Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Screening of Carbapenem Resistance *Klebsiella pneumoniae* and its MIC against Imipenem

Sarada Saud[1], Ashwani Agrawal[1], Soniya Pokhrel[1], Sushma Subedi[1], Sanjit Shrestha[2], and Niroj Man Amatya [1]
[1]Department of Medical Microbiology, Nobel College, Sinamangal, Kathmandu, Nepal Affiliated to Pokhara University.
[2]Pathology Department, B & B Hospital, Lalitpur, Nepal.

Received: 26 Feb 2022; Revised: 20 Jul 2022; Accepted: 26 Jul 2022; Published online: 30 Jul 2022

Abstract

Klebsiella pneumoniae is a common opportunistic pathogen causing a wide range of infections; pneumonia, urinary tract infection, bacteremia, and liver abscesses. It infects primarily immunocompromised and immunocompetent individuals. It presents itself as an antibiotic-resistant bacterium, especially for third-generation cephalosporins and carbapenems, creating serious global challenges. Therefore, this cross-sectional study was conducted in B & B Hospital, Lalitpur to screen the distribution of carbapenem resistance *K. pneumoniae* through ertapenem and to assess the minimum inhibitory concentration of imipenem for screened carbapenem resistance *K. pneumoniae*. From 3447 different clinical samples collected according to standard guidelines, *K. pneumoniae* was identified using standard microbiological techniques; staining and a panel of biochemical tests. The antibiotic susceptibility test of the isolates was performed by the Kirby-Bauer disc diffusion method as per CLSI 2018 guidelines. The screening of carbapenem resistance was assessed by using ertapenem disc and the MIC of imipenem for carbapenem resistance and intermediate was performed using an Epsilometer. A total of 85 *K. pneumoniae* were identified and their antibiotic susceptibility test revealed that ceftriaxone was the least effective antibiotic. The number of MDR, carbapenem-resistant and intermediate isolates was 51, 46, and 3, respectively. The MIC of imipenem through an Epsilometer from ertapenem resistant and intermediate revealed that 31, 5, and 13 isolates were resistant, intermediate, and sensitive respectively. These findings showed the inconsistency in the detection of carbapenem-resistant isolates in routine microbiology laboratories and further support the other tests for the detection of carbapenem resistance as suggested by CLSI.

Keywords: Carbapenem-resistant *Klebsiella pneumoniae*, Ertapenem, MIC

Corresponding author, e-mail: mahaju@gmail.com

Introduction

Klebsiella pneumoniae, an important member of the Enterobacteriaceae family; residing in the gastrointestinal tract of us, is not only the most clinically isolated opportunistic pathogen from immune-compromised individuals, neonates, critically ill patients, or patients with other risk factors in healthcare settings [1] but also from immunocompetent individuals causing a wide range of infections mostly urinary tract infection, pyogenic liver abscess, necrotizing pneumonia or other life-threatening infections [2]. Management of its infection becomes complicated after it is found to be not susceptible to the third-generation cephalosporin antibiotics, including monobactams [3]. This is further aggravated by the non-response of carbapenem antibiotics through either the expression of carbapenemase enzymes that make bacteria almost resistant to a β-lactam group antibiotic [4, 5] or alteration of permeability due to loss of porin or overexpression of the efflux pump [5, 6]. Therefore, WHO prioritizes extended-spectrum β-lactamase [ESBL] and carbapenem-resistant *Klebsiella pneumoniae* [CRKP] as a critical public health threat [7]. The epidemiological distribution of CRKP fluctuates in all countries [8] with significantly higher morbidity and mortality rate than those of carbapenem susceptible *K. pneumoniae,* which initiates devastating public health conditions [9].

This bacterium notoriety gained its name among antibiotic-resistant bacteria. The European Antimicrobial Resistance Surveillance Network (EARS-Net) showed that from 2005 the non-susceptibility rate of this bacterium had increased significantly against third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems that have greater variation in different countries of the European Union [10] and other parts of the world, increasing global public health concerns [11]. Hence, WHO recognized this bacterium with *Acinetobacter baumannii*, *Pseudomonas aeruginosa* as a WHO Priority Pathogen list for "Research and Development" of New Antibiotics [7]. Therefore, this study was carried out to find out the frequency of CRKP along with their antibiotic susceptibility profile and MIC

of imipenem for screened carbapenem-resistant and intermediate isolates.

