

Screening of *Klebsiella Pneumoniae* for Carbapenem Resistance and MIC of Imipenem

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Research

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Abstract

Background: *Klebsiella pneumoniae* is a common opportunistic pathogen causing a wider range of infections, pneumonia, urinary tract infection, bacteremia, and liver abscesses; primarily in immunocompromised as well as immunocompetent individuals. This bacterium presents itself as an antibiotic resistant one especially in third generation cephalosporins and carbapenem, creating serious global challenges. Therefore, this cross-sectional study was conducted in B & B Hospital, Lalitpur with the aim to screening the distribution of carbapenem resistance *Klebsiella pneumoniae* through ertapenem and assess the minimum inhibitory concentration of imipenem for screened carbapenem positive *K. pneumoniae*.

Methods: From 3447 different clinical samples collected according to standard guidelines, *Klebsiella pneumoniae* was identified through conventional microbiological techniques, staining and a panel of biochemical tests. The antibiotic susceptibility test of isolates was performed by the Kirby-Bauer disc diffusion method as per CLSI 2018 guidelines. Screening of carbapenem resistant was assessed by using ertapenem disc and the MIC of imipenem for carbapenem resistant and intermediate was done through epsilometer.

Results: A total of 85 nonduplicate *Klebsiella 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 epsilometer from resistant and intermediate ertapenem isolates revealed that 31, 5 and 13 isolates were resistant, intermediate and sensitive, respectively.

Conclusion: These findings showed the inconsistency in detection of carbapenem resistant isolates in routine microbiology laboratories and further support the other tests for detection of carbapenem resistance as suggested by CLSI.

Background

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 immunocompromised 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]. The management of its infection becomes complicated after it is found non-susceptible to third generation cephalosporin group of antibiotics including monobactams [3]. This is further aggravated by the nonresponse of antibiotic carbapenem through either expression of carbapenemases enzyme rendering bacteria almost resistant to β -lactam group of antibiotics [4, 5] or alteration of permeability due to loss of porin or over expression of efflux pump [5, 6]. Hence, 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 is fluctuating in all countries [8] with significantly higher morbidity and mortality rates than carbapenem susceptible *K. pneumoniae* initiating devastating public health conditions [9].

This bacterium notoriety gained its name among the antibiotic resistant bacteria. The European Antimicrobial Resistance Surveillance Network (EARS-Net) showed that in one decade from 2005, its non-susceptibility rate increased notably against third generation cephalosporin, aminoglycosides, fluoroquinolones and carbapenem having larger variation in different European Union countries (<http://atlas.ecdc.europa.eu/public/index.aspx?Instance>). Not only Europe, it also spread its arm around worldwide escalating global public health concerns[10]. Hence, WHO recognized this bacterium along 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 the frequency of CRKP along with their antibiotic susceptibility profile and MIC of imipenem for screened carbapenem resistant and intermediate isolates.

Methods

It is a trimester hospital-based prospective, cross-sectional study was carried out in the Microbiology Department of B and B Hospital, Nepal, from 15th July 2018. The target group of the study was irrespective of sex, all age groups of patients attending hospital for medical treatment. All collected data were entered and analyzed using SPSS V17.0; ethical consent was procured from 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[11]. Good quality specimens were accepted while unlabeled or mislabeled specimens, dry swabs, specimens leaking from a container, delay in transport of specimen, and inappropriately stored samples were excluded from this study. All specimens except blood were cultured on blood agar (HiMedia Laboratories, India), MacConkey agar (HiMedia Laboratories, India); incubated at 37⁰C overnight and follow biochemical tests for identification of *Klebsiella pneumoniae*. The BD™ BACTEC™ FX40 Automated Blood Culture System was used for blood culture and a positive culture bottle was further subcultured on blood agar and MacConkey as previously for identification [12].

