

Prevalence and Antimicrobial Susceptibility Pattern of *Klebsiella Pneumoniae* Isolated from Various Clinical Specimens in a Tertiary Care Hospital in Bangladesh

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Abstract

Introduction: *Klebsiella pneumoniae* are common causative agents of various infections and are of great concern for developing resistance against commonly prescribed antibiotics. This study gives an account of isolation of *K. pneumoniae* from various clinical specimens and their antimicrobial susceptibility, in a tertiary care hospital of Bangladesh. **Materials and Methods:** Various clinical specimens like urine, wound swab, sputum, blood and endotracheal aspirates were collected and processed for isolation of *K. pneumoniae* followed by their antimicrobial susceptibility testing. **Results:** Among the 316 samples that yielded culture positivity, *K. pneumoniae* were identified as second most common organism. The highest yield of *K. pneumoniae* (37.33%) were observed from wound swab followed by sputum (26.67%). Most of the isolates were resistant to sulphamethoxazole-trimethoprim (90.67%) and ceftriaxone (90.67%) followed by cefotaxime (89.33%), ceftazidime (89.33%) and cefuroxime (89.33%). The most sensitive antibiotic for the isolates was tigecycline. **Conclusion:** Isolated *K. pneumoniae* showed resistance to commonly prescribed antibiotics, which is very alarming and showing the importance on continuous monitoring and strict antimicrobial policy.

Keywords: Antimicrobial susceptibility, *Klebsiella pneumoniae*, Various clinical specimens, Strict antimicrobial policy.

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Introduction

Klebsiella spp. is the second most popular member of the aerobic bacterial flora of the human intestine of which *Klebsiella pneumoniae* is the medically most important species of the genus^{1,2}.

Klebsiella pneumoniae frequently causes lower respiratory tract infections (46.7%), bacteremia (27%), urinary tract infections (11.7%) and wound infections (11.36%)³⁻⁶. *K. pneumoniae* is the second most common cause (behind *Escherichia coli*) of community and hospital-acquired gram-negative bloodstream infection⁴ and 3-7% of hospital-acquired bacterial infections are related to *K. pneumoniae*⁷. Antimicrobial resistance among clinical isolates of *K. pneumoniae* has become an increasingly serious problem over the past twenty years⁸. Multidrug resistant strain of *K. pneumoniae* emerged due to indiscriminate use of various antibiotics⁹.

Klebsiella pneumoniae carbapenemases (KPCs) (a class A beta lactamase enzyme) are predominantly found in *K. pneumoniae* and KPCs are capable of efficiently hydrolyzing penicillins, cephalosporins, aztreonam and carbapenems^{10,11}. The increasing prevalence of carbapenem resistant *K. pneumoniae* (CRKP) has become a critical threat to human health¹².

Under this grave situation, tigecycline and colistin became the last-resort of treatment for infections by multidrug-resistant (MDR) *K. pneumoniae*¹³. Hence the present study was carried out to identify the prevalence of *K. pneumoniae* in various clinical specimens and antimicrobial susceptibility pattern of the isolates which will be beneficial for medical practitioners to select empirical antimicrobial therapy which will play an important role in minimizing the emergence rate of antimicrobial resistance.

Materials and Methods

This cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh, from July 2016 to June 2017. This research protocol was approved by the research review committee (RRC) and ethical review committee (ERC) of Dhaka Medical College.

Sample collection

A total of 500 clinical samples including urine, wound swab, sputum, blood and endotracheal aspirates were collected and processed for isolation of bacteria by following standard guidelines from adult hospitalized patients of Dhaka Medical College Hospital. Written consent was taken from all the participants for this study.

Microbiological methods:**Culture**

Sputum samples having >25 polymorphonuclear cells (PMNs) /HPF (100x) and <10 squamous epithelial cells /HPF (100x) in Gram stain¹⁴ and all the urine, wound swab, blood and endotracheal aspirates were inoculated in both blood agar and MacConkey agar media, for blood samples primary blood culture was done in Trypticase Soy Broth. After incubation at 37°C aerobically for 24 hours incubated plates were examined.

Isolation and identification of the organisms from culture

Phenotypic identification of the organisms were done by observing colony morphology, hemolytic criteria, staining character, pigment production and biochemical tests (oxidase and catalase tests and other biochemical reactions after inoculation in TSI, MIU, citrate agar media)¹⁵.