Materials and Methods

It is a hospital-based prospective cross-sectional study; carried out in the Microbiology Department of B and B Hospital, Nepal, from 15 July 2018 to 15 October 2018. The target group of this study was irrespective of sex, all age groups of patients attending the hospital for medical treatment. All collected data were entered and analyzed using SPSS V17.0. Ethical consent was obtained from the Nobel Institutional Review Committee (IRC).

Bacterial isolation and identification

Samples (blood, pus, urine, respiratory specimen, catheter tips, and joint fluid) were collected aseptically according to standard microbiological guidelines [12]. Good quality specimens were accepted, while unlabeled or mislabeled specimens, dry swabs, specimens that leak from a container, delay in specimen transport, and inappropriately stored samples were excluded from this study. All samples, except blood, were cultured on blood agar and MacConkey agar; incubated at 37^0C overnight, and identified as *K. pneumoniae* using Gram staining and conventional biochemical tests (Catalase, Oxidase, indole test, motility, citrate utilization, triple sugar iron utilization and Urease Test) [13]. The reagents and culture media were purchased from HiMedia Laboratories, India. The BD™ BACTEC™ FX40 Automated Blood Culture System was used for blood culture, and a positive culture bottle was further sub-cultured on blood agar and MacConkey as previously for identification [13].

Antibiotic susceptibility test

The antibiotic susceptibility test was performed following CLSI guidelines 2018 through the disc diffusion method. Eleven different antibiotics (HiMedia Laboratories, India); amikacin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), gentamicin (10 µg), nitrofurantoin (300 µg), piperacillin-tazobactam (100/10 µg), and ofloxacin (5µg) were tested in isolated *Klebsiella pneumoniae*. The bacterium showing resistance to at least one antibiotic from three or more than three different classes was categorized as multidrug resistance [14]. The resistance to carbapenem was screened using ertapenem (10µg) disc. According to zone size diameter, isolates were differentiated as sensitive, intermediate, and resistant with zone size inhibition ≥22 mm, 19-21mm, and ≤18 mm, respectively [15]. The minimum inhibitory concentration (MIC) of the imipenem was tested for isolates showing intermediate or resistance to carbapenem using the Imipenem Ezy MIC™ strip following the manufacture instructions (HiMedia Laboratories, India). The MIC of imipenem was interpreted and the isolates were differentiated as sensitive (≤1 µg/ml), intermediate (2 µg/ml), and resistant (≥4µg/ml) [15]. For quality control of the MIC test strip, carbapenem susceptible *Escherichia coli* ATCC 25922 was used.

Results

Patient Characterization

A total of 3447 specimens were received in the trimester period in which 771 samples showed culture-positive from which 815 bacteria were isolated. Among 815 bacterial isolates, 85 isolates were confirmed as *Klebsiella pneumoniae*. The rest data are shown in Table 1.

Table 1. Clinical and social demography of patients

S.N.	Status of patients	Number (%)
1	OPD	15 (17.65%)
2	Inpatients	70 (82.35%)

S.N.	Sex	Number
1	Male	50 (58.82%)
2	Female	35 (41.18%)

S.N.	Age group	Number
1	Below 20	12 (14.11%)
2	20-30	14 (16.47%)
3	30-40	12 (14.11%)
4	40-50	9 (10.59%)
5	50-60	14 (16.47%)
6	60 and above	24 (28.24%)

Antibiotic susceptibility test

The antibiotic susceptibility test showed that ceftriaxone (59, 69.42%) was the most non-susceptible antibiotic, followed by ciprofloxacin (49, 57.65%) and gentamicin (48, 56.47%). Furthermore, 60% (51 of 85) isolates were multidrug-resistant. The remaining data are shown in **Table 2**.

Carbapenem-resistant

The screening of carbapenem resistance showed that in 85 *K. pneumoniae* isolates, the carbapenem resistance and intermediate isolates were 46 (54.13%) and three (3.52%) respectively. The MIC test of both intermediate and resistant isolates was done as suggested by the CLSI guideline 2018. Among the 49 isolates, the MIC test of imipenem showed that 31 isolates were resistant, five intermediate, and 13 sensitives. Furthermore, in 85 isolates, 51 isolates were MDR *K. pneumoniae* isolates, in which 45 isolates were resistant to carbapenem and six isolates were susceptible to carbapenem.

