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Phenotypic detection of Amp C beta lactamases among the clinical isolates from a tertiary care hospital.

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ABSTRACT

Introduction: Antibiotic resistance among the Gram- negative pathogens is mediated by the production of extended spectrum beta lactamases (ESBLs) and Amp C □-lactamases, posing a serious threat in the management of infections. Amp C □- lactamases are the cephalosporinases that confer resistance to cephalothin, cefazolin, cefoxitin, penicillin and poorly inhibited by clavulanic acid. Although Amp C resistance is reported, their occurrence remains known in Enterobacteriaceae members.

Hence, present study is aimed to detect the Amp C □-lactamases in clinical isolates from a tertiary hospital, Nandyal.

Materials and methods: A total number of 320 clinical samples were included in the study, conducted at Santhiram medical college and general hospital, Nandyal, out of which 180(56.2%) are gram-negative isolates are tested for cefoxitin resistance and 47.7% are positive for Amp C production which were confirmed by phenotypic methods.

Results: The 180 samples which are screened for cefoxitin resistance,47.7% are positive. These are further confirmed for Amp C production by phenotypic methods. The most common organisms are Escherichia coli (23.6%) followed by Klebsiella (15.3%).

Conclusion: Amp C producers are found to be multi drug resistant and their detection by double disk synergy test is better than other phenotypic methods.

KEY WORDS: Amp C □ lactamases, Cefoxitin, Phenotypic methods

I. INTRODUCTION:

Amp C beta lactamases are the cephalosporinases that confer resistance to cephalothin, cefazolin, penicillin and poorly inhibited by clavulanic acid.

Drug resistance among gram-negative bacteria (enterobacterales, Acinetobacter baumanni and pseudomonas aeruginosa) has emerged and spread worldwide. Among the Enterobacteriaceae, Escherichia coli and klebsiella spp. are frequently isolated organisms that are multi drug resistant[1]. □-lactams are commonly used drugs to treat these organisms. □-lactams contains four major classes: Penicillin's; cephalosporins; carbapenems & monobactams[2]. Resistance to □-lactams is by production of □-lactamases, these enzymes hydrolyze the antibiotics before reaching the plasma membrane binding proteins located on the cytoplasmic membrane[3]. In gram negative pathogens, the production of □-lactamase enzymes remain the most important contributing factor for resistance to □-lactam antibiotics Major □-lactamases are plasmid mediated ESBLs, AMPC, Cephalosporinases and carbapenemases[1].

AMP C type cephalosporinases are Ambler class C □-lactamases. They hydrolyze penicillin's; cephalosporins; except the fourth-generation compounds & monobactams. In general, AMP C type enzymes are poorly inhibited by □-lactamase inhibitors; especially clavulanic acid. (4;5;6;7)

AMP-c □-lactamase resistance mechanisms can be

1)Inducible resistance via chromosomally encoded APM-C genes which is present in Citrobacter spp; Enterobacter spp. & Serratia spp.

2)Plasmid-meditated resistance; have clinical significance as plasmids not only transfer AMP C but also ESBL enzymes in the same plasmid. This type is seen in Klebsiella pneumoniae; Escherichia coli & proteus spp.[8]

Even though the AMP-C resistance is reported, but the accurate occurrence of AMP-C □-lactamases in Enterobacteriaceae members remain unknown.[8] Cefoxitin resistance is used for screening of AMPC □-lactamase producers in Enterobacteriaceae. Disc based assays like cefoxitin-cloxacillin double disc synergy test, disc approximation tests have been developed for detection of Amp c producing □-lactamases in the isolates.[10]

II. MATERIALS AND METHODS:

The present study is prospective study conducted at Santhiram medical college and general hospital, Nandyal for a period of 6 months

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from January 2022 to June 2022. A total of 320 samples received at Microbiology central laboratory at Santhiram medical college and general hospital, are cultured of which 180(56.2%) isolates are Gram Negative bacteria. These are screened for Amp C beta lactamase production using Cefoxitin disks and then confirmed by phenotypic methods like Cefoxitin -cloxacillin double disk synergy test, disk approximation test and E- strip test.

