DOI: NJLM/2013/5957:1992

Original Article

Microbiology Section

Study of Drug Resistance in Klebsiella Isolates in A Tertiary Care Centre

SMITA UDAY SHEVADE, GOPAL NANDLAL AGRAWAL

ABSTRACT

Context: Members of genus klebsiella cause various infections like urinary tract infection, septicemia, lower respiratory tract infection. Klebsiella has become resistant to numerous antibiotics.

Aims: The study was attempted to evaluate antimicrobial susceptibility pattern among klebsiella isolates.

Settings and design: *Klebsiella* isolates from samples of patients with various infections admitted to a tertiary care hospital.

Material and Methods: Klebsiella isolates from different clinical specimens were subjected to antibiotic susceptibility testing. Production of extended spectrum β-lactamase, AmpC β-lactamase and carbapenemase was tested.

Results: One hundred and three klebsiella strains were studied, of which 89 were Klebsiella *pneumoniae* and 14 were *Klebsiella oxytoca*. Twenty three strains were ESBL, four AmpC and 12 metallo-β-lactamase producers.

Conclusion: High drug resistance and β-lactamase production was observed in klebsiella isolates.

Keywords: Klebsiella, Drug resistance, β-lactamase production

INTRODUCTION

Members of genus *Klebsiella* cause various infections like urinary tract infection, septicemia, lower respiratory tract infection. The higher incidence of infections due to *Klebsiella* species during past decades probably reflects both an increase in nosocomial infections and a trend toward greater antibiotic resistance. In United States, Klebsiella accounts for 3-7% of all nosocomial infections, placing them among the eight most important infectious pathogens in hospitals [1]. Until recently, carbapenems were the choice for the therapeutic management of multidrug-resistant gram-negative bacterial infections. Currently, the spread of carbapenem-resistant bacteria has caused grave concern due to the limited choice in antibiotics for treating infections caused by them [2]. The resistance to carbapenems due to Metallo-β-Lactamases (MBLs) in *enterobacteriaceae* has been increasingly recognized [3]. Hence, aggressive surveillance of MBL producers will be extremely important. Recently reported New Delhi metallo-β-lactamase (NDM-1) producing *K. pneumoniae* has threatened the whole world [4]. This study was carried out to evaluate antibiotic susceptibility pattern among Klebsiella isolates.

METHODS

The study was conducted at a tertiary care hospital laboratory. One hundred and three Klebsiella strains were isolated from samples collected from various infections like urinary tract infection, suppurative infection, septicaemia, lower respiratory tract infection. These isolates were subjected to antibiotic sensitivity testing using disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) 2011 guidelines [5]. The isolates were further subjected to Minimum Inhibitory Concentration (MIC) testing for Imipenem (IPM) and Meropenem (MRP) by agar dilution method [5].

In β-lactamase detection tests, 0.5 McFarland inoculum of the test strain was lawn cultured on Mueller Hinton agar (MHA) and incubated at 37°C for 16-18 hours. Production of β-lactamase viz. Extended Spectrum β-Lactamase (ESBL), AmpC, carbapenemase and MBL were studied.

ESBL production was tested by initial screening suggested by CLSI followed by CLSI phenotypic confirmatory test using Ceftazidime and Ceftazidime Clavulanic Acid (CAZ-CAC) disks [5]. Additionally, Double Disk Synergy Test (DDST) using Amoxiclav-Cefotaxime (AMC-CTX) and Piperacillin-Tazobactum-Cefepime (PIT-CPM) was performed [6-8].

In the CLSI initial screening test, the test strain was exposed to a disk of ceftazidime (30 µg). The zone diameter ≤ 22 mm indicates ESBL production. In phenotypic confirmatory test, the test strain was exposed to disks of ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg). A ≥ 5-mm increase in a zone diameter for Ceftazidime-Clavulanic Acid (CAC) versus

its zone when tested alone Ceftazidime (CAZ) indicates ESBL production. In DDST, the test strain was exposed to disks of cefotaxime (30 µg) and of amoxiclav (20/10 µg) placed 20 mm apart, centre to centre and disks of piperacillin-tazobactam (100/10 µg) and of cefepime (30 µg) placed 25 mm apart, centre to centre. Plates were examined for enhancement of zone inhibition of Cefotaxime (CTX) and Cefepime (CPM) at the side facing amoxiclav (AMC) and Piperacillin-Tazobactam (PIT) disk respectively; the enhancement indicates ESBL production.

