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Detection of Extended Spectrum β - Lactamase & Amp C in Clinical Isolates of Klebsiella Species

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ABSTRACT

Clinical laboratories need to develop quick methods for detection of Extended Spectrum β- Lactamases (ESBL) & Amplified C (AmpC) β-Lactamase, so that the appropriate medication can be started without delay. Here, we reported the confirmatory methods for detection of ESBL & AmpC in Klebsiella species in Central laboratory MGM Hospital.We had isolated 201 Klebsiella from various clinical specimens and tested for the production of ESBL & AmpC followed by confirmatory methods as per Clinical Laboratory Standard Institute (CLSI) guidelines. Out of 3752 samples 201 klebsiella were isolated among them ESBL 26(12.93%), ESBL+AmpC 27(13.43%) amd only AmpC producer 14(6.94%) by confirmatory method (Inhibatory based method).The prevalence of ESBL & AmpC producing Klebsiella species is alarming and it requires strict implementation of infection control guidelines by safe hygiene practices, restricted use of broad spectrum antibiotics as empirical therapy.

KEYWORDS: ESBL, AmpC, Klebsiella species

INTRODUCTION

The β-lactamase are the most significant group of enzymes involved in conferring resistance to β-lactam antibiotics in gram-negative bacteria. They work by hydrolysing the β-lactam bond of any number of substrates thus rendering the antibiotic ineffective. Within months of the broader-spectrum β-lactamampicillin being released in Europe in 1964, the first incidence of resistance to ampicillin in Escherichia coli was described. Today there are many β-lactam antibiotics and many more β-lactamase enzymes, including Extended Spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases[1].

Newer β-lactamase that hydrolyze cephamycins, oxyimino and zwitterionic cephalosporin, monobactams, or carbapenems are of increasing concern because they restrict therapeutic options, cause treatment failures, and are increasing in occurrence[2]. *K. pneumoniae* is inherently resistant to penicillins and early cephalosporin due to constitutive production of chromosomally encoded class A group 2b β-lactamase. In addition to this enzyme, many *K. pneumoniae* strains produce one or more plasmid mediated β-lactamases. The most common belong to the enzyme families TEM, SHV Extended spectrum β-lactamase have been demonstrated in *K. pneumoniae* since 1983. Today, the most common SHV ESBLs worldwide are SHV-2, SHV-5 and SHV-12. New SHV variants still emerge. In recent years, reports on plasmid mediated enzymes belonging to the CTX-M family have become more and more frequent [3]. AmpC β- lactamase have gained importance since the late 1970s as one of the mediators of antimicrobial resistance in Gram negative bacilli. These enzymes are cephalosporinases capable of hydrolyzing all β–lactam to some extent. AmpC β-lactamases are of two types plasmid-mediated and chromosomal or inducible AmpC[4]. Plasmid-mediated AmpC β-lactamases produced by isolates of *Klebsiella pneumoniae* associated with decreased outer membrane permeability can even confer resistance to the carbapenems[5]. Plasmid-encoded AmpC genes have been found around the world since 1989, in nosocomial and non nosocomial isolates, have been most easily detected in those enterobacteria not expected to produce an AmpC β-lactamase[6]. Plasmid mediated AmpC-resistance has arisen through the transfer of chromosomal genes for the inducible AmpC β-lactamases onto plasmids. Plasmids with these genes can spread among other members of the family Enterobacteriaceae. [7]

MATERIAL METHODS:-

This study was carried out over a period of one year in the department of Microbiology, MGM Hospital Kamothe, Navi Mumbai. 201 clinical isolates of Klebsiella species were isolated from 3752 various clinical specimens by standard bacteriological methods.

ANTIBIOTIC SENSITIVITY:

By Kirby-Bauer method

A broth suspension of the test bacterium (0.5 Mc Farlandstandard) were prepared. Inoculation is performed by spreading with swabs on Mueller Hinton agar plates. After drying the plate (37°C for 30 minutes), antibiotic discs (4 – 6 per 10 cm plate) were applied with sterile forceps. After overnight incubation, the degree of sensitivity is determined by measuring the zones of inhibition of growth around the disc. Quality Control strains, *E.coli* ATCC 25922 (ESBL negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used. Antimicrobial susceptibility testing was performed according to CLSI guidelines.[8]

METHOD FOR ESBL :-

National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A disc of ceftazidime (30µg) alone and ceftazidime + clavulanic acid (30µg/10) were placed

at a distance of 25 mm center to center , on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37˚C. An increase in inhibition zone diameter of ≥5mm for a combination disc versus ceftazidime disc alone confirmed ESBL production.[9]

AmpC detection:

Inhibitor based test: All isolates were tested for AmpC β- lactamase production on discs containing boronic acid. A disc containing 30 µg of cefoxitin and another containing 30 µg of cefoxitin with 400 µg of boronic acid was placed on the agar. Inoculated plates incubated overnight at 37°C. An organism demonstrating a zone diameter around the disk containing cefoxitin and boronic acid ≥5 mm than the zone diameter around the disk containing cefoxitin alone were considered an AmpC producer.[10]

RESULT and DISCUSSION:-

Prevalence of 5.37% for Klebsiella species. The highest prevalence was shown by ET tube (20%) followed by sputum (14.14%). pus showed 5.56%, miscellaneous (3.33%), urine (3.08%), stool (1.17%) and blood (0.44%). Study by Shamweel Ahmad *et al.* showed the highest prevalence of Klebsiella in wound swabs (11.8%) followed by sputum (7.69%), Aspirates (7.54%). blood and urine showed 3.66%.[13]. In study by D. O. Acheamporg ***et al.*** showed the highest prevalence from sputum (14.0%) followed by urine (10.35%) and miscellaneous samples (8.8%) [14].