Amp C screening method:

Cefoxitin disk diffusion method: cefoxitin 30 µg disk is placed on the Muller Hinton agar inoculated with isolated organism and incubated over night at 37℃ and the bacterial isolates that yielded a zone diameter of less than 18 mm are considered as Amp C producers. Confirmation is done by various phenotypic methods[9].

Phenotypic confirmation methods:

a) Cefoxitin -Cloxacillin double disk synergy test:

Isolates are inoculated on Muller-Hinton agar, Cefoxitin-Cloxacillin disk(30µg-200µg) and cefoxitin disk (30µg) are placed at a distance of 20 mm and incubated overnight at 37^0 c. A zone difference of 4 mm diameter between the disks indicates Amp C production[13]. Table.3.

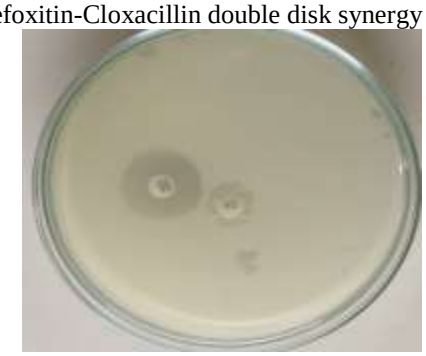
b) Disk approximation test: (Induction based)

The bacterial isolates are inoculated on Muller Hinton agar and a 30-µg ceftazidime disk was placed at the center of the plate then 10 µg imipenem, 30 µg cefoxitin, and 20/10 µg amoxicillin/clavulanate disks are placed at a distance of 20mm from the ceftazidime disk. The plate was inverted and incubated at 37^0 C. After overnight incubation, if there is any obvious blunting or flattening of the zone of inhibition between the ceftazidime disk and the inducing substrates is considered as positive result for Amp C production. Table.3.

c) E-strip test:

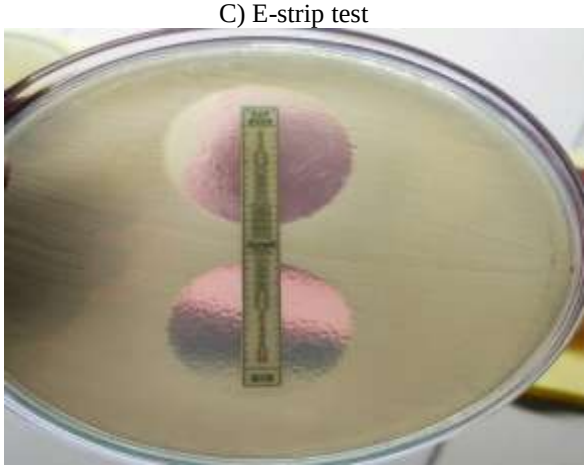
The Muller Hinton agar plates are inoculated with pure culture of the organism and the E- strip (MIX+: Ceftazidime, Cloxacillin +Clavulanic acid with 0.0032-4 and MIX: Ceftazidime and Cloxacillin with 0.125-16) are placed at the center using wooden applicator and incubated for overnight and observe of the zones and equal zones are obtained with ratio < 8 indicating Amp C production.

a) Cefoxitin-Cloxacillin double disk synergy test



b) Disk approximation test

C) E-strip test



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III. RESULTS:

Out of 180 isolates tested, 86 (47.7%) are positive for cefoxitin screening test, Escherichia coli 38(44.2%) is the most common organism. Phenotypic confirmatory methods by cefoxitin-cloxacillin double disk synergy test showed the best results when compared other tests with percentage of 46.5% (40) followed by disk approximation test 29.1% (25) and by E-strip test 15.1% (13) are positive for Amp C production.