AmpC production was tested by cefoxitin-cefotaxime disk antagonism test (CX-CTX) [9-10]. In this test, the test strain was exposed to a disk of cefotaxime (30 µg) and cefoxitin (30 µg) placed at a distance of 1.5 cm from edge to edge. Flattening of radius of zone of inhibition produced by cefotaxime (CTX) on the side nearest the cefoxitin (CX) disk is seen in case of AmpC β-lactamase producer organism.

Carbapenemase production was tested by initial screening test and phenotypic confirmatory test i.e. Modified Hodge Test (MHT) [5]. In the initial screening test, test strain was exposed to a disk of meropenem (10 µg). The zone diameter around 16-21 mm indicated carbapenemase production. In MHT, 0.5 McFarland standard suspension of E. coli ATCC 25922 was prepared in saline and diluted in 1:10 in saline and inoculated on MHA. A single disk of meropenem (MRP) was placed at the centre. Using a 10-µl loop, 3–5 colonies of test organism grown overnight on a blood agar were inoculated in a straight line out from the edge of the disk. After incubation, the plate was examined for enhanced growth around the test organism streak at the intersection of the streak and the zone of inhibition. The enhanced growth suggests carbapenemase production [Table/Fig-1].

MBL production was tested by combined disk test (CDT) [11-12] by using imipenem (10 µg) and MRP disks alone and with EDTA (750 µg). The difference of ≥7mm in zones of inhibitions of two disks indicated MBL production.

RESULTS

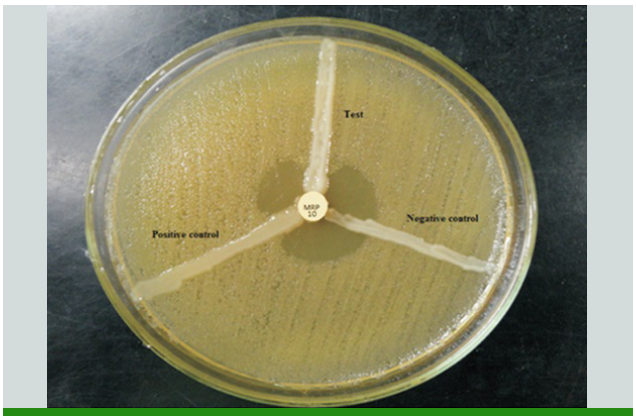
One hundred and three Klebsiella strains were isolated from samples collected from various infections like urinary tract infection (57.28%), suppurative infection (19.42%), septicaemia (16.50%) and lower respiratory tract infection (6.80%).

Klebsiella isolates were speciated. Antibiotic resistance of the species by disk diffusion method is shown in [Table/Fig-2].

Different β-lactamase production in *Klebsiella* isolates is shown in [Table/Fig-3]. Coproduction of β-lactamases was not seen in this study.

Of total 103 isolates, 23 (22.33%) were ESBL producers. ESBL production was detected in 22.33% isolates by CAZ-CAC, 5.83% by PIT-CPM and 2.91% by AMC-CTX method. The strains found to be ESBL producer by either of the DDST method were found to be ESBL producer by CAZ-CAC method.

Results of MHT using MRP and CDT using imipenem (IPM) and MRP are shown in [Table/Fig-4]. MHT detected carbapenemase production in two additional strains as compared to CDT. In MIC testing, MIC values of IPM and MRP were found to be parallel. Total 12 carbapenem resistant strains were detected by MIC testing.



