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Original Research Article

The outcome of different β lactamase production in *Klebsiella pneumoniae* subspecies pneumoniae isolated from different clinical specimen in tertiary care hospital: a study

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ABSTRACT

Background: *Klebsiella pneumoniae* subspecies pneumoniae is one of the most commonly isolated bacterial species in Microbiology laboratories and can cause Infection in hospitalised patients with strain resistant to many antimicrobials. Klebsiella pneumoniae is more frequently recovered from clinical specimen. The carrier rate as high as 20% may occur in hospitalized patient.
Methods: Isolates are screened for presumptive ESBL by reduced susceptibility to CAC, CAZ, Carbapenemase by Classical Hodge test and modified Hodge test, MBL by DP Test, AmpC production by testing their susceptibility to Cefoxitin using Kirby-Bauer disk diffusion method. KPC by combined Disc method using PBA.
Results: Out of total 300 Klebsiella pneumoniae strains studied, 98 (32.7%) were isolated from urine followed by pus and wound swab 74 (24.7%) and 63 (21%) were isolated from blood.
Conclusions: Maximum (23.5%) ESBL, (10.2%) AmpC β- lactamases and (10.2%) KPC producing strains were isolated from urine sample. Maximum 32.1% MBL producing Klebsiella pneumoniae strains were isolated from pus and wound swab.

Keywords: CRE, E-test, DP test, ESKAPE group, ESBL

INTRODUCTION

Klebsiella pneumoniae has been included in ESKAPE group of organisms, which are most commonly associated with antimicrobial resistance, especially in Hospitalized patients.[1] *Klebsiella pneumoniae subspecies pneumoniae* is one of the most commonly isolated bacterial species in Microbiology laboratories and can cause Infection in hospitalised patients with strain resistant to many antimicrobials. *Klebsiella pneumoniae subspecies pneumoniae* belongs to family Enterobacteriaceae and included in Tribe *Klebsiella.* Klebsiella species are widely distributed in nature, gastrointestinal tract of human and animal. *Klebsiella* species should be suspected when large colonies with mucoid consistency are recovered on primary isolation plates. *Klebsiella pneumoniae* is more frequently recovered from clinical specimen. It is rarely found in oropharynx of normal person and 1-6% carrier rate have been reported. The carrier rate as high as 20% may occur in hospitalized patient.[2]

Aim was to study the Outcome of different β- lactamase production in *Klebsiella pneumoniae subspecies pneumoniae* isolated from different clinical specimens in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (M.S.) from December 2015 to November 2016.

A total number of 300 *Klebsiella pneumoniae subspecies pneumoniae* strains were isolated from different clinical samples, received from the indoor patients departments (IPD) of Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Wardha (M.S) which is a tertiary care Hospital in rural setup. In the present study all 300 *Klebsiella pneumoniae subspecies pneumoniae* have been mentioned as *Klebsiella pneumoniae.*

All the specimens like urine, blood, pus and wound swab, body fluids, medical devices, tracheal aspirates etc. were cultured on Blood agar and Mac Conkey's agar. On Mac Conkey's agar.

METHODS

Detection of Extended spectrum β- lactamases (ESBL)

Screening tests

As per Clinical and Laboratory Standard Institute (CLSI) guidelines for Enterobacteriaceae, reduced susceptibility to Ceftazidime, Cefotaxime, Cefpodoxime, was observed as screening test and Phenotypic confirmatory tests were done.[3,4]

Confirmatory tests: As per Clinical and Laboratory Standard Institute (CLSI) guidelines for Enterobacteriaceae, the combined disc method was used as Confirmatory tests for detection of ESBL producing *Klebsiella pneumoniae* strains. ESBL producing strains were futher confirmed by E test.[5]

The disc containing Ceftazidime and Ceftazidime plus clavulanic acid respectively were used in this method. An increase in diameter of ≥5mm with Ceftazidime plus clavulanic acid (CAC) disc as compared to Ceftazidime (CAZ) disc alone was considered positive for ESBL production. The control strain used was *Klebsiella pneumoniae* ATCC 700603.

