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Research Article

MULTIDRUG RESISTANT BACTERIA CREEPING INTO A NEWLY SET UP TEACHING HOSPITAL-TIME TO INTERVENE

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Abstract- Introduction: Antimicrobial Resistance (AMR) has been on continuous rise and drug resistant bacteria are the commonest etiology in hospitalized and community acquired infections. The objective of this study was to detect multidrug resistant organisms isolated from various clinical specimen, and their antibiotic profile. **Materials and Methods**: 750 Clinical samples were cultured; organisms were isolated and identified. Antibiotic susceptibility test was done based on Kirby Bauer disc diffusion method. ESBL production was tested by phenotypic double disc potentiation test. AmpC ß-lactamse production was tested by disc antagonism method. Detection of serine carbapenamases and MBLs was performed by Modified Carbapenem Inactivation method and EDTA Modified Carbapenem Inactivation method respectively as described by new CLSI guidelines. Among the Gram positive cocci, Methicillin and Inducible Clindamycin resistance was detected by cefoxitin disc diffusion and D-test respectively. **Results**: Among the resistant gram-negative bacteria, 33(10.9%) were ESBLs, 11(3.65%) Amp C, 3(1%) each were ESBL+Amp C, inducible AmpC and MBLs. Out of 150 resistant staphylococcal isolates, 103(68.6%) showed methicillin resistance and 26 (17.3%) showed inducible clindamycin resistance. Multidrug resistance (MDR) was observed in 4.8% of Gram negative and 2% of Gram positive bacteria. Extremely drug resistant bacteria (XDR) were found in 2% and 1% of Gram negative and Gram positive bacteria. **Conclusion**: Rising levels of AMR mandates routine detection of various types of resistance patterns. Routine detection of ESBLs, screening for Amp C beta lactamases, inducible Amp C, and confirmation of MBLs will help in providing authentic antibiotic susceptibility testing reports.

Keywords- *Beta lactamases, Drug resistance, MRSA, Gram negative, Gram positive*

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Introduction

Antimicrobial Resistance (AMR) has been on continuous rise in the past decade. Drug resistant bacteria are the commonest etiology in hospitalized and community acquired infections. Drugs belonging to Beta lactam group are used as the main stay of treatment as well as for empirical therapy in infections caused by these bacteria. Antibiotic resistance among both gram positive and gram negative bacteria is a rapidly expanding problem and a matter of concern, as they are able to mutate, acquire and transmit plasmids and other mobile genetic elements encoding antibiotic resistance genes [1]. Inducible clindamycin and Methicillin resistance is common among gram positives. β-lactamases namely extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo β-lactamases (MBLs) are the major cause of β-lactam resistance among gram negatives [2]. Resistance genes for all these three enzymes are often carried on plasmids, facilitating rapid spread between bacteria [3]. ESBLs belong to Group 2be of Bush's functional classification and AmpC beta-lactamases are well defined enzymes with broad substrate specificity and classified as class C according to Ambler and group 1 by Bush-Jacoby-Medeiros [4]. Extended spectrum beta-lactamases (ESBL's) were first reported in 1983 and Amp C beta lactamases in 1988 [5]. While ESBLs can hydrolyze all penicillin's, extended spectrum cephalosporins and aztreonam ; the Amp C beta lactamases can hydrolyze extended spectrum cephalosporins as well as cephamycins [1,6]. Carbapenems and beta lactam with beta lactamase inhibitors are the drugs of choice for infections caused by bacteria producing ESBLs and Amp C beta lactamases. But with the increase in carbapenamase producing bacteria, the carbapenems are losing their importance as the highest order saviour antibiotics for multi drug or pan drug resistant bacteria. The carbapenemases are metallobetalactamases which belong to Class B type in Ambler classification and they can hydrolyse all classes of beta lactams [5,6]. Often all the three enzymes are co-expressed in the same isolate [7]. The presence of ESBLs and Amp-C- β-lactamases in a single isolate reduces the effectiveness of the β-lactam-β-lactamase inhibitor combinations, while MBLs and Amp-C-β-lactamases confer resistance to carbapenems [3]. ESBL producing isolates, in addition to being resistant to ß-lactam antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones thus making them multidrug resistant [8]. The reason being, carriage of resistance genes for multiple classes of drugs on the same plasmid. This further complicates the treatment of serious infections caused by these bacteria. These strains are associated with high morbidity, mortality, increased length of hospitalization and cost of health care. Among tropical countries, India has emerged as the focal point of antimicrobial resistance [6]. In the past decade multidrug resistant bacteria have been described in numerous pathogenic strains among members belonging to Enterobacteriaceae, Staphylococci, and non fermenters like Pseudomonas and acinetobacter in varying combinations [6,9,10-13]. With this background the above study was undertaken to detect multidrug resistant organisms isolated from various clinical specimen.