Table 2. Antibiotic Susceptibility Pattern of *K. pneumoniae*

S.N.	Antibiotic Susceptibility Pattern				Total Isolates
	Antibiotics used	Sensitive (%)	Intermediate (%)	Resistant (%)	
1	Amikacin	24 (28.23)	20 (23.53)	41 (48.24)	85
2	Gentamicin	34 (40)	3 (3.53)	48 (56.47)	85
3	Ciprofloxacin	28 (32.94)	8 (9.41)	49 (57.65)	85
4	Ofloxacin	33 (38.82)	5 (5.88)	47 (55.30)	85
5	Ceftriaxone	24 (28.23)	2 (2.35)	59 (69.42)	85
6	Ertapenem	36 (42.35)	3 (3.52)	46 (54.13)	85
7	Cefepime	11 (16.18)	8 (11.76)	49 (72.06)	68
8	Piperacillin-tazobactam	9 (14.06)	12 (18.75)	43 (67.19)	64
9	Chloramphenicol	38 (61.30)	11(17.74)	13 (20.96)	62
10	Cotrimoxazole	28 (50)	1 (1.78)	27 (48.22)	56
11	Nitrofurantoin	6 (13)	6 (13)	34 (74)	46

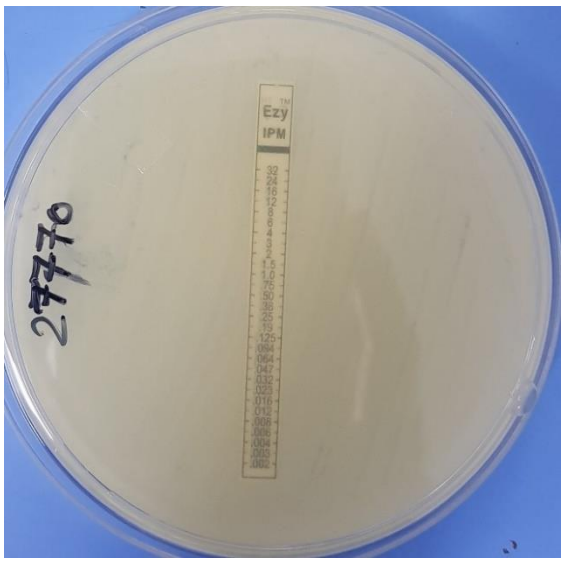

Figure 1. MIC Test

Discussion

Klebsiella pneumoniae is an opportunistic pathogen responsible for causing various community-acquired and healthcare-associated infections. Furthermore, the infection caused by this bacterium cannot be neglected, as it is included in ESKAPE pathogens and the increasing incidence of CRKP strains attracts attention to clinicians and other stakeholders.

In our study, the highest percentage of *K. pneumoniae* was obtained in the inpatient department than others. This higher incidence of *K. pneumoniae* in long-term hospitalized patients may be related to the immune status of the patients, as the bacterium was isolated from the surgery unit with the use of invasive devices and administration of immunosuppressive drugs. In hospital settings, the transmission of the pathogen increases drastically because the colonization rate increases with an extended stay in the hospital and prolonged antibiotic therapy. A similar study carried out in the United States also claimed a higher incidence of *K. pneumoniae* infections in long-term acute care hospitals than in the short-term hospital stay [16].

Antibiotic resistance is a common problem in *K. pneumoniae*. It is naturally resistant to the penicillin group of antibiotics [17] or acquires antibiotic resistance genes from mobile genetic cassettes called integrons, often carried out by transposons and transferable plasmids that transmit horizontally to receptor cells, integrated on plasmids or chromosomes through homologous recombination, expressing its fitness in the presence of antibiotics [18]. In this study, we evaluated 11 different antibiotics, in which amikacin, gentamicin, ciprofloxacin, ofloxacin, ceftriaxone, ertapenem were tested in all isolates, and the rest antibiotics were tested either as second-line antibiotics or depending on a source of clinical samples. Our results showed a mixed antibiotic resistance profile compared to others in terms of antibiotic use, time period, bacterium source and country.