E test

The ESBL E-test strip was placed onto the inoculated plate. After overnight incubation at 37^0C, the zone of inhibition was read from two halves of the strip. MIC ratio of Ceftazidime/ Ceftazidime plus clavulanic acid (TZ/TZL) ≥ 8 or deformation of ellipse or phantom zone present was considered as positive for ESBL production.

Detection of AmpC β- Lactamases

Screening test

The isolates were screened for presumptive AmpC production by testing their susceptibility to Cefoxitin and Cefotetan using Kirby- Bauer disk diffusion method.[6] The inhibition zone sizes were interpreted as per CLSI guideline.[3] All the isolates with an inhibition zone diameter of ≤18mm for Cefoxitin and ≤16mm diameter for Cefotetan respectively were labeled as screen positive.[7]

Confirmatory Test:

All 300 *Klebsiella pneumoniae* strains were studied for AmpC β- lactamases production and were confirmed by confirmatory test using Cefoxitin (CX) and Cefoxitin-Cloxacillin (CXX) discs.[7]

An increase in zone size of ≥4mm around the disc Cefoxitin plus Cloxacillin compared to Cefoxitin disc only was considered positive for AmpC production.

AmpC β- Lactamases production was further confirmed by disk potentiation test using Ceftazidime / Ceftazidime plus 3-aminophenyboronic acid (CAZ/CAZ+ 3-APB).[8]

Disk potentiation test: After overnight incubation at 37^oC, an increase in zone size of ≥5mm around the Ceftazidime plus 3-APB disc compared to Ceftazidime alone was considered as positive for AmpC β-lactamase production.

Confirmation of AmpC β- lactamase producing strains by E test

MIC ratio of Cefoxitin / Cefoxitin-Cloxacillin) ≥8 or deformation of ellipse or phantom zone present was considered as positive for AmpC β-lactamase production.

Detection of both ESBL and AmpC β- lactamase producing strains

Caudron et al recommended the use of boronic acid in combination with Clavulanic acid, for detection of ESBLs among AmpC β- lactamase producing strains.[9] To detect the strains producing both ESBL and AmpC β-lactamase, two discs containing Ceftazidime and Clavulanic acid (CAC) and the other 2 discs containing Ceftazidime (CAZ) only were placed widely apart. On one CAC disc 3- aminophenyl boronic acid (3-APB) solution was put.

Detection of Carbapenem resistant Klebsiella pneumoniae

All 300 *Klebsiella pneumoniae* strains were screened for Carbapenemase activity by Classical Hodge Test and Modified Hodge Test (MHT). All 300 *Klebsiella pneumoniae* strains were tested for Metallobetalactamases (MBL) production by disc potentiation test and MBL-E test strip (bioMerieux). The MBL-E test is considered as a standard Reference method for MBL detection.[10,11]

Detection of Klebsiella pneumoniae carbapenemases (KPC)

Producing strains was done by Combined disc method.

Classical Hodge test

The indicator organism, *Escherichia coli* ATCC 25922 (turbidity adjusted to 0.5 Mc Farland Standard) was used to inoculate the Mueller Hinton agar plate as lawn culture and the test strain was heavily streaked.

The plate was incubated overnight at 37^0C. The presence of distorted inhibition zone was interpreted as a positive result for Carbapenem hydrolysis screening.

Modified Hodge test (MHT)

The plate was incubated overnight at 37^0C. The presence of a cloverleaf shape zone of inhibition due to Carbapenemase production by the test strain was considered as positive Modified Hodge Test (MHT).

Imipenem-EDTA combined disc test for detection of MBL

The inhibition zone of the Imipenem disc and Imipenem EDTA disc were compared after 16-18 hours of incubation at 35^0C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥7mm than the Imipenem disc alone, it was considered as MBL positive.

