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Detection of Extended Spectrum Beta Lactamase and Carbapenemase Production in Klebsiella Pneumoniae in a Tertiary Care Hospital

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ESBL and Carbapenemase Detection in Klebsiella Pneumoniae

Abstract: ***Klebsiella species belonging to enterobacteriaceae family is both a community acquired and hospital based pathogen. Multidrug resistant Klebsiella species like extended-spectrum-β-lactamase (ESBL) producers and carbapenemase producers are increasing in Enterobacteriaceae family, it is worrisome as they are gut commensals and may spread to the community. Aims: To isolate, identify and antibiotic susceptibility pattern of Klebsiella pneumoniae (K. pneumoniae) and to detect the ESBL and Carbapenemase production. Methods and Material: K. pneumonia isolated from various samples was identified according to standard microbiological techniques. Antimicrobial sensitivity testing was performed by Kirby Bauer disk diffusion method. ESBL production was done by combined disc diffusion method and carbapenemase production was confirmed by Modified Hodge test (MHT). Results: Two hundred and fifty isolates of K. pneumoniae were isolated from various clinical samples. All ESBL positive isolates showed 100% sensitivity to imepenem and meropenem and all carbapenemase producing isolates were 100% sensitive to colistin, tigecycline and polymyxin B. Of 250 K. pneumoniae isolates, 52(20.8%) were carbapenemase producers, among them 44 (84.61%) were positive by MHT. Conclusions: As Modified Hodge test is a sensitive and rapid test for K. pneumoniae carbapenemase production, it can be recommended to screen all the isolates which are showing resistance to carbapenems.***

Keywords: Antimicrobial susceptibility, Carbapenemase, ESBL, *Klebsiella pneumoniae*, Modified Hodge test.

1. Introduction

Enterobacteriaceae family group of bacterias are normally present as human gut flora (1). They are one of the most important human pathogens isolated from the clinical samples accounting for majority of the infections (2, 3).

Klebsiella species which is one among the enterobacteriaceae family is both a community acquired and hospital based pathogen. It causes urinary tract infection, pneumonia, bacteremia, wound infection, cholecystitis, and catheter-associated bacteriuria etc. Multidrug resistant *Klebsiella* species causing hospital outbreaks are often caused by new types of strains i.e. extended-spectrum-β-lactamase (ESBL) producers (4).

ESBLs are a group of enzymes which hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. There are as many as 100 different ESBL enzymes, each with a preferential substrate. The genes responsible for the production of these enzymes are located on large plasmids which also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol. These isolates may be resistant to ceftazidime but susceptible to cefotaxime. Thus, susceptibility testing for third generation cephalosporins may not be able to detect ESBL-producing isolates. The Clinical Laboratory Standards Institute (formerly NCCLS) recommends susceptibility testing to several cephalosporins including cefpodoxime, cefotaxime, ceftriaxone and ceftazidime for routine screening for ESBL activity (5, 6, 7). *Klebsiella* spp. producing ESBLs such as SHV and TEM types have been a major cause of hospital-acquired infections since 1980s (8). ESBL-producing *K. pneumonia* has been consistently sensitive to Imipenem and meropenem of carbapenems group and cefoxitin and cefotetan of cephamycins (5).

Resistance to carbapenems is increasing in Enterobacteriaceae family, it is worrisome as they are gut commensals and may spread to the community. Also, carbapenemase enzyme can be easily transmitted via transposons and or integron resulting in widespread dissemination among susceptible gram negative bacilli rendering them resistant in the hospitals.This calls for an accurate diagnosis for effective therapeutic intervention (9).

Keeping this in mind, we conducted a study to know the antibiotic susceptibility patterns among *K. pneumoniae* and to detect ESBL and carbapenemase production in these organisms isolated from patients of our hospital.

