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Susceptibility of Colistin in Carbapenem Resistant *Klebsiella* Species in a Tertiary Care Hospital.

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ABSTRACT

Klebsiella accounts for 3 to 7% of all nosocomial infections particularly urinary tract infections, pneumonia, septicaemia and wound infections. Irrational use of antibiotics has led to the formation of multidrug resistant Klebsiella species. Scarcity of novel antimicrobials has led to reconsideration of polymyxins in particularly Colistin as a treatment option. Hence, this study was conducted to study the susceptibility of Colistin in carbapenem resistant Klebsiella isolates in a tertiary care hospital. This study was conducted in a tertiary care hospital over a period of 8 months. Carbapenem resistance among Klebsiella species was identified by disc diffusion test and modified Hodge test. Colistin susceptibility among carbapenem resistant isolates were determined by Epsilometer test (E test). Colistin resistant Klebsiella isolates were tested for the presence of most common genes such as mgrB, pmrK, phoQ by Pulse Field Gel Electrophoresis (PFGE). Of 1142 samples, Klebsiella species were isolated from 324 samples. Among them 138(43%) were multidrug resistant. The prevalence of carbapenem resistance among them was 24% and 55% by disc diffusion and modified hodge test respectively. Twenty one percent of carbapenem resistant Klebsiella species were resistant to Colistin. All 3 genes (mgrB, pmrK, phoQ) were expressed in 2 out 7 Colistin resistant strains while only 2 genes (mgrB, pmrK) were expressed in 2 Colistin resistant strains. Remaining strains did not express any of the three genes. Increasing incidence of multidrug resistant Klebsiella infections warrants urgent measures to strengthen antimicrobial usage policy and hospital infection control measures.

Keywords: Colistin, carbapenem resistance, klebsiella, antimicrobial resistance, PCR

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INTRODUCTION

Klebsiella has been identified as one of the most common causes of hospital acquired infections. It accounts for around 3 to 7% of all nosocomial bacterial infections. The most common nosocomial infections caused by Klebsiella are urinary tract infections, pneumonia, septicaemia and wound infections [1].

Over usage of extended-spectrum cephalosporins has led to the development of extended spectrum β-lactamase producing Klebsiella pneumoniae across the world [2]. Carbapenems were recommended as the treatment of choice for these multidrug-resistant strains. Nevertheless, its increased usage also led to the expansion of its resistance due to the production of carbapenemase enzyme [3,4]. Tigecycline, Colistin and fosfomycin were found to be effective for carbapenem resistant Klebsiella isolates. Despite in vitro susceptibility of tigecycline, it has very limited in vivo use in urinary and primary blood-stream infection. Polymyxins, in particular Colistin are highly effective for these resistant strains as it has wide spectrum of activity towards many bacteria which includes Klebsiella isolates [5].

However, resistance to Colistin has been reported worldwide due its increased usage in all ecosystems such as in animal husbandry as animal feeds, aquaculture, and for farming [6]. Colistin resistance in bacteria can occur through the process of mutation or several altered mechanisms. Genes responsible for these regulatory mechanisms are mgrB, pmrA, pmrB, pmrD, pmrK, phoP, and phoQ [7].

The increasing incidence of Colistin resistance among carbapenemase producing Klebsiella is of serious concern for both clinicians and patients since it increases the healthcare cost, duration of hospital stay and mortality. Hence it becomes utmost importance to study the extent of Colistin resistance among carbapenem resistant Klebsiella species in hospitals. However only limited data is available on susceptibility of Colistin among carbapenem resistant Klebsiella species particularly in South India [8,9,10]. Hence this study was conducted to determine the susceptibility of Colistin among carbapenem resistant Klebsiella species in a tertiary care hospital and to identify the determinants of Colistin resistance by molecular methods.

MATERIALS AND METHODS

This study was conducted in a tertiary care hospital of Tamilnadu for a period of eight months. All the clinical isolates with multidrug resistant Klebsiella species received at the microbiology laboratory during the study period were included in the study. A filled in proforma was also obtained from the patients with details like name, age, sex, ward, clinical diagnosis, risk factors, surgical intervention, hospital stay and other parameters relevant to the study.