Approximately 48.24% and 56.47% of the isolates were not susceptible to aminoglycosides, amikacin, and gentamicin, respectively. A range of studies shows a wide variation in resistance pictures that range from 1% to 86% for gentamicin. A study in the EU/EEA region showed that its resistance per cent ranges from below 1% to greater than 50 [19]. A comparative study from France and Algeria revealed that its resistance was 28% and 86%, respectively, [20] while in a study from Iran and India, the resistance rate was found to be 24% and 37.5%, respectively [21, 22]. A slightly lower percentage, 41%, was observed in Nepal [23] which is nearly equal to our study. Cephalosporins, such as ceftriaxone, are frequently used antibiotics for *K. pneumoniae* until they become ESBL producers. Our study showed that nearly 70% of the isolates were resistant to ceftriaxone. A similar finding was described from Ethiopia [24] and a four-year consecutive study from Greece [19]. Ciprofloxacin and

ofloxacin are alternative antibiotics of choice if the isolates are ESBL producers. The non-susceptibility rates for ciprofloxacin and ofloxacin were 57.65 % and 55.3 % in our study. Similar to aminoglycosides, a wider range of variations in the non-susceptibility of quinolones was observed, ranging from less than 1% to more than 90%. The study of Bulgaria, Italy, and Romania was in line with our study, while from Germany, Denmark, Iran, and India, the non-susceptibility rate was lower than in our study [19, 21, 22]. A few studies from Nepal showed that the resistance rate is more than 85 %, which is quite much higher than ours [23, 25]. In general, different studies conducted at different times revealed significant variability in antibiotic resistance patterns. Such a type of variation was also observed in other antibiotics mentioned in **Table 2**, which might be due to the low number of sample studies or how meticulously antibiotics were used in that country to mitigate antibiotic resistance problems. Hence, resistance to these first-line agents represents an unprecedented challenge to clinicians, scientists, and healthcare systems.

Carbapenems (imipenem, meropenem, and ertapenem) are the antibiotics of choice for those *K. pneumoniae* that are MDR and ESBL producers [26]. The phenotypic lab detection of carbapenem resistance is quite confusing and for confirmation of carbapenemase producer, different additional tests should be performed [27]. Therefore, CLSI recommends that ertapenem non-susceptibility is the most sensitive indicator for carbapenemase producers, and additional tests should not be performed for purposes other than epidemiological or infection control after breaking point evaluation of ertapenem, meropenem, and imipenem [15]. In our study, 49 (both intermediate and resistant) isolates showed positive screening through non-susceptibility to ertapenem. This suggests that resistance to carbapenem arises due to the formation of carbapenemase enzymes of classes A, B and D of the Amber class of β-lactamase, restricting the treatment option [27]. The CRKP was subjected to the E-test to determine the MIC of the imipenem. The test results showed that susceptible, intermediate and resistant were 13 [MIC ≤1µg/ml), 5 [4µg/ml ≤MIC≥1µg/ml) and 31 [MIC ≥4 µg/ml), respectively. This implies that results from phenotypic methods can vary, as suggested by CLSI.

Conclusions

The fast-growing antibiotic-resistant *Klebsiella pneumoniae* is a global problem, including in Nepal, such that the choice of an effective antibiotic is now chaotic. The continued emergence of CRKP has wreaked havoc on its routine diagnosis, as well as on its therapeutic treatment options. Susceptibility testing [disc diffusion and MIC) provides valuable information for therapeutic inference but does not adequately address carbapenem resistance, which is significant for infection control and epidemiological evidence necessary to curb the spread of carbapenem-resistant Enterobacteriaceae.

Authors' Contributions

The laboratory works were performed by Sarada Saud, Ashwani Agrawal, Soniya Pokhrel and Sushma Subedi. Research conceptualization, protocol selection and preliminary manuscript preparation were done by Sarada Saud, Ashwani Agrawal, Soniya Pokhrel, Sushma Subedi, Sanjit Shrestha and Niroj Man Amatya. Final manuscript preparation was done by Niroj Man Amatya. The study was supervised under the guidance of Sanjit Shrestha and Niroj Man Amatya. All authors read and approved the final manuscript.

Competing Interests

On behalf of all authors, the corresponding author states that there is no competing interest.

Financial disclosure

It is a self-funded pilot study and no funding is granted.

Acknowledgements

We would like to thank all the participants attained in this study and the Pathology Department of the B and B Hospital, Nepal.

Ethics approval

The ethical consent was procured from Nobel Institutional Review Committee [IRC) with reference number BCH IRC 161/2018.

