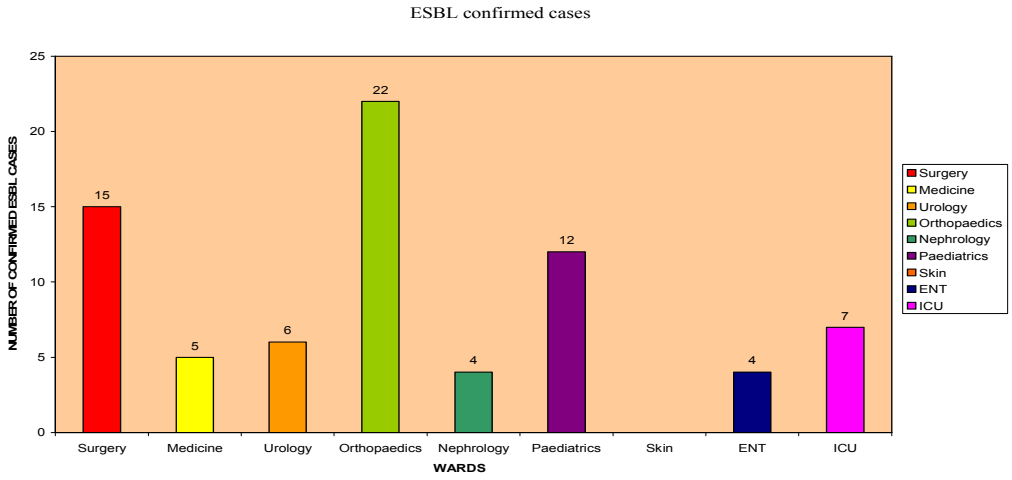


Chart (1-B)
Frequency of confirmed ESBL isolates from different wards.



Maximum number of AmpC producers were seen in *E.coli* followed by *Pseudomonas spp* and *Acinetobacter spp*. [TABLE 2.2]
From the various samples collected, confirmed AmpC isolates were maximum from pus (14 out of 30 samples) followed by urine (8 out of 30 samples), blood (4 out of 30 samples), body fluids (2 out of 30 samples), catheter tip (1 out of 30 samples) and sputum/ ET tip (1 out of 30 samples). [CHART (1-C)]
Most of the AmpC isolates were reported from patients who were hospitalised (26 out of 30 cases).The frequency of the AmpC isolates from different hospital wards showed that maximum cases were from Orthopaedics wards, followed by Surgery wards, Medicine ward, ICU, Urology ward and Paediatrics ward. [CHART (1-D)]

Out of the 11 isolates obtained from ICU, 4 showed to be positive for AmpC and 7 were ESBL producers. Thus showing a prevalence of 63.6% of ESBL and 36.4% of AmpC in our ICU. It also shows that the maximum number of isolates were *Acinetobacter spp* (4) followed by *E.coli* (3), *Klebsiella spp* (3) and *Pseudomonas spp* (1).ESBL and AmpC production was found to be maximum in *E.coli* isolates. [CHART (1-E)]

Table 2.2
Frequency of confirmed AmpC producers in different isolates.

ISOLATE	NUMBER OF AmpC	PERCENTAGE OF AmpC
Escherichia coli	14	46.7
Pseudomonas spp.	05	16.7
Acinetobacter spp.	05	16.7
Klebsiella spp.	04	13.3
Enterobacter spp.	-	-
Citrobacter spp.	01	3.3
Proteus spp.	01	3.3

CHART (1-C)
AmpC confirmed isolates from different samples.

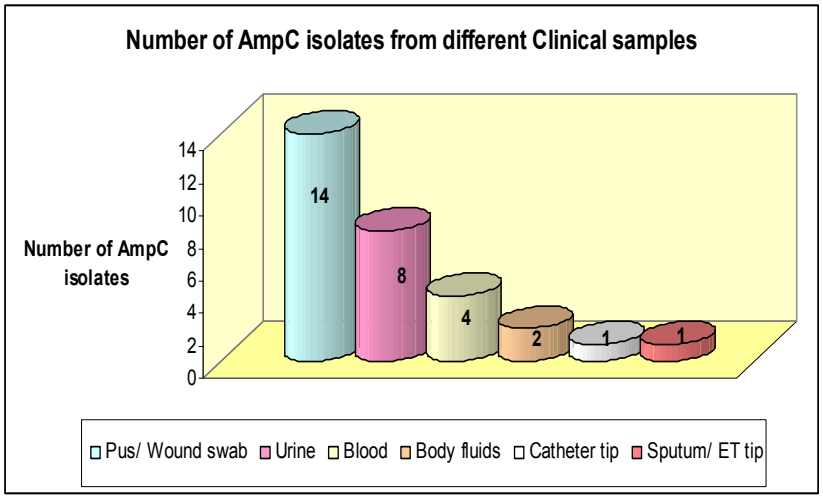


CHART (1-D)
AmpC confirmed isolates from different wards.