The multidrug resistant Klebsiella isolates were sub-cultured on to nutrient agar slope and stored at 2 to 8°C until testing. Carbapenem resistance was identified by disc diffusion test using Imipenem (10 µg), Ertapenem (10 µg). The Modified Hodge test for confirmation of suspected carbapenemase production was performed as recommended by the Clinical Laboratory Standard Institute guidelines [11]. A clover leaf-like indentation seen in the growth of Escherichia coli American type culture collection ATCC 25922 on the side of the test strain considered to be positive for carbapenemase synthesis. No growth denoted no carbapenemase production.

Susceptibility to Colistin among carbapenem resistant Klebsiella isolates was detected phenotypically by epsilometer test. Minimum inhibitory concentration of Colistin was determined by epsilometer test by using European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoint [12]. The Colistin resistant Klebsiella isolates were tested for the presence of most common genes such as mgrB, pmrK, phoQ by Pulse Field Gel Electrophoresis (PFGE) [13]. The kit was purchased from Helini Biomolecules, Chennai, India. Procedure for PFGE was followed according to the manufacturer's instructions. DNA was extracted according to the manufacturer's protocol. 5µl of extracted DNA is used for Polymerase Chain Reaction (PCR) amplification. Extracted DNA was then loaded in 1% agarose gel. Total volume 20µl was taken, mixed, spined briefly and kept inside the PCR machine and program was set as follows:

Denaturation was set at 95ºc for 5 min and second denaturation was set at 94ºC for 30s. Annealing was set at 58ºc for 30s and extension was set at 72ºc for 30s. Denaturation, annealing and extension were

repeated for 35 cycles. Final extension was set at 72º c for 5 min. Primers used for Polymerase chain reaction**:** mgrB: AAGGCGTTCATTCTACCACC mgrB: TTAAGAAGGCCGTGCTATCC (252bp)
pmrK: CGCTGAATATGCTCGACCCAGAAG pmrK: GCTGGCGGTAATCGTCTGTACG
(107bp) phoQ: ATATGCTGGCGAGATGGGAAAACGG phoQ: CCAGCCAGGGAACATCACGCT (137bp).

When the temperature reached 60ºc, 5µl of ethidium bromide was added. Warm agarose solution was added slowly into the gel platform. When the agarose solidified, gel was kept undisturbed. Buffer was then poured into the tank. Gel platform was placed carefully into tank. Level of buffer was 0.5cm above the gel. Then samples which was amplified by PCR were mixed with dye and loaded. Electrophoresis ran at 50V until the dye came to three fourth distance of the gel. The bands pattern in the gel were viewed in UV transilluminator. Appearance of band corresponding to the particular primer was taken as positive.'
Ethical clearance for the study was obtained from the Institutional Ethical Committee before the commencement of the study. Informed consent was obtained from reliable informants of patients and patients who participated inthe study. Data was analyzed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. The statistical procedures were performed by software Statistical Package for Social Sciences (SPSS) v.20. p value of less than 0.05 was considered statistically significant.

RESULTS

During the study period, a total of 1142 culture positive clinical specimens (urine, blood, pus, sputum, broncho alveolar lavage, pancreatic fluid) were received at the laboratory. Among them 324 clinical specimens were positive for Klebsiella. Out of which 138 isolates which were found to be multidrug resistant were included for the study.

The mean age of patients who had multidrug resistant Klebsiella isolates was 45 years. Almost 58% of such isolates were from males and remaining from females. Most of these strains were isolated from blood (30%), urine (28%), pus (27%), sputum (11%), vaginal swab (4%) (see Table/Figure 1). Nearly 24% of multidrug resistant Klebsiella isolates (n=33) were found to be resistant to carbapenem by disc diffusion method. Modified Hodge Test was found to be positive for carbapenemase production in 55% (n=18) of carbapenem resistant isolates.