2. Material & Methods

This study was conducted in the Department of Microbiology of a tertiary care hospital in south India from Feb 2014 to Jan 2015. Samples like blood, sputum, urine, tracheal aspirates/ broncho alveolar lavage, soft tissue samples and sterile body fluids which were received in the department of microbiology from patients attending out-patient and in-patient were chosen for the study.

K. pneumonia isolated from various samples was identified according to standard microbiological techniques (10). Antimicrobial sensitivity testing was performed on Mueller Hinton Agar (Hi-media, Mumbai) plates by disk diffusion method and the diameter of the zones of inhibition of growth was recorded and interpreted as sensitive, intermediate or resistant according to Clinical and laboratory standards institute (CLSI) 2014 guidelines. Organisms with intermediate levels of resistance to the antibiotics were included in the percentage of resistant organisms for final analysis.

Antimicrobial sensitivity to the following drugs was recorded: Penicillins: ampicillin10 µg, Cephalosporins: ceftriaxone 30 µg, ceftazidime 30 µg, cefotaxime 30 µg, cefoxitin 30 Carbapenems: imipenem 10 µg, meropenem 10 µg, Aminoglycosides: amikacin 30 µg, gentamicin 10 µg, Quinolones: Norfloxacin 5 µg, ciprofloxacin 5 µg, Tetracyclines: doxycycline 30 µg, Amoxyclav 30 µg, co-trimoxazole 25 µg, Aztreonam 30 µg, Nitrofurantoin 300 µg and Piperacilin /tazobactam 100/10 µg. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 strains were used as ESBL positive and negative controls respectively (11).

Detection of extended spectrum beta lactamase production (ESBL) (11)

All the *K. pneumoniae* isolated from these clinical samples were tested for ESBL production by combined disc diffusion method using two disks (concentration in µg) ceftazidime (30), ceftazidime/Clavulanic acid (30/10). The tests were interpreted according to 2014 CLSI guidelines and ≥ 5 mm increase in the zone of inhibition for ceftazidime/clavulinic acid disc when compared to ceftazidime disc alone was taken as ESBL producer.

Detection of carbapenemase production (11)

In all ESBL positive bacteria antibiotic susceptibility pattern to meropenem (Hi-media, Mumbai), and imepenem (Hi-media, Mumbai) was recorded for this study. By Kirby-Bauer method as per CLSI 2014 guidelines, isolates were considered as resistant to meropenem and imepenem if the zone of inhibition was <19 mm, intermediate if 20-22 mm and sensitive if >23mm. Organisms with intermediate levels of resistance to the antibiotics were included in the percentage of resistant organisms for final analysis. All the isolates which showed resistance to imepenem / meropenem were subjected to MHT on Mueller Hinton agar. *K. pneumoniae* ATCC 1705 and *K. pneumoniae* ATCC 1706 were used as MHT positive and negative strains respectively.

Procedure for Modified Hodge test

A Mueller Hinton Agar plate was inoculated with a 0.5Mc Farland's suspension of *Escherichia coli* ATCC 25922 and it was streaked to obtain confluent growth by using a swab. A 10 µg Imipenem disk was placed at its centre, and each isolate was streaked from the disk to the edge of the plate and plate was incubated at 37˚C overnight. After incubation, the plates were examined for a clover leaf type of indentation at the intersection of growth of the test organism and the *Escherichia coli* ATCC 25922, within the zone of inhibition of the carbapenem susceptibility disc (12). Interpretation of Modified Hodge test: A positive test shows a clover leaf like indentation of *Escherichia coli* ATCC 25922 which grows along the growth of test organism within the disc diffusion zone. A negative test shows no growth of *Escherichia coli* ATCC 25922 along the growth of test organism within the disc diffusion zone.

3. Statistical Analysis

Data was entered into a computerized Excel (Microsoft Excel 2009) spread sheet, and subsequently it was analyzed using SPSS (trial version 20) software. Descriptive statistics (means and percentages) were employed wherever necessary.