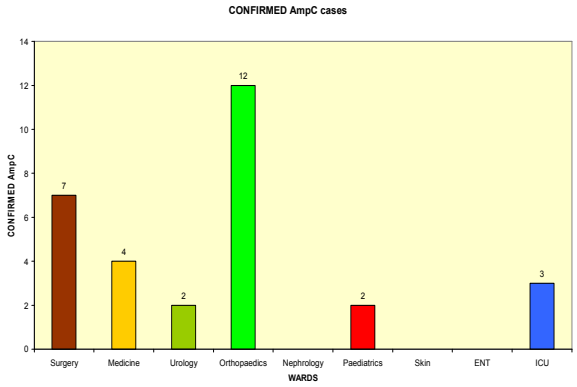
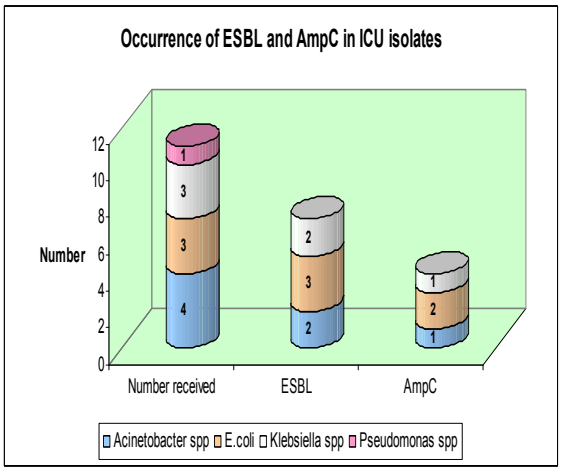


CHART (1-E)
Frequency of ESBL and AmpC isolates from the ICU.



DISCUSSION

The main objective of this study was to guide the clinician to check the emergence of ESBL and AmpC beta lactamase resistance by a retrograde analysis of the risk factors which were present in the clinical history of the patient. The prevalence of ESBL producers varies across continents and countries and also within hospitals. In the West, ESBL production varied from 3% in Sweden to 30-60% in Brazil,Colombia and Venezuela. In Asia it varied from 5% in Japan to 50% elsewhere.[3] In India, the prevalence rate varies in different institutions from 6.6% to 71.5%.[7,8] This difference may be due to geographical difference, different patterns of antibiotic use and differences in the selection of organisms for the study. The frequency of ESBL producers (58.1%) in our study is comparable to previous Indian studies as well to the study by *Shiju M P , Yashavanth R et al (2010)* (59.65%) from Mangalore. [9].The high incidence of ESBLs among *E.coli* may be peculiar to the Indian subcontinent as ESBLs have been predominantly reported among *K.pneumoniae* in Europe and USA. [10] However in contrast, our study showed a higher prevalence of ESBL producing *Klebsiella spp*.Most of these were multidrug ESBL producing strains showed resistance to routinely used and higher antimicrobials. High degree of resistance was observed in Ampicillin (97.8%), Cephotaxime (92%), Aztreonam (90.6%), Gentamicin (83%), Cotrimoxazole (78.7%),Ciprofloxacin (64%) and Cefepime (60%). The isolates were found to be less resistant to Imipenem (4%).These observations are in concordance with other Indian studies where similar resistance pattern was observed. [11, 12] AmpC screening test gave a positive result in only 3.1% of the isolates. When tested further with the confirmatory Modified three dimensional test by Manchanda and Singh 23.3% of the isolates tested positive. Most of the AmpC cases were reported from patients who were hospitalised (26 out of 30 cases).The frequency of the AmpC isolates from different hospital wards showed that maximum cases were from Orthopaedics wards, followed by Surgery ward, Medicine ward, ICU, Urology ward and Paediatrics wards. Out of the eleven isolates obtained from ICU, four showed to be positive for AmpC showing a prevalence of 36.6% in our ICU. From the Surgery ward , out of the 3 cases of burn 2 tested positive for AmpC showing a prevalence of 66.7% in burn cases.Maximum number of our AmpC isolates were *E.coli* - 46.7% (14 out of 30 cases) followed by *Pseudomonas spp* – 16.7% (5 out of 30 cases) and *Acinetobacter spp*- 16.7% (5 out of 30 cases). This result supports the findings of *Suranjana arora et al* who reported AmpC production in 47.8% of *E.coli* isolates but conflicts with the study results of *Manchanda and Singh et al* and *S*

Singhal et al who reported maximal incidence of AmpC producers among *Acinetobacter spp* (42.8% and 28.6% respectively). [13, 14]In our study we found that only 13.3% of AmpC producers were *Klebsiella spp*. This finding does not match with the view put forward by an Indian study where AmpC beta lactamases were most frequently isolated from *Klebsiella pneumoniae*. [14] This increased prevalence of bacterial pathogens producing both ESBLs and AmpC beta lactamases creates a requirement for laboratory testing methods that can accurately detect the presence of these enzymes in clinical isolates.

CONCLUSION

Prudent use of antibacterial drugs- using the appropriate drug at the appropriate dosage and for the appropriate duration – is one important means of reducing the selective pressure that helps resistant organisms emerge. The concept of "presumptive antimicrobial therapy" based on common pathogens, known susceptibility patterns and host factors would be useful in monitoring resistance trends in a region over time and assessing the effects of interventions to reduce antimicrobial resistance.

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