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Original Research Article

Prevalence of multi drug resistant *Klebsiella pneumoniaee* infections, causing a HAVOC in intensive care units of a tertiary care hospital

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Abstract: Multi drug resistance *Klebsiella pneumoniae*e are rapidly emerging as life threatening nosocomial infections. The ICUs are considered as epicenter of infection mainly because of severe clinical conditions, increasing use of invasive diagnostic procedures lapses in sterilization and disinfections. Beta-lactamase which include ESBL, AMPC, CARBAPENAMASES, MBL have emerged as the most worrisome mechanism of resistance amongst the gram negative bacteria which pose a therapeutic challenge to health care institutes. This study aims to find the prevalence of MDR *Klebsiella pneumoniaee* isolates from various clinical specimens of ICU patients and to evaluate their sensitivity patterns. The present study was a retrospective study of a 50 bedded ICU in tertiary care hospital from August 2014 to May 2015. Out of total 500 different clinical specimens studied 288 (57.6 %) specimens showed growth of *Klebsiella species.* Phenotypic characterizations of Beta lactamases present in *Klebsiella* isolates were carried out by disc diffusion methods (CLSI guidelines). The prevalence of Beta lactamase genes in 25 strains of *Klebsiella* was also studied using multiplex PCR. Antibiotic susceptibility was performed according to the CLSI guidelines. Among the total of 500 isolates from ICU 288 (57.6 %) isolates were *Klebsiella* maximum being isolated from tracheal specimens. The most common βlactamase identified was AMPC 151 (52.43%), followed by carbapenames 45 (15.62%), ESBL 31 (10.76%), (7.29%), MBL 5 (1.73%), unknown 25 (%), Coproduction of ESBL +AMPC 17(5.90%), AMPC+CARBAPENAMSES 8 (2.77%), AMPC +MBL 4 (1.38%) was detected phenotypically and similar results were found genotypically.
Keywords: Beta lactamases, MDR, tertiary care, ICUs, *Klebsiella pneumoniaee*.

INTRODUCTION

The aim of the present study was to conduct surveillance in the Intensive care Unit of a tertiary care hospital in Indore to study the prevalence of multi drug resistant *Klebsiella pneumoniae* [2]. The Intensive care unit's is called the epicenter of infection, due to its extremely vulnerable population which are associated with severe clinical conditions along with the impaired immunity increased risk of becoming infected through multiple procedures and use of invasive devices distorting the anatomical integrity, lapses in infection control practices and indiscriminate use of antibiotics. Multi drug resistant *Klebsiella pneumoniae* is increasingly being reported worldwide [1]. Multi drug resistant *Klebsiella pneumoniae* is associated with high morbidity and mortality [7]. The *Klebsiella pneumoniae* species in addition to its virulences and ability to acquire antibiotics resistance determinants are able to survive on skin and water surface and resist desiccation which adds to its pathogencity [7]. Multi drug resistant *Klebsiella pneumoniae* sepsis has serious implications because of limited choices of antibiotics available [7]. *Klebsiella pneumoniae* is a well described health care associated pathogen and a cause of sepsis, urinary tract infections, Pneumonia, and soft tissues infection in patients in the Intensive care unit's [8]. The most common reservoir for these pathogens appears to be the gastrointestinal tract of colonized patient, and patient-to-patient transmission is facilitated by transient or resistant hand carriage of health care workers (HWCs) [8]. Antibiotics resistance is a major world wide problem in the Intensive care units [6]. The treatment of multi drug resistant bacterial infection such as the MBL, AMPC, ESBL, CARBAPENAMASE, producing. *Klebsiella pneumoniae* is a major concern to clinicians and continues to be problematic. Clinically these pathogens are becoming more and more resistant to the old and some of the more recently developed antimicrobial agents due to non availability of mechanisms to fight resistances [3]. These strains are

difficult to control because they spread easily within and between hospitals and treatment options for multi drug resistant infections are extremely limited [1]. Strategies to control outbreaks have included antibiotic control policies , cohorting infected and colonized patients, transmission- precautions, surveillance cultures of patients, the environments and HCW's and improving hand hygiene [2].