4. Results

Two hundred and fifty isolates of *K. pneumoniae* were isolated from various clinical samples. Sample wise distribution of *K. pneumoniae* was 36%, 11.2%, 24%, 23.2%, and 5.6% from blood, sputum, urine, miscellaneous and pus respectively as shown in Graph 1.

Antimicrobial susceptibility pattern of *K. pneumoniae* isolates

Of the 250 *K. pneumoniae* isolates tested for their antibiogram, 68% showed susceptibility to 3rd generation cephalosporin and 32% were resistant. Amoxyclav (48.5%) showed highest percentage of resistance followed by cotrimaxazole and ciprofloxacin. Similarly, a highest percentage of isolates were susceptible to imepenem (79.2%) and meropenem (79.2%) followed by piperacillin/tazobactam, norfloxacin, nitrofurantoin, ceftazidime and ceftriaxone as shown in Table 1. All ESBL positive isolates showed 100% sensitivity to imepenem and meropenem and all carbapenemase producing isolates were 100% sensitive to colistin, tigecycline and polymyxin B.

Sample wise distribution of ESBL positive *K. pneumoniae*

Of 250 *K. pneumoniae* isolates, 78 (31.2%) were ESBL producers. Sample wise distribution was 38.5%, 25.6%, 23%, 10.3% and 2.6% from blood, urine, miscellaneous, sputum and pus respectively as shown in Table 2.

Sample wise distribution of carbapenemase producers

Of 250 *K. pneumoniae* isolates, 52 (20.8%) were carbapenemase producers. Sample wise distribution was 30.7%, 26.92%, 19.23%, 15.38% and 7.69% from blood, miscellaneous, pus, sputum and urine respectively as shown in Table 3.

Carbapenemase producers that were positive by MHT
Of 250 *K. pneumoniae* isolates, 52 were carbapenemase producers, among them 44 (84.61%) were positive for MHT as shown in graph 2.

5. Discussion

In the present study highest number of *K. pneumoniae* were reported from blood sample 90/250 (36%) followed by urine 60/250 (24%), miscellaneous 58/250 (23.2%), sputum 28/250 (11.2%) and pus 14/250 (5.6%).This agrees with the study of Saroj Kothari et al who has found highest prevalence of *K. pneumoniae* in blood (31%) (13).

In the present study, 31.2% ESBLs are reported from patients admitted in the hospital. This is in co ordinance with the study conducted by Gupta V et al who has also reported 30.18% ESBL *K. pneumoniae* from various clinical samples (14). A study conducted by Ananthan and Subha (15) from Chennai reported 23.6% of ESBL *K. pneumoniae* from clinical isolates. In other studies, Menon et al. (16) from Chennai and Supriya et al. (17) from Nagpur have also reported the prevalence of ESBL producing *K. pneumoniae* were 21.2% and 25.65%, respectively.

In the present study, resistance to carbapenem was 20.8% which correlated with the study of Manoharan and Premalatha et al who reported 17% resistance to carbapenems in Enterobacteriaceae strains (18). On the contrary, Priya dutta, Varsha Gupta et al (9), Wattal C et al (19) and Gupta E et al (2) showed 7.87%, 13-57% and 17-22% resistance to carbapenems respectively.
Based on our antibiotic susceptibility testing, imipenem and meropenem were the most effective antibiotics against ESBL-producing *K. pneumoniae* (100%), followed by amikacin (78.4%), piperacillin/ tazobactam (76%) and norfloxacin (72.8%). *K. pneumoniae* isolates, which were ESBL producers, were reported to be 100% sensitive to imipenem in the study conducted by Jones et al. (20). Cefoxitin resisted hydrolysis by ESBLs and showed moderate activity against isolates producing ESBL in our study. This could be due to the concomitant presence of a plasmid-mediated AmpC-type β-lactamase that effectively hydrolyses this antibiotic or to porin-deficient mutants .

Imipenem and meropenem are the drugs of choice for life-threatening infections due to ESBL-producing Enterobacteriaceae or in an outbreak setting. However, to preserve the therapeutic value of carbapenems, based on institutional patterns of susceptibility results, piperacillin/tazobactam, fluoroquinolones or an aminoglycoside would be preferable.

