International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 8, 2019, pp.-1694-1696.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

PREVALENCE OF CARBAPENAM RESISTANT *KLEBSIELLA PNEUMONIAE* IN A TERTIARY CARE HOSPITAL, HYDERABAD, TELANGANA STATE

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Received: August 10, 2019; Revised: August 24, 2019; Accepted: August 26, 2019; Published: August 30, 2019

Abstract- Background: Irrational and indiscriminate use of Carbapenam antibiotics has resulted in emergence of extremely drug resistant bugs. The carbapenam resistance among the Enterobacteriaceae has increased enormously in the last decade which is of particular concern. Carbapenam resistant *Klebsiella pneumoniae* account for about 26-44% of the deaths. Materials and methods: The study was conducted for a period of 6 months. The specimens received were endotracheal aspirates, pus, urine, blood and sputum and were processed by standard microbiological methods. 60 isolates of *Klebsiella* spp from inpatient specimens which were resistant to carbapenam antibiotics were further subjected to genotypic characterization for the detection of carbapenamase enzymes. Results: The mean age of the patients was 43.7yrs. Risk factors identified were prolonged ICU stay, repeated hospitalisations, prior exposure to antibiotics and presence of indwelling catheters/tubes. The source of infection in majority of the cases was nosocomial, attributed to presence of invasive devices. The isolates were moderately susceptible to carbapenams and highly susceptible to Polymixins. Carbapenamases are detected in about 44 (73.33%) isolates. Among these, Co expression with one or more enzyme subclasses was noted in about 22 (50 %) isolates. The mortality rate was 54% in our hospital. Conclusion: This intimidating situation can be addressed by strict adherence to infection control practices in conjunction with effective use of diagnostic methods in the clinical microbiology laboratory to identify the *CRKP* along with education of health care providers, patients and laypersons to limit the abuse or overuse of antibiotics.

Keywords- *Carbapenemases, carbapenam resistant Klebsiella pneumoniae, Klebsiella pneumonia, Antimicrobial susceptibility testing, Infection control, Multiplex PCR*

Citation: Tipparthi S.K., *et al*., (2019) Prevalence of Carbapenam Resistant *Klebsiella Pneumoniae* in a Tertiary Care Hospital, Hyderabad, Telangana State. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 8, pp.-1694-1696.

Academic Editor / Reviewer: Dr Ranjana Hawaldar

Introduction

Carbapenams are one of the extended spectrum antibiotics used in severe life-threatening infections. Irrational and indiscriminate use of these potent antibiotics has resulted in emergence of extremely drug resistant bugs. The bacteria have evolved several mechanisms to overcome the effect of the carbapenams like alterations in structure of drug target, loss of porin channels, over expression of efflux pumps and production of enzymes called carbapenamases [1]. The last mechanism *i.e*., production of carbapenamases is the most common observed mechanism for the development of drug resistance [2]. The common carbapenamses documented in Enterobacteriaceae belongs to class A carbapenemase (KPC), class B metallo-β lactamases (IMP, VIM, NDM) and class D oxacillinase (OXA-48 like) [3]. Among these, NDM and OXA-48 like group are frequently reported in various studies in India [4-6]. The carbapenem resistance among the Enterobacteriaceae has increased from 9% in 2009 to about 80% in 2017 [7,8]. This increasing trend of carbapenem resistance is of particular concern. Among the *Enterobacteriaceae, Klebsiella pneumoniae* is one of the emerging extensively drug resistant pathogens. Carbapenam resistance was reported in *Klebsiella Pneumoniae* (CRKP) in 1996 and since then there are a greater number of cases reported to cause infection by these bacteria in susceptible hosts lead to higher rates of mortality [9]. This pathogen is most commonly associated with hospital acquired infections like pneumonia, urinary tract infections and blood stream infections [10]. Carbapenam resistant *Klebsiella pneumoniae* account for about 26-44% of the deaths [11]. So, the therapeutic options to treat the infections by this dreadly pathogen would be a combination therapy with last resort antibiotic, Colistin [7]. Even though there is high disease prevalence by this bacterium in India, the reports on the mechanisms of resistance from South India are very few. So, we aim to do phenotypic and genotypic characterization of Carbapenam resistant Enterobacteriaceae with special reference to *Klebsiella pneumoniae* in our hospital.