MBL E-test

MIC ratio of Imipenem/ Imipenem- EDTA (IP/IPI) of ≥8 or deformation of ellipse or phantom zone indicate MBL production.

Detection of Klebsiella pneumoniae carbapenemases (KPC)

Detection of KPC producing *Klebsiella pneumoniae* carbapenemases (KPC) was done by Combined disc method. The test should be considered positive when growth inhibitory zone around the disc containing Imipenem plus Phenyl boronic acid was ≥5mm compared to zone diameter of Imipenem alone.

Detection of Metallobetalactamase (MBL) and Klebsiella pneumoniae Carbapenemase (KPC)

The production of both KPC and MBL were considered when the growth inhibitory zone diameter seen around Imipenem disc with both PBA+ EDTA had increased to ≥5mm, as compared to the growth inhibitory zone diameter seen around the Imipenem disc alone.

RESULTS

isolation of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Table 1 showing the isolation of *Klebsiella pneumonia* strains isolated from different clinical specimens (n=300).

Out of total 300 *Klebsiella pneumoniae* strains studied, 98 (32.7%) were isolated from urine followed by pus and wound swab 74 (24.7%) and 63 (21%) were isolated from blood. Amongest these 98 *Klebsiella pneumoniae* strains isolated from urine 23 (23.5%) strains were only ESBL, 10 (10.2%) strain was only AmpC β-lactamases, 5 (5.1%) were only MBL and 10 (10.2%) were only KPC producers.

Out of 98 *Klebsiella pneumoniae* strains isolated from urine specimens, 75 (76.5%) strains produced different types of β-lactamases either alone or in combinations and 1 (1.02%) strain produced all the four types of β-lactamases i.e. ESBL, AmpC β-lactamases, MBL and KPC in combinations. Out of 74 *Klebsiella pneumoniae* strains isolated from pus and wound swab 15 (20.3%) were only ESBL, 3 (4.1%) were only AmpC β-lactamases, 7 (9.5%) were only MBL and 3 (4.1%) strains were KPC producers respectively. From pus and wound swab 1 (1.4%) *Klebsiella pneumoniae* strains produced three types of β-lactamases i.e. ESBL, AmpC and MBL in combination. Out of 63 blood cultures positive *Klebsiella pneumoniae* strains 20 (31.7%) were found to be only ESBL, 3 (4.8%) were only AmpC β-lactamases, 7 (11.1%) were only MBL, 5 (7.9%) were only KPC producers respectively.

From blood 1 (1.6%) strains produced all three types of β-lactamases i.e. ESBL, AmpC, and MBL in combination. Out of 29 *Klebsiella pneumoniae* strains isolated from sputum specimens, 12 (41.4%) strains were only ESBL producers and 8 (27.6%) were ESBL and AmpC β-lactamases producers in combination. 15 *Klebsiella pneumoniae* strains isolated from Tracheal aspirates 10 (66.7%) strains were β-lactamases producers.

Out of these 15 *Klebsiella pneumoniae* strains, 1 (6.7%) was only ESBL producers, 2 (13.3%) were only MBL producer, 3 (20%) strains produced ESBL and AmpC in combination and 1 (6.7%) produced all 3 types of β-lactamases i.e. ESBL, AmpC, MBL in combination.Out of total 136 ESBL producing *Klebsiella pneumoniae* strains detected in the present study, 33 (24.3%), 41 (32.4%) and 28 (20.6%) ESBL producing strains were isolated from blood, urine, pus and wound swab respectively.

Total 75 AmpC β- lactamases producing *Klebsiella pneumoniae* strains were detected and out of these 75 strains, 15 (20%), 27 (36%) and 18 (24%) were isolated from blood, urine, pus and wound swab respectively either alone or in combination with other β-lactamases. Out of total 56 MBL producing strains 11 (19.6%), 18 (32.1%) and 18 (32.1%) were isolated from blood, urine, pus and wound swab respectively.