Table 1.Prevalence of Klebsiella strains in various clinical Specimens.

Specimen	Total no of Specimen	No of Klebsiella isolates (%)
sputum	693	98 (14.14)
pus	675	38 (5.65)
urine	875	27 (3.08)
ET	120	24 (20)
blood	899	5 (0.55)
miscellaneous	150	5 (3.33)
stool	340	4 (1.17)
Total	3752	201 (5.37)

Table 2.Prevalence of ESBL &AmpC Producers among Klebsiella species.

Sample	No of strain	ESBL (%)	ESBL + AmpC (%)	AmpC (%)
Sputum	98	10 (10.2)	16 (16.32)	7 (7.14)
Pus	38	8 (21.05)	6 (75)	2 (52.63)
Urine	27	6 (22.22)	7 (25.92)	2 (7.40)
ET	24	7 (29.16)	8 (33.33)	3 (12.5)
Blood	05	2 (40)	0 (00)	0 (00)
Stool	04	1 (25)	0(000	0 (00)
Miscellaneous	05	2 (40)	0 (00)	0 (00)
Total	201	26 (12.93)	27 (13.43)	14 (6.96)

Table 3.Maximum sensitivity and resistance pattern in clinical specimens.

Specimen	Antibiotic showing maximum sensitivity (%)	Antibiotic showing maximum resistance (%)
sputum	NET(92.85%), AK(94.89%),-LOM(91%)	PF(15.3%), CS(14.28%),CF(11.2%)
pus	OF(74%),GEN(71%),-LOM(47%)	CTX(48%),CPZ(48%),-CAZ(48%)
urine	GF(82%), BA(78%),CH(72%)	CI(55%),TE(38%),AS(34%)
ET	PF(80%),OF(80%),LOM(70%)	CXM(80%),CTX-(75%),CPZ(66%)
blood	LOM(100%),P-F(100%),OF(100%)	CXM(40%),CTX(40%),-CAZ(40%)
Stool	NET(100%), GEN(100%),AK(100%)	CXM(50%),CP-Z(50%),CIP(50%)
Miscellaneous	OF(100%), PF(100%) LOM(80%)	CXM(60%),CP-Z(60%),CIP(60%)

In 201 isolates, the highest number of *Klebsiella species* were isolated from sputum (48.75%) followed by pus (18.9%), urine (13.43%), endotracheal tube (11.9%). Blood and miscellaneous samples showed 2.48% of the growth. However in a study by A.O.Okesola *et al.* 88 isolates of *Klebsiella species* were identified from various clinical specimens which included sputum32(36.4%), wound swabs 22(25%), urine 18(20.5%), wound biopsy 4(4.6%), ear swabs 6(6.8%), blood 2(2.3%), eye swabs 2(2.3%) and catheter tips 2(2.3%).[15] In the study by Iffat Javed *et al,* 60 isolates of *Klebsiella species* were identified from various clinical specimens which included sputum 7(11.2%), high vaginal swabs 3(4.83%),urine 23(37.1%), blood 3(4.83%), pus 17(27.4%), endotracheal aspiration 3(4.83%),CSF 4(6.45%), body fluid 2(3.22%).[16] R. Sarathbabu *et al.* isolated Klebsiella from sputum 65(29.55%), urine 81(20.66%), pus 36(20.81%).[17]

Prevalence of ESBL and AmpC was found in Klebsiella species isolated from the various clinical specimens. Prevalence of total ESBL was 12.93%. The highest prevalence was seen in endotracheal tubes (29.16%), followed by urine (22.22%), pus (21.05%), sputum (10.2%), blood (40%), miscellaneous (40%), stool (40%). Prevalence of both ESBL and AmpC was 13.43% in total. The highest prevalence was in pus (75%) followed by ET (33.33%), Urine (25.92%), sputum (16.62%). Blood, stool and miscellaneous didn't show any ESBL+AmpC positive. The total AmpC prevalence was 6.96%. It was highest in Pus (52.63%) followed by ET tube (12.5%), urine (7.40%), sputum (7.14%). Shamweel Ahmad *et al.* showed 10.4% prevalence of ESBL producing Klebsiella. The highest prevalence of ESBL was seen on aspirtes (25%) followed by sputum (20%), Blood (18.2%), wound swabs (13.9%) and urine (9.2%). [18] Laghawe Avinash R. *et al* showed 11.7% prevalence of AmpC in Klebsiella species.[19]

CONCLUSION:-

The highest prevalence of *Klebsiella species* was observed in ET tubes (20%) followed by sputum (14.14%). The least growth was seen in blood. 12.93% of Kebsiella isolates were ESBL producers. 6.98% of Klebsiella showed AmpC production. Maximum AmpC production was observed in pus (52.63%) and lowest in blood and stool. Prevalence of both ESBL and AmpC was 13.43% in total. The highest prevalence was in pus (75%) followed by ET (33.33%), Urine (25.92%), sputum (16.62%). Maximum sensitivity against *Klebsiella species* was observed against lomefloxacin (84.48%) followed by gentamicin (80%), ciprofloxacin (80.45%), netilin (78.73%), amikacin (75.85%) cefaperazone (68.9%), cefotaxime 62.64% and least sensitivity was cefuroxime.

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