Table.1. Screening test with Cefoxitin

	Number	Percentage
Cefoxitin resistance <18 mm	86	47.7%
Cefoxitin sensitive	94	52.3%
Total	180	100

Result: screening test of 180 samples for Amp C production by using Cefoxitin disk is 86(47.7%).

Table.2. Distribution of organisms according to Cefoxitin resistance for Amp C production

S.NO.	Organisms	Number	Percentage
1.	Escherichia coli	38	44.2%
2.	Klebsiella pneumoniae	26	30.3%
3.	Acinetobacter	12	13.9%
4.	Citrobacter	10	11.6%
	Total	86	100

Result: Among the 86 samples which are screened with Cefoxitin, Escherichia coli is the predominant organism 44.2% followed by Klebsiella pneumoniae 30.3%.

Table.3. Distribution of organisms according to different phenotypic methods

S.NO.	Organism	Amp C screening by Cefoxitin	Cefoxitin-Cloxacillin double disk synergy test	Disk approximation test	E-TEST	Amp C producers
1.	Escherichia coli	38(44.2%)	21(55.2%)	13(34.2%)	9(23.6%)	9(23.6%)
2.	Klebsiella pneumoniae	26(30.3%)	11(42.3%)	9(34.6%)	4(15.3%)	4(15.3%)
3.	Acinetobacter spp.	12(13.9%)	5(41.6%)	2(16.6%)	--	2(16.6%)
4.	spp.	10(11.6%)	3(30.0%)	1(10.0%)	--	1(10.0%)
	Total	86	40(46.5%)	25(29.1%)	13(15.1%)	16(65.5%)

Result: In the phenotypic methods, Cefoxitin-Cloxacillin double disk synergy test showed best results46.5% followed by Disk approximation test29.1%.

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Table.4. Sample wise distribution of Amp C beta -lactamase producers

S.NO.	Sample type	Number	Amp C producers	Percentage
1.	Pus	54	10	62.5%
2.	Urine	42	6	37.5%
3.	Sputum	36	-	-
4.	Blood	30	-	-
5.	Ascitic fluid	18	-	-
	Total	180	16	100

Result: Among all the samples tested for Amp C production, Pus samples are maximum in number 62.5% followed by Urine 37.5%.

Table.5. Department wise distribution of Amp C beta lactamase producers

S.NO.	Department	Amp C producers	Percentage
1.	Surgery	7	43.75%
2.	Orthopedics	5	31.25%
3.	Obstetrics & Gynecology	3	18.75%
4.	Medicine	1	6.25%
	Total	16	100

Result: Amp C producers are maximum in the samples received from Surgery department 43.75% followed by Orthopedics 31.25%.