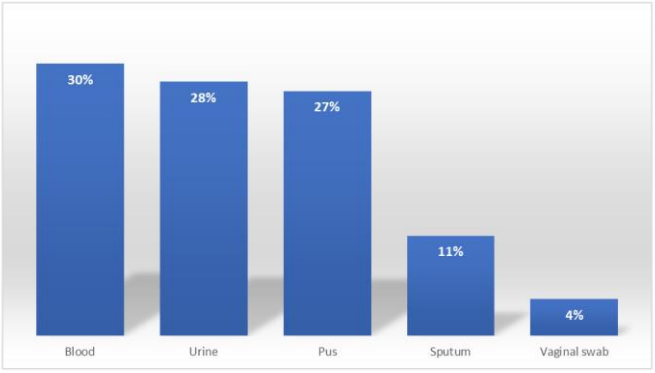

Figure 1: Various specimens showing multidrug resistant Klebsiella isolates

Seven out of 33 carbapenem resistant isolates (21%) were found to be resistant to Colistin by Epsilometer test (E test). Polymerase Chain Reaction was performed to all Colistin resistant strains detected by E test and then agarose gel electrophoresis was run to identify the expression of 3 common genes (mgrB, pmrK, phoQ). All 3 genes were expressed in 2 out 7 Colistin resistant strains while only 2 genes (mgrB, pmrK) were expressed in other 2 Colistin resistant strains. Three strains did not express any of the above-mentioned genes.

The mean age of patients harboring Colistin resistant strains was 40 years. Four of these strains were isolated from male patients and remaining from female patients. Four strains were from intensive care units (Intensive Medical Care Unit and Sick Neonatal ward) (see Table/Figure 2). Three of the patients harboring Colistin resistant strain had blood stream infection. Remaining four patients had respiratory tract infection, surgical site infection, wound infection and urinary tract infection.

Table 1: Distribution of Colistin resistant Klebsiella isolates among various wards

Wards	Number of Colistin resistant Klebsiella isolates	Percentage (%)
Intensive Medical Care Unit	3	43
Sick Neonatal Ward	1	14
Burns ward	1	14
Thoracic medicine	1	14
Surgery	1	14
Total	7	

Four patients with Colistin resistance strains were on mechanical ventilation and others were on central venous catheterization. The mean duration of stay in hospitals among patients harboring Colistin resistant strains was 29 days. There was no statistically significant association between Colistin resistance and duration of stay in hospital (p value > 0.05). All Colistin resistant strains were sensitive to tigecycline. One patient who had Colistin resistant Klebsiella died.

DISCUSSION

The increasing prevalence of multidrug-resistant pathogens heralds the dawn of post antibiotic era and makes physicians left with no treatment options. In this present study, nearly 43% of Klebsiella isolates were multidrug resistant. A study from New Delhi found nearly 54% of the Klebsiella isolates to be multidrug resistant [14]. In this study, nearly 30% and 28% of multidrug resistant Klebsiella isolates were from blood and urine samples respectively. A study done in Intensive Care Unit of a tertiary care hospital in India also reported that nearly 30% of Klebsiella isolates were from blood and urine [15]. However, similar study done in Iran reported that nearly half of the multidrug resistant isolates were from urine samples and only 5% of isolates were from blood samples [16].

Current study reported nearly 24% of multidrug resistant Klebsiella to be resistant to carbapenem. India has shown the highest prevalence of carbapenem resistant Klebsiella pneumoniae (54%) when compared to other parts of the world [17]. A 10-year retrospective study done at a tertiary care hospital in New Delhi has shown that the prevalence of carbapenem resistance in K. pneumoniae increased from 2.4% to 52% over a period of ten years [4].

Modified Hodge test found that 18 out of 33 carbapenem resistant Klebsiella isolates (55%) were carbapenemase producers. Though Modified Hodge test was a simple, cheap and easy to perform test, it has a low sensitivity and specificity when compared to inhibitor-based methods [18,19]. Hence, clinical microbiological laboratory cannot fully rely only on this test for the identification of carbapenemase producing bacteria. Modified Hodge test is capable of identifying only the production of carbapenemases while the Inhibitor-based method is capable of identifying and differentiating the types of carbapenemases. In this study, Epsilometer test detected that nearly 21% of carbapenem resistant Klebsiella strain were resistant to Colistin. E test has been evaluated to be having good concordance with broth microdilution method [20]. A similar study done in a tertiary care hospital in Nothern India showed that almost 6% of carbapenem resistant Klebsiella isolates were resistant to Colistin [21]. Another study from Tamilnadu found that nearly 45% of Klebsiella isolates from two different centres were resistant to both Colistin and carbapenem [22]. Strict infection control practices, including active surveillance and stringent antibiotic usage policies partcularly in intensive care units can halt the emergence of resistant strains.