MATERIALS AND METHODS

The present study was conducted in the department of microbiology of tertiary care hospitals in Indore. A total of 500 specimens were included in the study from August 2014 to MAY 2015. The specimens included blood, sputum, pus, endotracheal secretions, urine, fluids etc. 288 specimens showed growth of *Klebsiella pneumoniaee*. All the isolates were characterized to species level using standard procedures. Organism collection, transport, confirmation, of organism identification and development and management of a centralized data base were coordinated by microbiology department of Bombay hospital Indore India.

BactT alert 3D (biomerivex, france) automated system was used to culture all the blood sample received. Blood culture bottled were incubated for seven day before being reported negative, identification and antibiotics susceptibility testing of all these isolates were done using routine biochemical tests and standard Kirby-baurers methods for antibiotics susceptibility and subsequently and interpreted as per CLSI guidelines. Isolates from positive culture showing growth of *Klebsiella pneumoniae* were included in the study.

Antimicrobial susceptibility study

The antimicrobial susceptibility listing of the drugs were determined by the disc diffusion method according to the clinical laboratory standards institute methods (CSLI). Quality controls (QC) were performed on each day of testing using ATCC standard strains as the reference strains throughout study [2]. The strains were typed on the basis of antibiogram.

Phenotypic Detection of enzymes producing organisms

All the antibiotics disc were procured from Hi-media Mumbai. The strains were tested for ESBL production by phenotypic confirmatory disc diffusion test (PCDDT) AMPC production by AMPC disk test and carbapenamases production by modified Hodge test. MBLs the metallo –ß-lactames production was detected by the imepanem EDTA double disk synergy test.

Detection of ESBLs

Each strains was screened for the ESBL producing against cefotaxime, ceftazidime .The strains which were resistant to these third generation cephalosporins were confirmed by these phenotypic test i.e the disc potentiation test (by using ceftazidime & ceftazidime –clav, cefotaxime and cefotaxime clav discs), the double disc synergy test as per the CLSI guideline [4]. The strains showing a difference of >/= 5mm were considered as ESBL producing.

Detection of AMPc Beta- Lactames

All the strains were screened for the AMPC –ß-LACTAMES production by the disc antagonism test. Using cefoxitin and cefoxitin+cloxacillin disc diffusion method [4]. A difference of 4mm or more was considered as positive for AmpC production.

Detection of Metallo-Beta-Lactamases (MBLs)

The metallo-Beta-lactamese production was detected by the Imipenam-EDTA double disc synergy test. The organism were considered to be MBL Producers if they showed a difference of >7mm in the zone size of Imipenem=Imipenem EDTA.[4].

Modified Hodge Test

The strains were subject to modified Hodge test for detection of carbapenamase. An overnight culture suspension of E. coli ATCC 25922 adjusted of 0.5 MC.FARLAND standard was inoculated using a sterile cotton swab on the surface of a Muller-Hinton agar. After drying 10mg meropenems disk was placed at the center of the plate and the test strains were streaked from the edge of the disk to the periphery of the plate in four different directions. The plates were incubated overnight at 37c. The presence of a (clover leaf shaped) zone of inhibition due to carbapenames production by the test strains was considered as positive [7].

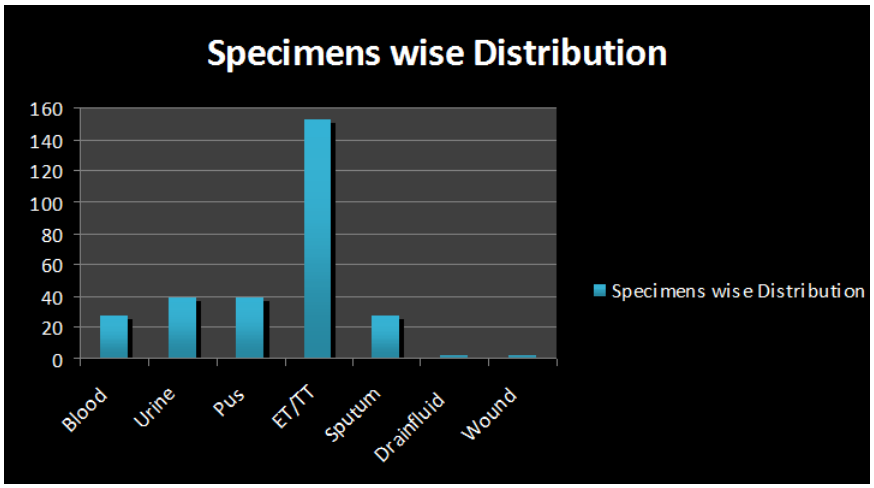
Genotypic Detection of Beta Lactamase Genes:

For the genotypic detection 25 strains of MDR *Klebsiella pneumoniaee* were outsourced where Multiplex PCR test was performed for the detection of ESBL (SHV,TEM and CTX-M type genes), AmpC gene and MBL gene(blaIMP, blaVIM, blaNDM-1).

Statistical analysis

The results obtained were subjected to statistical analysis for the detection of Mean absolute error for MDR *Klebsiella pneumoniaee* producing different Beta lactamases and the value was 0.989 which is </= to 1.

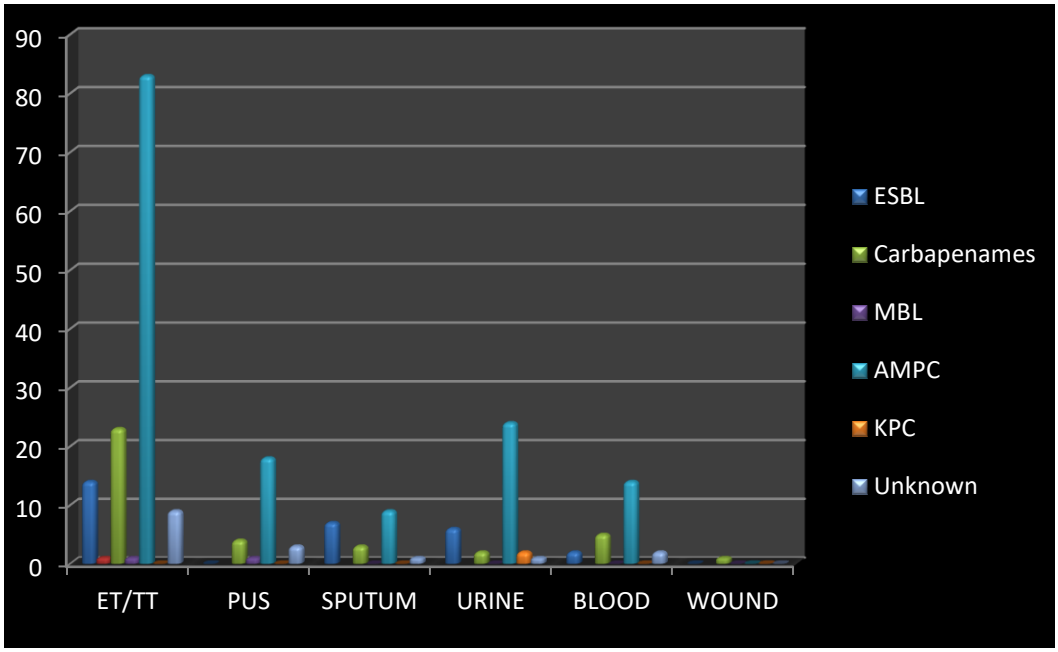
RESULTS



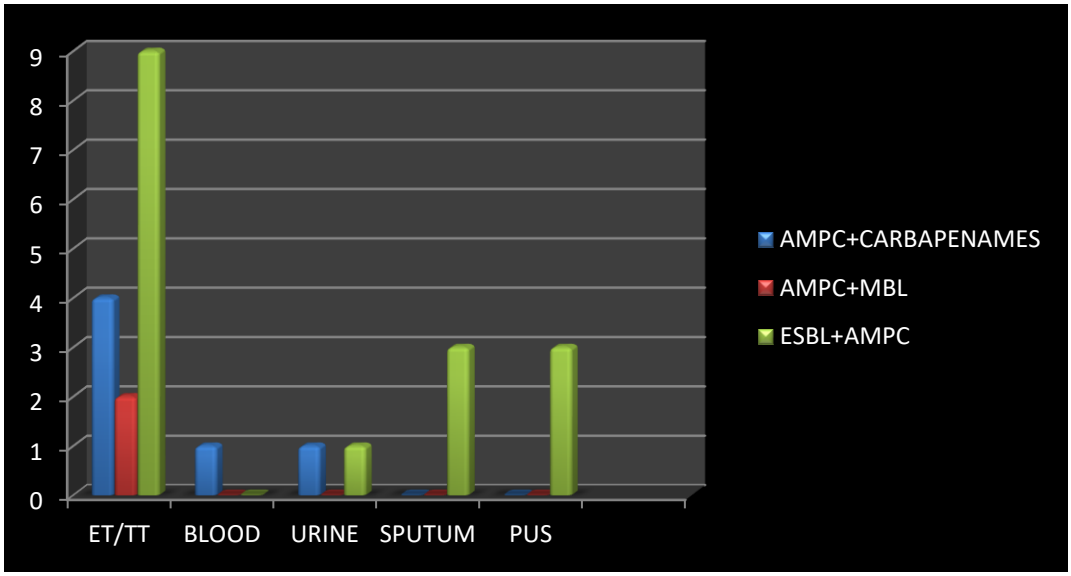
Graph-1: Specimens wise distribution.

Table-1: Specimen Wise Distribution.

SERIAL NUMBERS	SPECIMENS NAME	TOTAL ORGANISMS	*KLEBSIELLA PNEUMONIAE*
1	BLOOD	42	27
2	URINE	121	39
3	PUS	56	39
4	ET/TT	234	152
5	SPUTUM	34	27
6	BAL	1	0
7	DRAIN FLUID	1	2
8	STOOL	8	0
9	UNNECLS	1	0
10	WOUND	1	2
11	DRAIN TIP	1	0



Graph –2



Graph-3:

Table-2: Phenotypic Detection of Beta Lactamses

S. No	Enzymes	Value	Percentege
1	AMPC	151	52.43
3	MBL	5	1.73%
4	CARBA	45	15.62%
5	ESBL	31	10.76%