Out of total 40 KPC producing strains 7 (17.5%), 18 (45%) and 11 (27.5%) were isolated from blood, urine, pus and wound swab respectively either alone or in combination.

Table 1: Isolation of *Klebsiella pneumonia* strains isolated from different clinical specimens (n=300).

Specimen n=300	Negative for β-Lactamases n=79	ESBL n=78	Ampc n=18	MBL n=24	KPC n=20	ESBL + Ampc n=49	MBL + KPC n=19	ESBL + MBL n=5	Ampc + MBL n=4	ESBL+ Ampc+ MBL n=3	ESBL+ Ampc+ MBL + KPC n=1
Blood n=63	13	20	3	7	5	11	2	1	0	1	0
Urine n= 98	23	23	10	5	10	14	7	3	2	0	1
Pus and Wound swab n=74	23	15	3	7	3	12	8	0	2	1	0
Stool n=2	0	2	0	0	0	0	0	0	0	0	0
Medical devices= 11	7	2	1	0	0	1	0	0	0	0	0
Sputum n=29	4	12	0	2	1	8	1	1	0	0	0
Tracheal Aspirate n=15	5	1	1	2	1	3	1	0	0	1	0
Body Fluid n=7	3	3	0	1	0	0	0	0	0	0	0
Nasal pack n=1	1	0	0	0	0	0	0	0	0	0	0

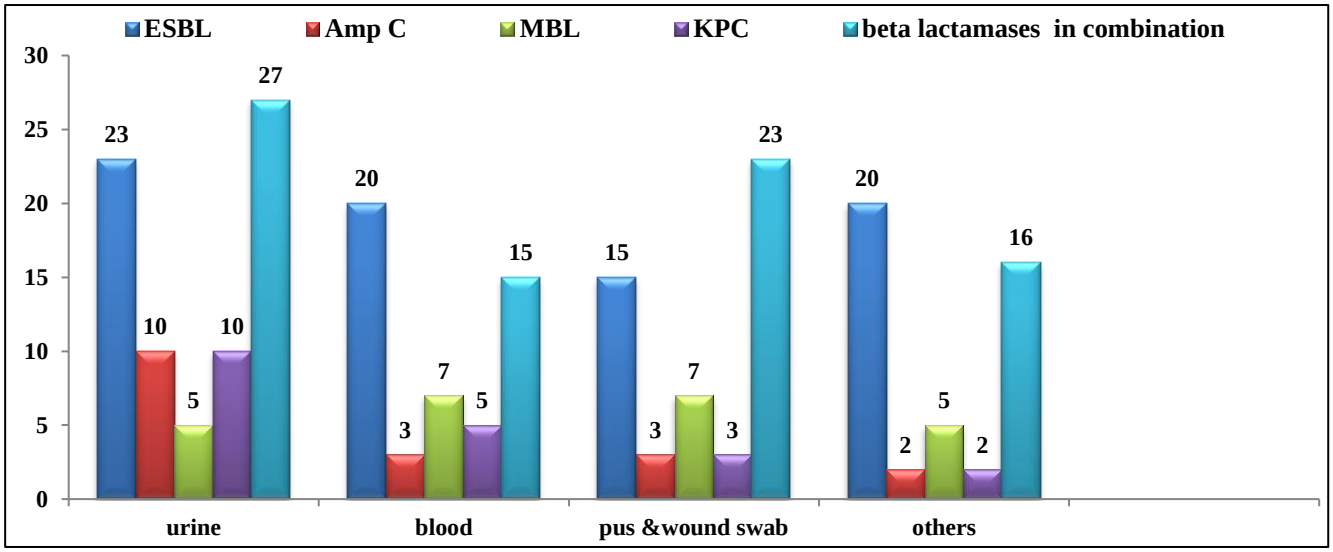

Figure 1: Isolation of β- lactamases producing *Klebsiella pneumoniae* strains from different clinical specimens.