It was found that 4 out of 7 Colistin resistant strains (57%) harboured both mgrB gene and pmrK gene. Previous research also reported mgrB gene chromosomal mutation involving modification of lipopolysaccharide to be the most common mechanism for Colistin resistance [8,23]. The mechanism of resistance in 3 strains (43%) which did not detect any of the three genes might be due to other genes such as pmrA, pmrB which were not screened for as reported in previous studies [24,25].

Nearly 57% of patients harboring Colistin resistant Klebsiella strains were from intensive care units. The rate of acquiring carbapenem resistant carriers was 14.5% and this rate was augmented in patients admitted in intensive care units [26]. Present study reported that 43% of Colistin resistant strains were causing blood stream infection. Previous similar studies from India and other countries also showed blood stream infection to be the most infection caused by Colistin resistant Klebsiella [5,15,27]. Almost 14% of Colistin resistant Klebsiella strains were from patients having urinary tract infection in this study.

This is similar to a study done in Chennai in which 22% of patients with pan drug resistant gram-negative bacteria had urinary tract infection [28].

The major risk factors associated with Colistin resistance were mechanical ventilation and central venous line insertion. The risk of developing infection from resistant strains also increased with increase in hospital stay [27,29]. Only 14% of patients harboring Colistin resistant strains had known exposure to Colistin in this study. This could be due to the excessive use of antibiotics across all ecosystems over the past few years including humans, animals and agriculture [30]. This study revealed that the mortality rate due to infections caused by these resistant strains was 14%. This is in accordance with previous study which showed similar mortality rate with pandrug-resistance gram negative bacterial infections [31].

CONCLUSION

This prospective study reported the increasing incidence of Colistin resistance among carbapenem resistant Klebsiella species in a tertiary care hospital. Paucity of newer effective antimicrobials alarms us to rationalize the usage of Colistin, one of the last available options for treating these infections. Surveillance of antibiotic prescription, strengthening of antimicrobial usage policy and hospital infection control must be implemented to prevent the exploitation of antibiotics and to halt the emergence of resistance.

REFERENCES

[1] Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11(4):589-603.

[2] Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 2015;22(1):90-101.

[3] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18(4):657-86.

[4] Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli & Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Res 2012;135(6):907-12.

[5] M Shawky, S, Abdallah A, & El Kholy M. Antimicrobial activity of Colistin and Tigecycline against carbapenem resistant Klebsiella pneumoniae clinical isolates in Alexandria, Egypt Int J Curr Microbiol App Sci 2015;4(2):731-742

[6] Global Antibiotic Resistance Partnership (GARP) - India Working Group. Rationalizing antibiotic use to limit antibiotic resistance in India+. The Indian Journal of Medical Research 2011;134(3):281-294.

[7] Yi-Hsiang C, et al. Colistin Resistance Mechanisms in Klebsiella pneumoniae Strains from Taiwan. Antimicrobial Agents and Chemotherapy 2015;59(5):2909–2913.

[8] Pragasam AK, et al. Molecular Mechanisms of Colistin Resistance in Klebsiella pneumoniae Causing Bacteremia from India—A First Report. In Frontiers in Microbiology 2017;7.

[9] Satyajeet K, Geeta S, Ravindra V, & Vaishali S. Emergence of Colistin Resistant Gram Negative Bacilli, in a Tertiary Care Rural Hospital from Western India. In Indian Journal of Microbiology Research 2021;2021:2594).

[10] Goel G, Hmar L, Sarkar De M, Bhattacharya S, Chandy M. Colistin-Resistant Klebsiella pneumoniae: Report of a Cluster of 24 Cases from a New Oncology Center in Eastern India. Infection Control & Hospital Epidemiology. Cambridge University Press; 2014;35(8):1076–7.

[11] CLSI. Performance standards for antimicrobial susceptibility testing for bacteria that grow aerobically, Approved standard M100-S17, Clinical and Laboratory Standards Institute ,Seventeenth Informational supplement,Wayne, Pennsylvania, USA: 27 – 42,2016.

[12] Galani I, et al. Colistin susceptibility testing by Etest and disk diffusion methods. International Journal of Antimicrobial Agents 2008;31(5):434–439.

[13] Choi M.-J, & Ko KS. Mutant prevention concentrations of Colistin for Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella pneumoniae clinical isolates. Journal of Antimicrobial Chemotherapy 2014;69(1), 275–277.