Antibiotic susceptibility test

The antibiotic susceptibility test was performed in accordance with the recommendation of CLSI through the disc diffusion method. Twelve different antibiotics (HiMedia Laboratories, India) were tested in isolated strains: amikacin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), colistin (10 µg), gentamicin (10 µg), nitrofurantoin (300 µg), piperacillin-tazobactam (100/10 µg), and ofloxacin (5 µg). The bacterium showing resistance to

at least one antibiotic from three or more than three different classes was categorized as multidrug resistance [13]. The carbapenem resistance was screened from ertapenem (10 µg) disc (HiMedia Laboratories, India). According to zone size diameter, isolates were differentiated as sensitive, intermediate and resistant with zone size inhibition ≥ 22 mm, 19–21 mm and ≤ 18 mm, respectively [14]. Isolates that were carbapenem intermediate or resistant, the minimum inhibitory concentration (MIC) of imipenem were tested using the Imipenem Ezy MIC™ Strip following manufacture (HiMedia India) instructions. The MIC of imipenem was interpreted and isolates were differentiated as sensitive (≤ 1 µg/ml), intermediate (2 µg/ml) and resistant (≥ 4 µg/ml) [14]. 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 of which 815 bacteria were isolated. Among 815 bacterial isolates, 85 isolates (50 from male and 35 from female) were confirmed as *Klebsiella pneumoniae* after excluding duplicate samples. Most of the isolates were from in-patient department (82.58%) than out-patient department (17.42%). Age-wise data showed that the highest growth of isolates was obtained from the age groups 20–30 and 50–60 each having 14 (17.42%).

Antibiotic susceptibility test

The antibiotic susceptibility test showed that among the first-line antibiotics, ceftriaxone (59, 69.42%) was the most non-susceptible antibiotic followed by ciprofloxacin (49, 57.65%) and gentamicin (48, 56.47%). Further, 60% (51 out of 85) isolates were multidrug resistant. Notably, no resistance was observed in antibiotic colistin, but it was tested only for 46 isolates because it was used as a second-line antibiotic. The rest data are shown in Table 1.

Table 1
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
12	Colistin	46 (100)	0	0	46

Carbapenem resistant

Among the 85 *K. pneumoniae* isolates, 46 (54.13%) and 3 (3.52%) isolates were found as carbapenem resistant and intermediate, respectively determined phenotypically by using ertapenem discs. The MIC test of both intermediate and resistant isolates was done as suggested by CLSI guideline 2018. Among the 49 isolates, the MIC test of imipenem showed that 31 isolates were resistant, 5 were intermediate and 13 were sensitive.

Similarly, in 51 MDR isolates, 45 isolates were carbapenem resistant and 6 isolates were carbapenem susceptible. Among the 34 isolates that were non-MDR, only one was carbapenem resistant and rest 33 were carbapenem susceptible.

Discussion

Klebsiella pneumoniae is an opportunistic pathogen responsible for causing various community acquired and healthcare associated patients. Further infection caused by this bacterium cannot be neglected as it is included in ESKAPE pathogens and the growing incidence of CRKP strains creates attention to clinicians and other stake holders.

In our study, the highest percentage of *K. pneumoniae* was obtained from the in-patient 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 from surgery unit having use of invasive devices and administration of immunosuppressive drugs. In hospital settings, the transmission of the pathogen increases drastically because colonization rate increases with extended stay in hospital and prolonged antibiotic therapy. A similar study carried out in the United States also claimed a higher incidence of *K. pneumoniae* infections in the long-term acute care hospitals than in the short-term hospital stay[15].

Antibiotic resistance is a common problem in *K. pneumoniae*. It is naturally resistant to penicillin group of antibiotics[16] or acquires resistance gene 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 [17]. In this study, we evaluated 12 different antibiotics in which amikacin, gentamicin, ciprofloxacin, ofloxacin, ceftriaxone and ertapenem were tested in all isolates and rest antibiotics were tested either as second-line antibiotics or depend on a source of clinical samples. Our results showed a mixed antibiotic resistance profile comparison with others in terms of using antibiotic use, time period, source of bacterium and country.