Additional disclosure

This manuscript has been already published in preprint form with title "Screening of*Klebsiella pneumoniae*for Carbapenem Resistance and MIC of Imipenem" with weblink: https://www.researchsquare.com/article/rs-36584/v1

Reference

1. Broberg CA, Palacios M, Miller VL. Klebsiella: a long way to go towards understanding this enigmatic jet-setter. F1000Prime Rep. 2014;6:64.
2. Paczosa MK, Mecsas J. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol Mol Biol Rev. 2016;80[3):629-61
3. WHO. Antimicrobial resistance: global report on surveillance. Geneva, Switzerland: World Health Organization; 2014.
4. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clinical microbiology reviews. 2007;20(3):440-58
5. Martínez-Martínez L, Pascual A, Hernández-Allés S, Alvarez-Díaz D, Suárez AI, Tran J, et al. Roles of β-lactamases and porins in activities of carbapenems and cephalosporins against Klebsiella

pneumoniae. Antimicrobial agents and chemotherapy. 1999;43(7):1669-73
6. Padilla E, Llobet E, Doménech-Sánchez A, Martínez-Martínez L, Bengoechea JA, Albertí S. Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrobial agents and chemotherapy. 2010;54(1):177-83
7. WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. February 27, 2017. 2018.
8. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. The Lancet infectious diseases. 2013;13(9):785-96
9. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. Annals of clinical microbiology and antimicrobials. 2017;16(1):18.
10. European Centre for Disease Prevention and Control. Surveillance Atlas of Infectious Diseases: ECDC; [Available from: http://atlas.ecdc.europa.eu/public/index.aspx?Instance.
11. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clinical Microbiology and Infection. 2014;20(9):821-30
12. Leber AL. Clinical microbiology procedures handbook. Washington, DC, USA: American Society for Microbiology; 2016.
13. Tille P. Bailey & Scott's diagnostic microbiology: Elsevier Health Sciences; 2015.
14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3):268-81
15. CLSI. Performance standards for antimicrobial susceptibility testing. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
16. Lin MY, Lyles-Banks RD, Lolans K, Hines DW, Spear JB, Petrak R, et al. The importance of long-term acute care hospitals in the regional epidemiology of Klebsiella pneumoniae carbapenemase–producing Enterobacteriaceae. Clinical infectious diseases. 2013;57(9):1246-52
17. Mandell G, Dolin R, Bennett J. Mandell, Douglas, and Bennett's principles and practice of infectious diseases: Elsevier; 2009.
18. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clinical microbiology reviews. 2018;31(4):e00088-17
19. European Centre for Disease PC. Surveillance of antimicrobial resistance in Europe 2016. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). 2017.
20. Berrazeg M, Diene SM, Drissi M, Kempf M, Richet H, Landraud L, et al. Biotyping of multidrug-resistant Klebsiella pneumoniae clinical isolates from France and Algeria using MALDI-TOF MS. PloS one. 2013;8(4):e61428.
21. Estabraghi E, Salehi TZ, Aminiq K, Jamshidian M. Molecular identification of extended-spectrum β-lactamase and integron genes in Klebsiella pneumonia. Journal of the Nepal Medical Association. 2016;54(202).
22. Shilpa K, Thomas R, Ramyashree A. Isolation and Antimicrobial sensitivity pattern of Klebsiella pneumoniae from sputum samples in a tertiary care hospital. Int J Biomed Adv Res. 2016;7(2):53-7.
23. Subedi S, Maharjan J, Shrestha B. Antibiotic Susceptibility Test of Klebsiella pneumoniae and K. oxytoca Isolated from Different Clinical Samples and Perform Random Amplified Polymorphic DNA among K. pneumoniae. Microbiology Research Journal International. 2016:1-11.
24. Gashe F, Mulisa E, Mekonnen M, Zeleke G. Antimicrobial resistance profile of different clinical isolates against third-generation cephalosporins. Journal of pharmaceutics. 2018;2018
25. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrobial Resistance & Infection Control. 2017;6(1):67
26. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Clinical Microbiology and Infection. 2000;6(9):460-3.
27. Miller S, Humphries RM. Clinical laboratory detection of carbapenem-resistant and carbapenemase-producing Enterobacteriaceae. Expert review of anti-infective therapy. 2016;14(8):705-17