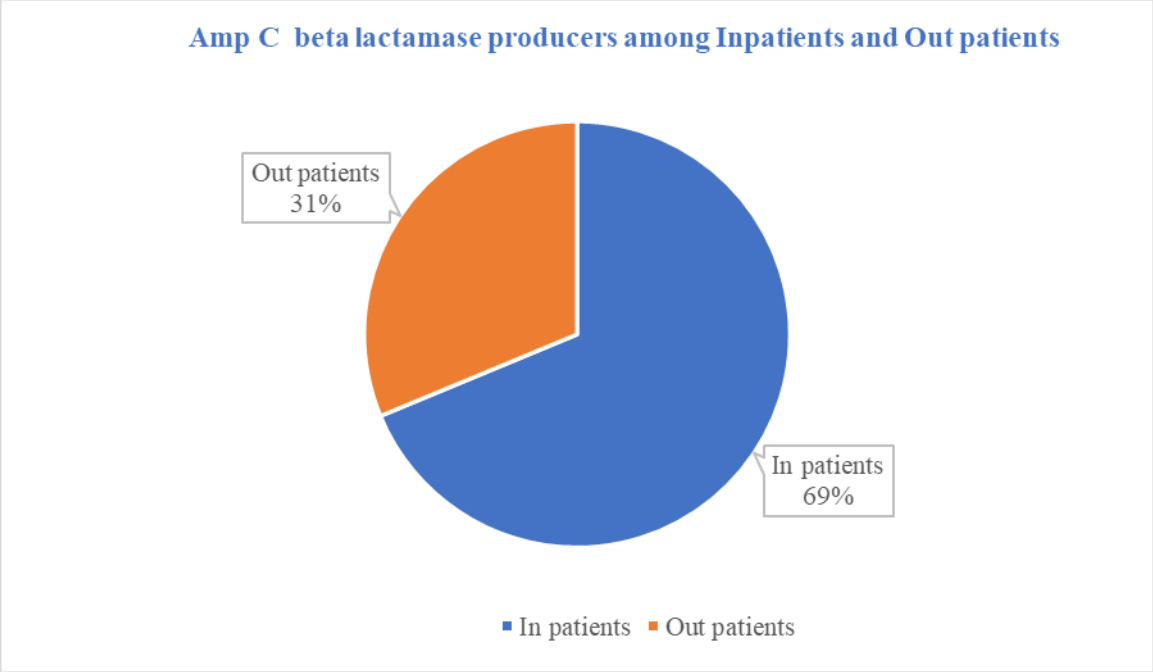

Table.6. Age and Gender wise distribution of Amp C beta lactamse producers

Gender/Age	1-10yrs	11-20 yrs	21-30yrs	31-40yrs	41-50yrs	51-60yrs	TOTAL
Male	--	--	1	2(12.5%)	2(12.5%)	5(31.25%)	10(62.5%)
Female	--	1	--	3(18.75%)	1(6.25%)	1(6.25%)	6(37.5%)

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Total	--	1(6.25%)	1(6.25%)	5(31.25%)	3(18.75%)	6(37.50%)	16

Result: Amp C producers are common in Males 62.5% and females it is 37.5%.

IV. DISCUSSION:

In the present study, 320 samples were processed out of which 180 are Gram negative bacteria belong to family Enterobacteriaceae which are screened for Cefoxitin resistance and (86) 47.7% are positive which is similar to Ronni Mol P et.al, that is 46.8%. and 57% in Anuradha et.al. study. The most common organism in this study is Escherichia coli 44.2% followed by Klebsiella pneumoniae 30.3% this is similar with the study conducted by Shagufta et.al in which Escherichia coli is the most common organism with 48%. In other study conducted by Parveen et.al, Pseudomonas 42.8% is the common organism.

In phenotypic confirmation tests, organisms are maximum diagnosed by Cefoxitin-cloxacillin disk diffusion test 46.5% which is similar to in Inamdar &Anuradha et.al study it is 47.5%. By Disk approximation test 29.1% showed indentation but it is 37.5% in Inamdar& Anuradha et.al study. E-strip method showed the positivity of 15.1%.

The maximum number of Amp C producers are from pus samples 62.5% followed by urine 37.5% which similar to Shagufta et.al with pus samples 48.5%and urine 24.4 %. The highest number of Amp C producers is from Surgery department 7(43.75%) followed by Orthopedics 5(31.25%).

In the present study, isolates confirmed to be Amp C producers are significantly higher in the age group of
51-60 years 37.5% similar to Shagufta et.al study 37.7%. Also 62.5% of samples belonging to males and samples from female patients are 37.5% are Amp C producers this is consistent with the previous study by Shagufta et.al 62.6% from males and 37.7% from female patients. The higher prevalence of Amp C producers in the females is between age group is 31 to 40 years 31.25% are from urine samples because urinary tract infections are common among the females.

In the present study Amp C beta lactamase producers are predominant in the In patients 69% than Out patients 31% similar to Shagufta et.al 80% isolates were from In patients this may be due to longer duration of stay in the hospital indicating Nosocomial infection. It is concluded that Cefoxitin-Cloxacillin double disk synergy test showed better results than other phenotypic tests.

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