Approximately 48.24% and 56.47% isolates were non-susceptible to aminoglycoside amikacin and gentamicin, respectively. A range of studies show greater variation in resistant pictures ranging below 1–86% for gentamicin. A study in the EU/EEA region showed that its resistant percent ranges from below 1% to greater than 50 [18]. A comparative study from France and Algeria revealed that its resistance was 28% and 86%, respectively[19] while study from Iran and India, its resistance was found as 24% and 37.5%, respectively [20, 21]. A slightly higher percentage, 41%, was pictured from Nepal [22] that is nearly equal to our study. Cephalosporins like ceftriaxone are frequently used antibiotics for *K. pneumoniae* until unless they are ESBL producers. Our study showed that nearly 70% of isolates were ceftriaxone resistant. A similar finding was reported from Ethiopia [23] and 4 year consecutive study from Greece [18]. Ciprofloxacin and ofloxacin are alternative antibiotic of choice if the isolates are ESBL producers. The non-susceptibility rates of ciprofloxacin and ofloxacin were 57.65% and 55.3% in our study. Similar to aminoglycosides, a wider range of variation was observed in the non-susceptibility of quinolones ranging from below 1% to greater than 90%. The study from Bulgaria, Italy and Romania was in line with our study, while from countries like Germany, Denmark, Iran, and India, the non-susceptibility rate was lower than our study [18, 20, 21]. Few studies from Nepal showed that the resistance rate is more than 85%, which is quite much than ours [22, 24]. Over all different studies on different times showed marked variation in antibiotic resistance patterns. Such type of variation was also observed in other antibiotics mentioned in Table 1, 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.

Carbapenem (imipenem, meropenem, and ertapenem) are the antibiotics of choice for MDR and ESBL producing *K. pneumoniae* [25]. The phenotypic lab detection of carbapenem resistance is quite confusing

and should perform different test panels [26]; hence, CLSI recommends that non-susceptibility to ertapenem is the most sensitive indicator of carbapenemase producers and those addition tests need not be done other than epidemiological or infection control purposes after breakpoint evaluation of ertapenem, meropenem and imipenem [14]. In our study, 49 (both intermediate and resistant) isolates showed screening test positive through ertapenem nonsusceptibility. This suggests that carbapenem resistance 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 [26]. The CRKP was subjected to E-test to determine MIC of imipenem. The test results showed susceptible, intermediate and resistant were 13 (MIC \leq 1 μ g/ml), 5 (4 μ g/ml \leq MIC \geq 1 μ g/ml) and 31 (MIC \geq 4 μ g/ml), respectively. This implies that results from phenotypic methods can vary, as suggested by CLSI.

Conclusion

The fast growing antibiotic resistant *Klebsiella pneumoniae* is a global problem, including Nepal, such that the choice of effective antibiotic is now chaotic. Further emergence of CRKP havoc on its routine diagnosis as well as therapeutic treatment option. The susceptibility testing (disc diffusion and MIC) delivers valuable information for therapeutic inferences but does not properly answer about carbapenem resistance, which is significant for infection control and epidemiological evidence necessary to curb the spread of carbapenem resistant Enterobacteriaceae.

Abbreviations

CLSI: Clinical and Laboratory Standards Institute

CRKP: Carbapenem resistant *Klebsiella pneumoniae*

ESBL: Extended Spectrum Beta Lactamase

MDR: Multi drug resistant

MIC: Minimum inhibitory concentration

Declarations

Ethics approval and consent to participate

The ethical consent was procured from Nobel Institutional Review Committee (IRC). Before sample collection, the objectives of the study were clearly told to the patient and written consent was taken.

Consent for publication

Not applicable.

Availability of data and materials

The data are confidential and could not be sharable.

Competing interests

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

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Authors' contributions

All 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.

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