Material and Methods

The study was carried out at a newly set up tertiary care teaching hospital at Bangalore, India over a period of 3 months.

750 Clinical samples which were received by our microbiology laboratory, like pus, urine, sputum, stool, blood and body fluids were cultured, organisms were isolated and identified by conventional method [14]. Antibiotic susceptibility test was done based on Kirby Bauer disc diffusion method according to CLSI guidelines [15]. Antibiotics used for Gram positive organisms were Penicillin, Erythromycin, Clindamycin, Cefoxitin, Cotrimaxazole, Chloromphenicol, Amikacin, Vancomycin and Linezolid. For gram negative organisms, Ampicillin, Amikacin, Amoxclav, Cefazolin, chloramphenicol, Cotrimaxazole, Ciprofloxacin, Tetracycline, Ceftazidime, Ceftriaxone, Cefipime, Piperacillin, Piperacillin with Tazobactam(PIT), Imipenem, and Meropenam. High level gentamycin 120mg (120mg) was used for Enterococci. For urine samples, Nitrofurontin and Norfloxacin were used. ATCC *E.coli* 25922 and *Klebseilla pneumoniae* ATCC 7006003 were used controls.

Detection of ESBL

Strains resistant to 3rd gen cephalosporins were selected for phenotypic confirmation of ESBL production. Combined disc method was used where in ceftazidime 30µg and piperacillin Tazobactam (PIT) discs were used and widening of the inhibition zone from ceftazidime, towards PIT was looked for [Fig-1]. Increase in zone of more than 5mm around the PIT disc or widening of zone of inhibition towards PIT was considered as ESBL producer [15].

Fig-1 Detection of ESBL

Detection of AmpC ß-lactamse

Screening test

The isolates were screened for presumptive AmpC production by testing their susceptibility to 30µg cefoxitin disc (Cx) (Himedia, Mumbai) using Kirby Bauer disk diffusion method. Isolates with an inhibition zone diameter of ≤18mm for cefoxitin were labelled as AmpC positive and were subjected to confirmatory test by AmpC disc test.

AmpC disc test

Strains which were resistant to Cefoxitin (<18mm) were tested for AmpC ß-lactamse production. Disc antagonism method was used where in a sterile disc smeared with the test organism was placed beside Cx disc, over a lawn culture of ATCC *E.coli.* Indentation along the disc with test organism was an indicator of AmpC [16] [Fig-2].

Fig-2 Amp C disc test

Inducible AmpC production

Disc approximation method described by Sander et al was followed. The test isolate was lawn cultured and exposed to ceftazidime 30 µg discs and Imipenam disc 10µg placed 20mm apart. Flattening of zone of inhibition around Ceftazidime indicated inducible Ampc production [17] [Fig-3].

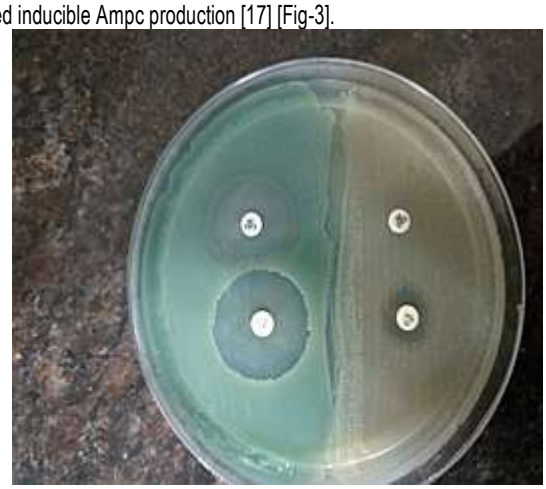

Fig-3 Inducible AmpC

Detection of Serine Carbapenamase and MBL production

Screening test

Isolates were screened for Serine Carbapenamase and MBL production by testing their susceptibility to Imipenem (Imp) (10µg) and Meropenem ((Mrp) 10µg) antibiotic discs (Himedia, Mumbai) using Kirby Bauer disk diffusion method. Strains which were resistant to Imp and Mrp (<19mm) were considered to be probable producers of serine carbapenamases or MBLs, and tested for Serine Carbapenamase production by Modified Carbapenem Inactivation method (mCIM). Production of MBL was detected by EDTA Modified Carbapenem Inactivation Method (Ecim) method as described by new CLSI guidelines [18].