Materials and Methods

Institutional ethical clearance was obtained prior to the study. Patient informed consent was taken stating that the isolates obtained from the samples would be used for research purpose. The study was conducted in the Department of Microbiology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, Telangana state. The study was conducted for a period of 6 months *i.e*., June 2018 to December 2018. Specimens received from inpatients included endotracheal aspirates, blood, urine, sputum and pus. About 60 isolates of *Klebsiella* spp were obtained. All types of non-repetitive specimens from inpatients, showing growth of *Klebsiella* spp, resistant to two or more classes of antimicrobial agents were included in the study. Repetitive isolates, Isolates from patients with transfer to our hospital from other hospitals were excluded from the study.

Phenotypic characterization

The specimens received were processed by standard microbiological methods. [12, 13].

Antimicrobial susceptibility testing was performed by Kirby Bauer Disc diffusion method for different classes of antimicrobials such as Fluoroquinolones-Ciprofloxacin (5µg), Aminoglycosides- Amikacin (30µg), Gentamicin(5µg);Cephalosporins - Cefotaxime (30µg), Ceftazidime(30µg); β-lactam/β-lactamase inhibitors - Piperacillin/Tazobactam(100/10µg), Cefoperazone/Sulbactam (75/30 µg); Carbapenems-Meropenem (10µg) ; Polymixins-Colistin Ezy MIC TM strip (0.016- 256 µg/ml) as per the recommendations by Clinical Laboratory Standards Institute (CLSI) and zones of inhibition were interpreted according to CLSI 2018 guidelines.[14]ATCC E. coli 25922 was used as the quality control strain for susceptibility testing. The isolates resistant to carbapenams were further tested for confirmation with Modified Hodge test as per the CLSI guidelines [14].

Genotypic characterization

Bacterial DNA was extracted from 18hr cultures by boiling method [15]. Carbapenemase genes detected by multiplex PCR were blaNDM, blaVIM, blaIMP, blaOXA-23, blaOXA 48, and blaKPC and were performed as per established protocol using published primers [16, 17]. Known positive controls for each gene were used with every run.

Clinical data collection

Data from eligible subjects was collected retrospectively. Patient data included age, gender, admission date, admission diagnosis and antimicrobial susceptibility test results. Data was entered in WHONET 5.6 to prevent duplicate entry.

Results

60 isolates of *Klebsiella* spp were obtained from various specimens from inpatients in our hospital during the study period [Fig-1]. The mean age of the patients was 43.7yrs. Majority of the patients were immunocompromised (72%). Risk factors identified were prolonged ICU stay, repeated hospitalizations, prior exposure to antibiotics and presence of indwelling catheters/tubes. The source of infection in majority of the cases was nosocomial, attributed to presence of invasive devices either presence of Foleys catheter (46.6%), presence of endotracheal tube (33.33%), presence of central or peripheral line. The antimicrobial susceptibility of *Klebsiella* spp is shown in [Fig-2]. The isolates were least susceptible to fluoroquinolones, third generation cephalosporins; moderately susceptible to aminoglycosides, betalactam and betalactam inhibitor combination, carbapenams and highly susceptible to Polymixins. The results of multiplex PCR for blaNDM, blaVIM, blaIMP, blaOXA-23, blaOXA 48, and blaKPC are shown in [Table-1] and [Table-1a]. The gel electrophoresis image of the products of multiplex PCR is show in [Fig-3]. Carbapenamases are detected in about 44 (73.33%) isolates. Among these, Co expression with one or more enzyme subclasses was noted in about 22 (50 %) isolates. Out of the 44, 14 were carbapenam susceptible isolates and 28 isolates were carbapenam resistant. 2 carbapenam resistant isolates did not show presence of any carbapenamase enzyme. The mortality rate was 54% in our hospital.