DISCUSSION

Praveen et al from Puducherry in 2011 have reported 97% of ESBL producing *Klebsiella pneumoniae* from blood culture, whereas Varaiya et al. in 2008 have reported 35.3% of *Klebsiella pneumoniae* strains in their study were ESBL producers which were isolated from clinical specimens.[12,13] In another study in 2013, it has been reported that 34.3% isolates of *Klebsiella pneumoniae* were AmpC β- lactamase producers.[14]

Recently Carbapenem resistant Enterobacteriaceae (CRE) pose a real threat to Medical fraternity as the increased frequency with which Enterobacteriaceae cause infection and the increased mortality associated with infections caused by CRE. Metallo betalactamases (MBL) has gained importance in recent years as MBL genes located

on integron that resides on mobile genetic elements and can disseminates in hospital. Bansal et al. have reported in their study that 62% of *Klebsiella pneumoniae* strains were *Klebsiella pneumoniae* Carbapenemases (KPC) producing, 24% were MBL producing and 14% were both MBL and KPC producing.[15]

Others include, Stool, Medical devices, Sputum, Tracheal aspirates, Body fluids, Nasal pack.

Figure 1 shows the isolation of β- lactamases producing *Klebsiella pneumoniae* strains from different clinical specimens. From urine sample (n=98), 23 (23.5%) strains produced only ESBL and 27 (27.6%) produced different β- lactamases in combination. Out of these 27 strains 14 (51.9%) strains produced both ESBL and AmpC, 3 (11.1%) strains produced both ESBL and MBL and 1 (3.7%) strain produced all four types of β- lactamases i.e. ESBL, AmpC, MBL and KPC in combination. Hence total ESBL producing strains isolated from urine sample were 41 (41/136 i.e.30.1%). In 2015, Singh et al. reported maximum 41.5% of ESBL producing *Klebsiella pneumoniae* were isolated from urine.[16] In 2005, Kader et al, from Saudi Arabia reported that in their study 57.5% of ESBL producing *Klebsiella pneumoniae* strains were isolated from urine.[17] In the present study, 41 (30.1%) the maximum number of ESBL producing *Klebsiella pneumoniae* strains were isolated from urine followed by blood 33 (24.3%), pus and wound swab 28 (20.6%) and 34 (25%) from other specimens.

Highest number of total AmpC β-lactamase producing strains, 27 (27/75 i.e. 36%) were also isolated from urine sample and out of these 27 strains, 10 strains (10/27 i.e. 37%) produced only AmpC β-lactamases. But highest number 17 (17/56 i.e.30.4 %) were MBL producing *Klebsiella pneumoniae* strains and were isolated from pus and wound swab followed by urine 12 (12/56 i.e.21.4%). Singh et al, 2015 reported maximum isolation of MBL from endotracheal secretion i.e. 62.5%.[16] If the total KPC producing *Klebsiella pneumoniae* strains are considered, the highest number 18 (18/40 i.e. 45%) were again isolated from urine. Hence it was found that the maximum number of total ESBL, AmpC and KPC producing strains were isolated from urine sample whereas maximum number of total MBL producing strains were isolated from pus and wound swab. This may be because the number of urine sample were more compared to other sample and 33 (33/98 i.e. 33.7%) strains were isolated from patients who were catheterized.

CONCLUSION

Maximum (23.5%) ESBL, (10.2%) AmpC β- lactamases and (10.2%) KPC producing strains were isolated from urine sample. Maximum 32.1% MBL producing *Klebsiella pneumoniae* strains were isolated from pus and wound swab.

Hence, to conclude, *Klebsiella pneumoniae* strains which are one of the most common isolates from different clinical specimens must be tested for detection of different types of β-lactamases by confirmatory phenotypic tests like combined disc methods for ESBL, AmpC β-lactamases, KPC and Disc potentiation tests for MBL by Clinical Microbiology Laboratory to prevent the delay in detection of these β-lactamases producing strains to get a good therapeutic outcome for the patients and to prevent the spread of these β-lactamases producing strains in the Health care set up by taking proper Infection control measures.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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