The MHT is a phenotypic screening test for carbapenemases which is used for epidemiological purposes, and its use is currently proposed by the Clinical and Laboratory Standards Institute (CLSI). The MHT is easy to perform, but divergent specificity values have been reported, so should be aware of false-positive results (21).

In the present study, the sensitivity of MHT was calculated to be 95.65% and specificity was 72.72%. Ana Paula Cury et al (22) in their study found 100% sensitivity and 98% specificity. A similar study by Anderson et al (23) who had also evaluated the MHT for detection of KPC-mediated resistance proposed that the test demonstrated 100% sensitivity and specificity for detection of KPC activity. Diana Doyle et al (24) in her study showed that MHT had a sensitivity of 98% for detecting KPC producers. In our study, specificity was less compared to other studies. This can be improved by standardization of interpretation of the results for the KPC detection.

The MHT may detect the presence of carbapenemases, disadvantage is that it is not specific for KPC and may have false-positive results due to non-carbapenemase enzymes, such as AmpC and/or extended-spectrum beta-lactamases (ESBLs), combined with porin loss. Anna paula cury et al (22) showed in their study that, positive MHT had 98% agreement with the molecular bla KPC results, highlighting the good positive predictive value of KPC detection among Enterobacteriaceae when a standardized method for interpretation is practised. Enterobacteriaceae bacterias that not susceptible to imipenem/meropenem but have a negative MHT result are not KPC producers.

6. Conclusion

ESBL have become widespread throughout the world and indiscriminate use of antibiotic is one of the main causes. Carbapenems are the drug of choice for ESBL producers. So it becomes important to detect the carbapenem resistance. As MHT is a sensitive and rapid test to detect *K. pneumoniae* carbapenemase production, it can be recommended to screen all the isolates which are showing resistance to carbapenems.

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Table 1: Antibiotic susceptibility pattern of *K. pneumoniae* isolates

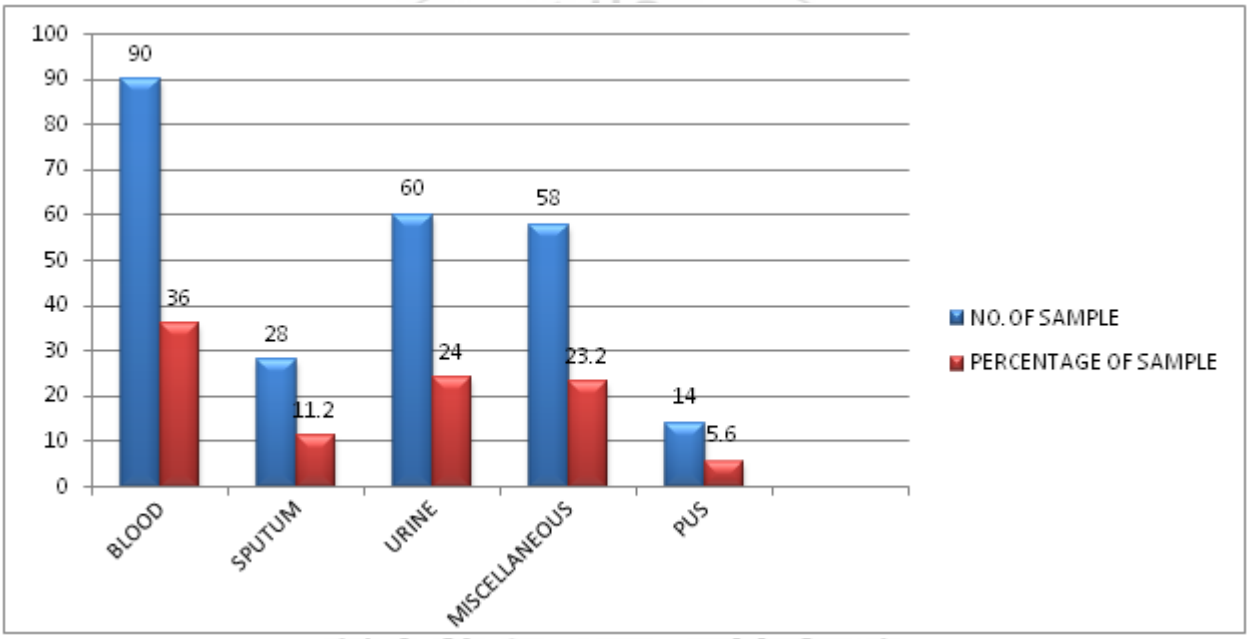
Antibiotic	*Sensitive (N0.)*	*Percentage%*
Ampicillin	0	0
Amoxyclav	128	51.2
Gentamicin	160	64
Amikacin	196	78.4
Ciprofloxacin	135	54
Norfloxacin	182	72.8
Nitrofurantoin	179	71.6
Ceftazidime	170	68
Ceftriaxone	170	68
Cefotaxime	167	66.8
Cefoxitin	160	64
Aztreonam	154	61.6
Co-trimaxazole	130	52
Piperacillin/Tazobactam	190	76
Imipenem	198	79.2
Meropenem	198	79.2
Tigecycline	250	100
Colistin	250	100
Polymyxin B	250	100