mCIM for Serine Carbapenemase Production

1. A loopful of test bacteria from an overnight agar plate was transferred to a test tube containing 2ml trypticase soy broth (TSB). The suspension was vortexed.
2. 10µg meropenem disc was added to the suspension.
3. The TSB+ disc suspension was incubated for 4hrs at 35º C.
4. Prior to completion of 4hrs, 0.5 Mc Farland suspension of *E.coli* ATCC 25922 was lawn cultured onto a MHA plate.
5. The meropenem disc was removed from TSB suspension and placed on the MHA plate with *E.coli*. This plate was incubated at 35º C overnight. (Fig 4)

Interpretation

Following incubation, the zone around meropenem disc was measured. No zone of inhibition or presence of colonies within the zone of 15mm around the meropenem disc was considered due to carbapenemase production and the test isolate was considered to be a producer of serine carbapenemase. A zone size of >19mm was considered a negative test.

Ecim for MBL production

The procedure followed was same as above, in addition 20µl EDTA from a 0.5 molar solution was added to the TSB broth containing meropenem disc.

Interpretation

Following incubation, a zone size of >5mm compared to zone size in mCIM test was considered to be due to production of MBL. If the isolate was only mCIM positive and eCIM negative, then it was considered as serine carbapenamase producer.

Resistance patterns in Gram positive bacteria

Methicillin resistance: The gram-positive cocci resistant to cefoxitin(30µg) disc with a zone size of <21 mm was considered to be methicillin resistant according to CLSI guidelines.

Table-3 *Distribution of various beta lactamase among various clinical isolates*

Organism isolated	n	ESBL	%	AmpC	%	Ind AmpC	%	AmpC+ESBL	%	Carbapenamase	%	MBL	%
E.coli	120	23	19	5	2					3	2		
K.pneumoniae	64	5	8	3	5	2	3	1	2				
Pseudomonas	52	1	2		0	1	2	1	2	1	2	1	2
Citrobacter	23	2	9	1	4			1				1	4
K.oxytoca	21	1	5	2	0								
Proteus	12		0		0								
Acinetobacter	11		0		0							1	9
E.cloacae	4	1	25		0								

Table-1 *Prevalence of Beta lactamases in the hospital*

Organism	GNB	Beta lactamases
E.coli	120	28
K.pneumoniae	64	11
Pseudomonas	52	5
Citrobacter	23	5
K.oxytoca	21	5
Proteus	12	0
Acinetobacter	11	0
E.cloacae	4	1
Total GNB	307	55

Isolates that were Erythromycin (E) resistant, and Clindamycin sensitive, were tested for Inducible Clindamycin resistance. Erythromycin 15 microgram disc was placed at a distance of 15mm from CD disc. They were identified by the presence of a D zone around clindamycin disc.

Fig-4 eCIM positive (MBL)

Result

Total 307grams negative isolates and 200 grams positive isolates from various clinical samples were studied. Fifty five gram negative bacteria were found to produce beta lactamases, [Table-1]. Among the resistant gram negative bacteria, 33(10.9%) were ESBLs, 11(3.65%) Amp C, 3(1%) ESBL+Amp C, Inducible AmpC 3(1%) and 3(1%) MBLs [Table-2].

Table-2 Distribution of Betalactamase and carbapenamase

Beta lactamases	No of isolates	%
ESBL	33	10.9%
AmpC	11	3.65%
Inducible AmpC	3	1%
ESBL+AmpC	3	1%
MBL	3	1%
Carbapenamases	4	1.3%

Our study revealed that, ESBLs were predominantly produced by *E.coli (19%), Citrobacter(9%), Klebsiella pneumoniae(8%), Klebsiella oxytoca(5%) and pseudomonas* (2%). One isolate among the four Enterobacter cloacae produced ESBL(25%). Amp C beta lactamases were mainly produced by *Klebsiella pneumoniae, E.coli,* and *Citrobacter* species [Table-3]. Out of 200 grams positive isolates, distribution of resistance pattern is as given in [Table-4]. Out of 150 Staphylococcus isolates, 56 isolates showed Erythromycin resistance, among which 35 were resistant to both indicating constitutive MLSb phenotype, and 21 showed the characteristic D zone and hence were positive for inducible CDR (Table-5). To sum up, the rate of MRSA in our study was 68.6%, Betalactamases 17.9%, MDR and XDR rates were 4.8%, 2% and 2%, 1% among gram negative and gram positives respectively [Table-6].