Discussion

The epidemiological pattern of Carbapenam resistant *Klebsiella pneumoniae* is highly variable. The highest rate is reported by Europe (68%) while lowest rates are reported by Africa (4%) probably due to lack of active surveillance. USA has rates of 11% carbapenam resistance. The main carbapenamases reported in Europe are KPC followed by OXA-48-like and NDM while in USA, KPC followed by NDM and minimal due to OXA-48-like [18]. Adherence to dedicated infection control measures and regular surveillance programme instituted by developed countries such as Australia and New Zealand have recorded prevalence as low as 1% of KPC in hospital acquired infections. Few studies From India have been reported in this change of trend of carbapenemases (Table 2). In India, the predominant carbapenamases are NDM and OXA-48-like and KPC is rarely reported [19,20]. But in our study, we found about 6 isolates having bla KPC alone and in 4 isolates, coexpression with other carbapenamase genes like blaNDM, blaVIM and blaOXA 48. Due to less patient population, the data available on the effectiveness of monotherapy vs combination therapy is less.

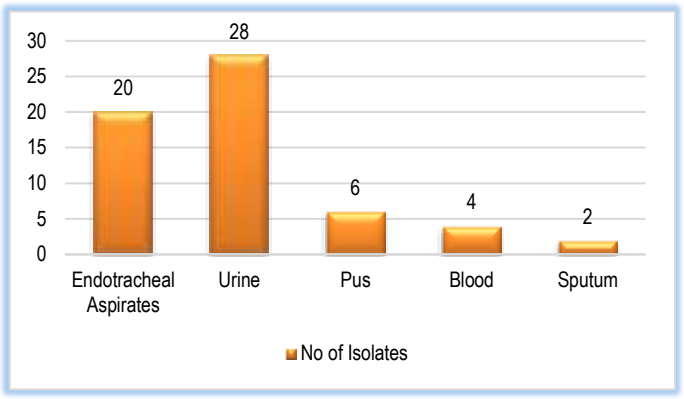

Fig-1 Types of samples and number of isolates of *Klebsiella* spp

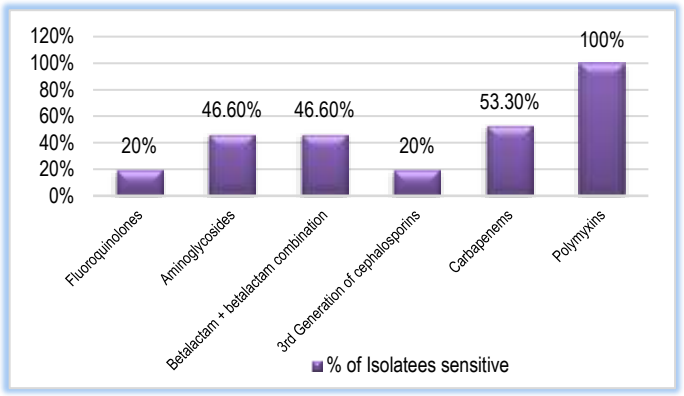

Fig-2 Antimicrobial susceptibility of *Klebsiella* spp

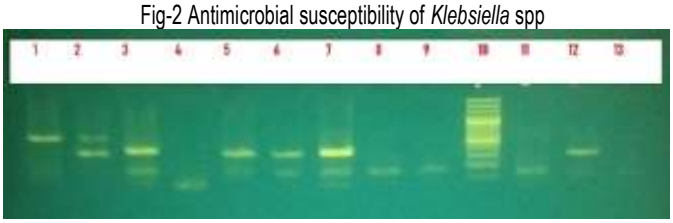

Fig-3 Gel electrophoresis picture showing 100bp ladder- lane 10; blaNDM (439 bp) - lane 5, 6, 7, blaIMP (183bp) - lane 11, blaOXA 48 (736bp) - lane 1,2