6	UNKNOWN	25	8.68%
7	AMPC+CARBA	8	2.77%
8	AMPC +MBL	4	1.38%
9	ESBL+AMPC	17	5.90%

Table-3: Genotypic detection of Beta lactamases by Multiplex PCR:

SHV	ESBL(12) TEM	CTX-M	AmpC(8)	NDM-1	MBL(5) VIM	IMP
9	10	1	8	3	2	0

Table-4: Sensitivity Pattern of MDR Klebsiella SP

Antibiotics	ESBL-31	AMPC-151	MBL-5	CARBAPENAMES-45	UNKNOWN-25
COLISTIN	31(100%)	151(100%)	5(100%)	35(77.77%)	25(100%)
MEROPENEM	26(83.87%)	115(76.15%)	0	0	23(92%)
IMEPENEM	24(77.41%)	138(91.39%)	0	27(60%)	14(56%)
TIGECYCLINE	31(100%)	151(100%)	5(100%)	45(100%)	0
PIP+TAZO	22(70.96%)	0	0	0	0
AMIKACIN	22(70.96%)	56(37.08%)	2(40%)	9(20%)	9(36%)
CEFO+SULBACTUM	23(74.19)	0	0	0	0
CHLORAMPHENICOL	0	83(54.96%)	0	0	22(88%)
TOBRAMYCIN	0	42(27.81%)	0	10(22.23%)	0
ERTAPENEM	0	0	0	0	13(52%)
LEVOFLOX	0	0	0	0	8(32%)
TETRACYCLINE	0	0	0	15(33.33%)	0
NETILMYCIN	0	0	1(20%)	8(17.77%)	0
POLYMXIN B	0	0	5(100%)	0	0

During the study period a total 288 *Klebsiella pneumoniaee* were isolated from a range of clinical specimens of patient's hospitalized in ICU's of a tertiary care hospital.