Table-4 Resistance among gram positive isolates

Type of resistance	N	% n=150
Methicillin resistance	103	68.6%
Inducible CDR	26	17.3%

Discussion

The present study showed a high prevalence of drug resistance among Gram positive as well as Gram negative bacteria. Occurrence of ESBLs and Amp C production among enterobacteriaeceae was in close concordance with a study by Vijaya Shivanna *et al* [2]. 3 out of 11 that were screened positive for Amp C production, were inducible Amp C producers. This high prevalence highlights the need for being vigilant over the production of inducible Amp C beta lactamases. Clavulanic acid may act as an inducer of high level AmpC production resulting in false negative result in ESBL confirmatory test. MBLs were predominantly produced by Acinetobacter, Pseudomonas and Citrobacter species. Pseudomonas species were found to produce 2% each of ESBLs, Amp C and MBLs which is similar to a study by Jayakumar *et al* [10]. Various studies have shown ESBL prevalence rates ranging from15%, 18%, 54%, 73%, 14% to 73 %, AmpC occurrence varying from 9%, 20%,19%, 34%,18 % MBL 24%,27%,17%,17% and the presence of multiple enzymes varied from 1%-6% [1, 8, 12]. This difference could be due to the factors like antibiotic usage pattern in a not very old hospital like ours (<4yr old). The additional factor could be variations in the normal flora due to cultural, nutritional and ethnic difference in various populations. Continuous monitoring of the drug resistant patterns could give an insight into the development of resistance and aid in treatment as well as infection control measures. In our study, chromosomally mediated (inducible AmpC) resistance was 3(1%) and Plasmid mediated (uninducible AmpC) was 11%. In study by Ashok *et al*, Waseff *et al* and Nasir *et al*, uninducible Amp C rates were 4%, 5.8% and 22% respectively and inducible AmpC rates were 4.8% and 72% respectively [17,19,20]. Though there is no gold standard for these tests, these phenotypic tests are inexpensive, and highly sensitive and specific [17]. To sum up production of Beta lactamases was more common among *E.coli* and *Klebseilla* and least or none among Proteus and Acinetobacter (Table 1). In our study, MRSA prevalence rate was quite high with rate of 68.6% (Table 4). It is comparable to studies by Dar *et al* (54.85%) and Borg et al (65%) [21,22]. But studies by Sasirekha *et al* , Shittu and Lin *et al*, and Mehta *et al* have reported lower rates like 27.45%, 26.6%, 26.9%, respectively [23-25]. The overall Inducible Clindamycin resistance was 17.3% among total gram positives isolated. Among Methicillin resistant strains the rate was more among Staphylococcus aureus, compared to epidermidis species. MSSA isolates were observed to have a higher inducible Clindamycin resistance, compared to Methicillin resistant strains. This is in concordance with study by Sasirekha *et al*, Schreckenberger *et al* and Levin *et al* [23,26,27]. In contrast, inducible clindamycin resistance was more among MRSA compared to MSSA in a study by Ajantha *et al* [28]. Multidrug resistant (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. Extensively drug resistant (XDR) was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (*i.e.*, bacterial isolates remain susceptible to only one or two antimicrobial categories). Pandrug resistant (PDR) was defined as nonsusceptibility to all agents in all antimicrobial categories. In our study, among 307 grams negative isolates, 15 were MDR and 6 were XDR isolates, and among 200gram positive isolates, 4 were MDR and 2 were XDR. The study was limited by the fact that molecular characterization of the various β-lactamases could not be performed due to financial constraints.

Ours is a teaching hospital, which is still in the stage of developing into a full fledge hospital. Nevertheless Methicillin resistance rates are quite high, though beta lactamases are still in the lower range.

Table-5 *Distribution of Methicillin resistance*

Organism isolated	N	% (n=150)	Inducible CDR	% n=150
MRSA	75	50%	17	11.3%
MRSE	28	18.6%	4	2.6%
MSSA	47	31.3%	5	33.3%

Conclusion

Rising levels of AMR mandate the routine detection of various types of resistance patterns. Routine detection of ESBLs, screening for Amp C beta lactamases, looking for inducible Amp C resistance and confirmation of MBLs will help in providing authentic AST reports. This shall also help in optimal patient management and better infection control practices.

Application of research: It is necessary to implement screening tests for detection of various beta lactamases and their co-existence as a routine during laboratory investigation. This will help in providing authentic AST reports and optimal patient management as well as better infection control practices.

Research category: Medical Microbiology.

Abbreviations:
AMR- Antimicrobial Resistance
ESBL- Extended spectrum-ß- lactamase
MBL- Metallo-ß- lactamase
EDTA- Ethylenediamine tetraacetic acid

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