[Table/Fig-1]: Modified Hodge test showing carbapenemase production (MRP – meropenem)

Drugs	No. of resistant isolates (%)		
	K. pneumoniae n = 89	*K. oxytoca* n = 14	Total
Ampicillin	89 (100)	14 (100)	103 (100)
Amoxiclav	89 (100)	14 (100)	103 (100)
Cephalothin	89 (100)	14 (100)	103 (100)
Cefuroxime	89 (100)	14 (100)	103 (100)
Cefoxitin	63 (70.79)	9 (64.29)	72 (69.90)
Cefotaxime	74 (83.15)	11 (78.57)	85 (82.52)
Cefepime	74 (83.15)	11 (78.57)	85 (82.52)
Piperacillin	65 (73.03)	10 (71.43)	75 (72.82)
Piperacillin-tazobactam	31 (34.83)	5 (35.71)	36 (34.95)
Imipenem	10 (11.24)	0	10 (9.71)
Meropenem	10 (11.24)	0	10 (9.71)
Gentamicin	55 (61.80)	8 (57.14)	63 (61.65)
Amikacin	34 (38.20)	5 (35.71)	39 (37.86)
Tobramycin	62 (69.66)	9 (64.29)	71 (68.93)
Netilmicin	30 (33.71)	4 (28.57)	34 (33.01)
Ciprofloxacin	71 (79.78)	111 (78.57)	82 (79.61)
Nitrofurantoin*	27 (30.34)	3 (21.43)	30 (29.13)
Norfloxacin*	79 (88.76)	12 (85.71)	91 (88.35)
Cotrimaxazole*	79 (88.76)	12 (85.71)	91 (88.35)

[Table/Fig-2]: Antibiotic resistance by disk diffusion in Klebsiella isolates (n = 103). *Urinary antibiotics

Klebsiella species (n)	No. of klebsiella isolates producing (%)			
	ESBL*	AmpC	Carbapenemase	Total
K. pneumoniae (89)	20 (22.47)	4 (4.49)	12 (13.48)	36 (40.45)
K. oxytoca (14)	3 (21.43)	0	0	3 (21.43)
Total (103)	23 (22.33)	4 (3.88)	12 (11.65)	39 (37.86)

[Table/Fig-3]: β-lactamase production in Klebsiella isolates (n = 103). *Extended spectrum β-lactamase

Method	Combined disk test for MBL production using			
	Imipenem		Meropenem	
	+	-	+	-
No. of modified hodge test positive strains for carbapenemase production using meropenem	10	2	10	2

[Table/Fig-4]: Comparison of modified hodge test and combined disk test in Klebsiella isolates

DISCUSSION

Klebsiella is a successful opportunistic pathogen associated with various ailments; a linear increase in drug resistance has being witnessed in the bug over the time scale [13].

Out of 103 strains of Klebsiella, 89 were *Klebsiella pneumoniae* and 14 were *Klebsiella oxytoca* [Table/Fig-2]. *Klebsiella pneumoniae* is the most important Klebsiella species from a medical standpoint, causing community-acquired and nosocomial infections [14].

All the Klebsiella isolates were resistant to ampicillin, amoxiclav, first and second generation cephalosporins. Resistance to cephamycins, third and fourth generation cephalosporins varied from 70–83%. Amongst aminoglycosides, amikacin and netilmicin showed better susceptibility for Klebsiella isolates. Amongst urinary antibiotics, nitrofurantoin showed good susceptibility [Table/Fig-2].

In the study, 23 (22.33%) Klebsiella isolates were ESBL, 4 (3.88%) AmpC and 12 (11.65%) carbapenemase producers [Table/Fig-3].

To detect ESBL production, CAZ-CAC method was found to be a better method as compared to two DDST methods viz. AMC-CTX and PIT-CPM. It is a simple method and can be performed by a small scale peripheral microbiological laboratory.

Results of IPM and MRP susceptibility were similar by disk diffusion test. CLSI [5] has listed these two drugs together in a single box designating clusters of agents for which interpretive results (susceptible, intermediate, or resistant) are similar. Results of IPM and MRP susceptibility by MIC method were also similar. But, MIC testing was able to detect carbapenem resistance in two additional strains as compared to disk diffusion test.

Carbapenemases are classified into class A, B and D. Class B carbapenemases consist of MBL only. MHT detects carbapenemase production which includes MBL too. CDT detects only MBL production. In this study, MHT detected carbapenemase production in two additional strains as compared to CDT [Table/Fig-4]. These two strains might be producer of non-MBL carbapenemase or the test for carbapenemase detection (MHT) might be more sensitive for detection of MBL as compared to the test for MBL production (CDT).