Table 2: Sample wise distribution of ESBL positive isolates

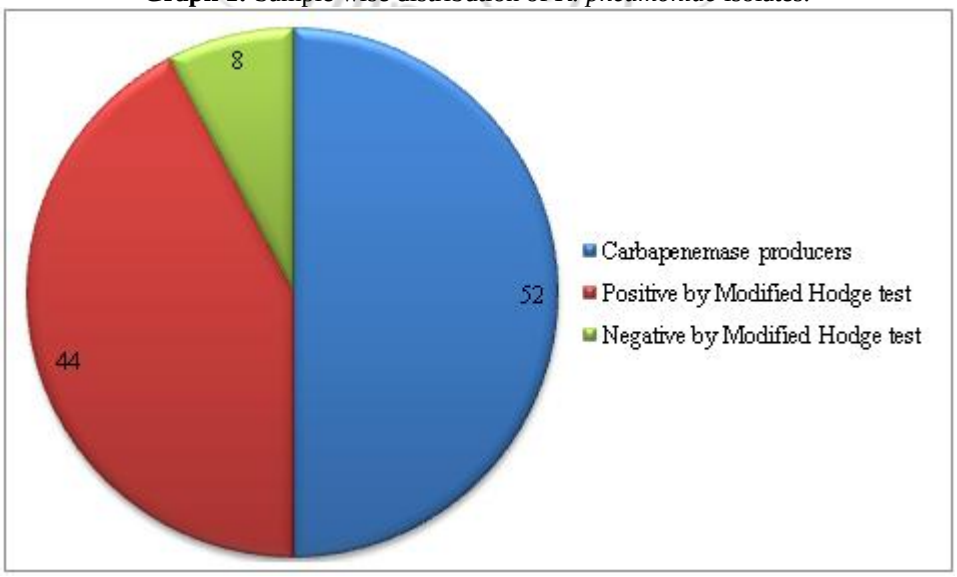
Type of Sample	*Number*	*Percentage%*
Blood	30	38.5
Urine	20	25.6
Miscellaneous	18	23
Sputum	8	10.3
Pus	2	2.6
Total	78	100

Table 3: Sample wise distribution of Carbapenemase positive isolates

Type of Sample	*Number*	*Percentage%*
Blood	16	30.7
Miscellaenous	14	26.92
Pus	10	19.23
Sputum	8	15.38
Urine	4	7.69
Total	**52**	**100**



Graph 1: Sample wise distribution of *K. pneumoniae* isolates.



Graph 2: Detection of Carbapenemase production by Modified Hodge Test

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