Klebsiella pneumoniae isolates were frequently isolated from respiratory specimens ET/TT (152), followed by Urine(39), Pus(39), Blood(27), Sputum(27), Fluid (2),Wound(2) shown in Graph 1.

As showns table 2 phenotypic detection of beta lactamases revealed high AMPC production 151/288 (52.43%), followed by Carbapenemases 45/288(15.62%), ESBL31/288(10.76%), UNKNOWN 25/288(8.68%), MBL5(1.73%),co-prodution of ESBL+AMPC was found in 17(5.90%) , followed by AMPc+Carbapenamase 8/288(2.77%), AMPc+MBL 4/288(1.38%) as shown in Graph 2&3.

Genotypic detection of resistant genes by multiplex PCR in 25 selected strains revealed a higher ESBL production with SHV and TEM types of ESBLs more prominent as shown in Table no4. A striking feature was a high rate of co production of more than 2 genes i.e ESBLs+AmpC +MBL which is a matter of concern.

Sensitivity pattern of the MDR *Klebsiella* as shown in Table no 5 indicates a higher resistance towards 3rd generation cephalosporins, quinolones. Common sensitivity for ESBLs was Meropenem, Imipenem, Colistin Amikacin and Pip+Tazo. Similarly for AmpC were Imipenem, Meropenem, Chloramphenicol, Colistin and Tobramycin. For MBLs the sensitivity was towards Colistin, Tigecycline, Polymyxin B.

DISCUSSION

The infection caused by multidrug resistant *Klebsiella pneumoniae* that produced various beta lactamase enzymes have been reported with in increasing frequency in the ICU's and are associated with a significant increases morbidity and mortality [4, 2]. *Klebsiella pneumoniae* is a recognized of hospital acquired infection worldwide [16] & becomes a global challenge as this organisms is resistant to cephalosporine , Amyglucosides, fluro-quilones, and Now emergines of carbapename, resistance in this species is off considerable concern, living relatively in limited treatment options from ICU's infection [2]. The patient's in ICU are more prone to colonization and infection by the various pathogens [9] and *Klebsialla pneumonia* species conmenst isolates which cause infection in such patients [2]. The numerous beta lactames are encoded by either by the chromosomal gene or transferable are encoded which are located on the plasmids or the transponzones [4]. In our study nearly 57.6% of ICU infections were caused by *Klebsiella pneumoniae*. Similarly study reported the incident of *Klebsiella pneumoniae* 55-77% [15, 14, 9, 16]. The beta-lactames producing in this *Klebsiella species* were AMPC 52.45%, CARBAPENAMES 15.62%, ESBL-10.76%, ESBL+AMPC- 5.90%, AMPC+CARBAPENAMES-2.77%, AMPC+MBL-1.38%, UNKNOWN-8.68%, MBL-1.38%.

Similar report were seen by Bandlekar *et al*. [18] (22.9%) of AMPC production and by Battarcharji *et al*. showing 22% production [17]. The high prevalence of AMPC production is our study may be due to differences in the geographical distribution resulting in variations.

In the prevalence of Beta-lactameses co-production detected phenotypically was ESBL+AmpC 17(77%), AMPC+MBL (1.38%).

Similarly results were seen by Loweena *et al*. [4]. The co-existances of different class of beta-lactameses may pouse diagnostic and treatment challenges the AMPC producing organisms' can act as resumious and high level expressions of AMPC may mask the recorganized of ESBL's resulting in fatal and in appropriate antimicrobial therapy.

CONCLUSION

In the present study *Klebsiella pneumoniaee* was a predominant multi drug resistant organism isolated from the ICUs of the tertiary care center. A high prevalence of ESBL and AmpC producing strains were observed and an increasing prevalence of coexistence of genes was also observed in both phenotypic and genotypic methods which result in failure of antimicrobial therapy. In this respect, antimicrobial stewardship and infection control measures are urgently needed for controlling the spread of MDR infections in ICUs.

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