All the 12 carbapenemase producers; detected positive by MHT were resistant to IMP and MRP by MIC testing. Two strains additionally detected resistant to IMP and MRP by MIC and not by disk diffusion test were the same which were found to be carbapenemase producer by MHT and MBL non-producer by CDT. Thus carbapenem resistance in Klebsiella isolates in this study was as high as 11.65%. In our setting, *K. pneumoniae* isolates showed carbapenem resistance as high as 11.24%.

There is a high drug resistance and β-lactamase production in Klebsiella in our tertiary care set up. Drug resistant Klebsiella strains might facilitate the spread of antibiotic resistance among different members of *enterobacteriaceae*. It is necessary to have the knowledge of drug resistance in the bugs so that its control strategy can be more effectively planned.

REFERENCES

[1] Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998; 11:589-603.

[2] Walsh TR. Emerging carbapenemases: A global perspective. *Int J Antimicrob Agents.* 2010; 36 Suppl 3:8-14s.

[3] Tato M, Morosini M, Garcia L, Alberti S, Coque MT, Canton R. Carbapenem heteroresistance in VIM-1-producing *Klebsiella pneumoniae* isolates belonging to the same clone: consequences for routine susceptibility testing. *J Clin Microbiol.* 2010; 48: 4089-93.

[4] Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla (NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother.* 2009; 53:5046-54.

[5] Performancestandards for antimicrobial susceptibility testing; Twenty first informational supplement. *Clinical Labortory Standards Institute (CLSI).* 2011.

[6] Pitout JDD, Reisbig MD, Venter EC, Church DL, Hanson ND. Modification of the double-disk test for detection of enterobacteriaceae producing extended-spectrum and AmpC β-lactamases. *J Clin Microbiol.* 2003, 41:3933-5.

[7] Shobha KL, Ramachandra L, Rao G, Majumder S, Rao SP. Extended spectrum beta-Lactamases (ESBL) in gram negative bacilli at a tertiary care hospital. *J Clin Diag Res.* 2009; 3:1307-12.

[8] Shobha KL, Gowrish Rao S, Sugandhi Rao, Sreeja C.K. Prevalence of extended spectrum beta-lactamases in urinary isolates of *Escherichia coli*, *Klebsiella* and Citrobacter species and their antimicrobial susceptibility pattern in a tertiary care hospital. *Indian Journal for the Practising Doctor.* 2007; 3: No. 6 (2007-01 - 2007-02).
[9] Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, Mehta A. Detection of β-lactamases in nosocomial gram negative clinical isolates. *Indian J Med Microbiol.* 2004; 22:247-50.
[10] Sanders CC, Sanders Jr WE, Goering RV. In vitro antagonism of beta-lactam antibiotics by cefoxitin. *Antimicrob Agents Chemother.* 1982; 21: 968-75.
[11] Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-β-Lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J Clin Microbiol.* 2008; 46:2028-37.
[12] Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas spp.* and *Acinetobacter spp. J Clin Microbiol.* 2002; 40:3798-3801.
[13] Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. *Indian J Med Microbiol.* 2002; 20:92-95.
[14] RussoTA, Johnson JR. Diseases caused by gram-negative enteric bacilli. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J (eds.). Harrison's Principles of Internal Medicine, Vol. 1, 18th Ed, Mc Graw Hill, New Delhi, 2012, p 1246-57.

AUTHOR(S):
1. Dr. Smita Uday Shevade
2. Dr. Gopal Nandlal Agrawal

PARTICULARS OF CONTRIBUTORS:
1. Resident, Department of Microbiology, Indira Gandhi Government Medical College, Central Avenue, Nagpur, Maharashtra – 440018, India.
2. Assistant Professor, Department of Microbiology, Indira Gandhi Government Medical College, Central Avenue, Nagpur, Maharashtra – 440018, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Gopal N. Agrawal,
Assistant Professor, Department of Microbiology,
Indira Gandhi Government Medical College, Central Avenue,
Nagpur, Maharashtra – 440018, India.
Email: gnagrawal_1@yahoo.co.in
Ph: 9890450488

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Dec 31, 2013**