Table-1 Results of multiplex PCR for Carbapenemase enzymes

Carbapenamase enzymes	No. of isolates	%
Class A	6	13.64
Class B	10	22.7
Class D	6	13.64
Class A+B	2	4.55
Class A+D	0	0
Class B+D	18	41
Class A+B+D	2	4.55
Total	44	

Table-1a Results of multiplex PCR for subfamily of Carbapenemase enzymes - blaNDM, blaVIM, blaIMP, blaOXA-23, blaOXA 48, and blaKPC *ND- Not Detected

Carbapenamase enzyme sub family	No. of isolates	%
NDM	2	4.55
IMP	6	13.6
OXA 23	2	4.55
VIM	2	4.55
KPC	6	13.6
OXA 48	4	9.1
NDM+OXA 23	4	9.1
NDM+OXA 23+VIM	2	4.55
NDM+OXA 48+VIM	2	4.55
NDM+OXA 23+OXA 48	2	4.55
NDM+OXA 48+VIM+KPC	2	4.55
OXA 23+VIM	4	9.1
OXA 48+VIM	4	9.1
VIM+KPC	2	4.55
Total	44	

Table-2 Comparison between different studies on Carbepenamase producing *Klebsiella* spp from India

	Class A(bla_{KPC})%	Class B (bla_{NDM}, bla_{VIM}, bla_{IMP})%	Class D ($bla_{OXA\ 48}$, $bla_{OXA\text{-}23}$)%	Class A+B%	Class A+D%	Class B+D%	Class A+B+D%
Present study	13.64	22.7	13.64	4.55	0	41	4.55
Veeraraghavan *et al* [7]	0	24.1	13	19	0	28	ND
Chaudhary *et al* [21]	7.9	23.55	ND	0	ND	0	ND
Anandan *et al* [20]	0	34	44	0	0	16	ND
Pragasam *et al* [22]	0	24	55	0	0	16	ND
Sharma *et al* [23]	0	27	36	0	0	5	ND

The existing evidence shows that combination therapy is associated with better outcomes compared to monotherapy. Successfully tried combination therapies against CR K. pneumoniae infections include tigecycline - colistin, tigecycline - gentamicin and carbapenem-colistin [21-23]. Few studies have demonstrated that meropenem-colistin-tigecycline combination works well against CRKP possibly due to colistin-carbapenem synergy [24-26]. But FDA has issued a black box warning against the usage of tigecycline as it leads to treatment failure due to low serum concentration and hence is associated with high mortality rates. Colistin attains sufficient serum levels, hence are used as synergistic agents along with carbapenams despite of their serious side effects like nephrotoxicity and neurotoxicity. In our hospital most of the CRKP infected patients were treated with a combination of colistin with meropenem. Inspite of this the mortality rate in our hospital is very high (54%) which correlates with the study conducted by Falagas *et al*.[9]. As per the saying "old is gold", there is a need to evaluate the effectiveness of certain antibiotics like minocycline, gentamicin in CRKP infections. If the susceptibilities of these agents are similar to the carbapenams, they can be used as carbapenam sparing antibiotics.

Conclusion

This intimidating situation can be addressed by strict adherence to infection control practices in conjunction with effective use of diagnostic methods in the clinical microbiology laboratory to identify the CRKP along with education of health care providers, patients and laypersons to limit the abuse or overuse of antibiotics.

Application of research: Health care organizations should adopt mechanisms which can capture the changing trends of the prevalence of antimicrobial resistant organisms so that the true picture is seen and national policies for containment of infection can be done.

Research Category: Medical microbiology

Acknowledgement / Funding: Authors are thankful to Department of Microbiology, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, 500068, Kaloji Narayana Rao University of Health Sciences, Warangal, 506007, Telangana, India

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Research project name or number: Clinical case research

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, 500068, Telangana

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, 500068, Telangana.
Ethical Committee Approval Number: Nil

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