[14] Perween N, Prakash SK, Siddiqui O.Multi Drug Resistant Klebsiella Isolates in Burn Patients: A Comparative Study. J Clin of Diagn Res 2015; 9(9):DC14-DC16.

[15] Sodhi K, Mittal V, Arya M, Kumar M, Phillips A, & Kajla B. Pattern of Colistin resistance in Klebsiella isolates in an Intensive Care Unit of a tertiary care hospital in India. Journal of Infection and Public Health 2020; 13(7): 1018–1021.
[16] Moini AS, Soltani B, Taghavi Ardakani A, Moravveji A, Erami M, Haji Rezaei M, Namazi M. Multidrug-Resistant Escherichia coli and Klebsiella pneumoniae Isolated From Patients in Kashan, Iran. Jundishapur J Microbiol 2015;8(10):e27517.
[17] Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathog Glob Health 2017;111(5):240-246.
[18] Arif B, Khan E, Hussain F, Nasir A, & Irfan S. Effectiveness of Modified Hodge Test to detect NDM-1 Carbapenemases: An experience from Pakistan. Journal of the Pakistan Medical Association 2013;63:955–960.
[19] Azimi L, Lari AR, Talebi M, Owlia P, Alaghehbandan R, Asghari B, Lari ER. Inhibitory-based method for detection of Klebsiella pneumoniae carbapenemase Acinetobacter baumannii isolated from burn patients. Indian J Pathol Microbiol 2015;58(2):192-5.
[20] Behera B, et al. Evaluation of susceptibility testing methods for polymyxin. International Journal of Infectious Diseases 2010;14(7): e596–e601.
[21] Behera H, Tarai B, & PD. (2016). Incidence of Colistin-resistant Klebsiella pneumoniae and its antibiotic susceptibility pattern from a tertiary care hospital, northern India.
[22] Ramesh N, et al. Colistin susceptibility of gram-negative clinical isolates from Tamil Nadu, India. Asian Biomedicine. 2016;10(1): 35-39.
[23] Cannatelli A, D'Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, Gaibani P, Rossolini GM. In vivo emergence of Colistin resistance in Klebsiella pneumoniae producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. Antimicrob Agents Chemother 2013;57(11):5521-6.
[24] Jayol A, Poirel L, Brink A, Villegas MV, Yilmaz M, Nordmann P. Resistance to Colistin associated with a single amino acid change in protein PmrB among Klebsiella pneumoniae isolates of worldwide origin. Antimicrob Agents Chemother 2014;58(8):4762-6.
[25] Jayol A, Nordmann P, Brink A, Poirel L. Heteroresistance to Colistin in Klebsiella pneumoniae associated with alterations in the PhoPQ regulatory system. Antimicrob Agents Chemother 2015;59(5):2780-4.
[26] Armand-Lefèvre L, Angebault C, Barbier F, Hamelet E, Defrance G, Ruppé E, Bronchard R, Lepeule R, Lucet JC, El Mniai A, Wolff M, Montravers P, Plésiat P, Andremont A. Emergence of imipenem-resistant gram-negative bacilli in intestinal flora of intensive care patients. Antimicrob Agents Chemother 2013;57(3):1488-95.
[27] Garbati MA, Bin Abdulhak A, Baba K, & Sakkijha H. Infection due to Colistin-resistant Enterobacteriacae in critically-ill patients. The Journal of Infection in Developing Countries, 2013;7(10 SE-Original Articles): 713–719.
[28] Ghafur A, Lakshmi V, Kannain P, Murali A, Ma T. Emergence of Pan drug resistance amongst gram negative bacteria! The First case series from India. Journal of Microbiology and Infectious Diseases 2014; 4(03): 86-91.
[29] Antoniadou A, Kontopidou F, Poulakou G, Koratzanis E, Galani I, Papadomichelakis E, Kopterides P, Souli M, Armaganidis A, Giamarellou H. Colistin-resistant isolates of Klebsiella pneumoniae emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother 2007;59(4):786-90.
[30] Carlet J, et al Society's failure to protect a precious resource: antibiotics. The Lancet 2011; 378(9788): 369–371.
[31] Falagas ME, Bliziotis IA, Kasiakou SK, Samonis G, Athanassopoulou P, & Michalopoulos A. Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. BMC Infectious Diseases 2005; 5(1):24.