Antimicrobial susceptibility test

The antimicrobial susceptibility of the isolated *K. pneumoniae* was done by Kirby-Bauer modified disc-diffusion technique¹⁶ using Mueller Hinton agar plates following Clinical and Laboratory Standard Institute (CLSI) guidelines¹⁷ and United States Food and Drug Administration (FDA) guideline for tigecycline¹⁸.

Control strain

Escherichia coli ATCC 25922 was used as control strain to assess the performance of the method.

Statistical analysis

Data were analyzed by using Microsoft Office Excel (2013) software (Microsoft, Redmond, WA, USA).

Role of funding source

There is no involvement of any funding source.

Results

A total of 500 samples were included in the present study. Of which, 170 were urine, 162 were wound swabs, 71 were sputum, 52 were endotracheal aspirates and 45 were blood samples. Culture positivity among the samples is shown in table I.

Table-I: Culture positivity from various clinical samples (N=500).

Samples	Number of samples	Culture positive n (%)
Urine	170	81 (47.65)
Wound swab	162	131 (80.86)
Sputum	71	42 (59.15)
ETA*	52	41 (78.85)
Blood	45	21 (46.67)
Total	500	316 (63.20)

* ETA= Endotracheal aspirates.

N= Total number of samples.

n= Culture positive samples.

The microorganisms isolated are shown in table II. Most frequently isolated microorganism was *E. coli* (32.28 %), followed by *K. pneumoniae* (23.73 %).

Table-II: Distribution of organisms isolated from different samples (N =316).

Organisms	n (%)
<i>Escherichia coli</i>	102 (32.28)
<i>Klebsiella pneumoniae</i>	75 (23.73)
<i>Klebsiella oxytoca</i>	5 (1.58)
<i>Pseudomonas spp.</i>	41 (12.97)
<i>Acinetobacter spp.</i>	17 (5.38)
<i>Proteus spp.</i>	12 (3.80)
<i>Enterobacter spp.</i>	10 (3.16)
<i>Citrobacter spp.</i>	6 (1.90)
<i>Salmonella spp.</i>	2 (0.63)
Gram positive bacteria	46 (14.56)
Total	316 (100.00)

N= Total number of bacteria.

n= Total number of bacterial species.

The highest yield of *K. pneumoniae* (37.33%) was observed from wound swab followed by sputum (26.67%). These facts may be referred from table III shown below.

Table-III: Distribution of *Klebsiella pneumoniae* isolated from different samples (N=75).

Samples	n (%)
Urine	14 (18.67)
Wound swab	28 (37.33)
Sputum	20 (26.67)
ETA*	7 (9.33)
Blood	6 (8.00)
Total	75 (100.00)

* ETA= Endotracheal aspirates.

N= Total number of *K. pneumoniae*.

n= Number of *K. pneumoniae* isolated from different samples.

The result of the antimicrobial susceptibility pattern of the isolated *K. pneumoniae* is depicted in table IV. Most of the isolates were resistant to sulphamethoxazole-trimethoprim (90.67%) and ceftriaxone (90.67%) followed by cefotaxime (89.33%), ceftazidime (89.33%) and cefuroxime (89.33%). The most sensitive antibiotic for the isolates was tigecycline.

Table-IV: Antibiotic resistance pattern of isolated *Klebsiella pneumoniae* (N=75).

Antimicrobial drugs	Resistance n (%)
Amikacin (30 µg)	54 (72.00)
Amoxicillin-clavulanic acid (20/10 µg)	56 (74.67)
Cefepime (30 µg)	65 (86.67)
Cefotaxime (30 µg)	67 (89.33)
Ceftazidime (30 µg)	67 (89.33)
Ceftriaxone (30 µg)	68 (90.67)
Cefuroxime (30 µg)	67 (89.33)
Ciprofloxacin (5 µg)	66 (88.00)
Gentamicin (10 µg)	57 (76.00)
Imipenem (10 µg)	28 (37.33)
Meropenem (10 µg)	28 (37.33)
Piperacillin-Tazobactam (100/10 µg)	54 (72.00)
Sulphamethoxazole-Trimethoprim (1.25/23.75 µg)	68 (90.67)
Tigecycline (15 µg)	11 (14.67)

N= Total number of isolated bacteria.

n= Total number of resistant bacteria.

Discussion

In the present study, culture positivity among the collected samples was 63.20% which is closer to a study in India reporting 56.9% culture positivity⁵. In the present study, among the culture positive samples *Escherichia coli* (32.28%) was the most common organism followed by *K. pneumoniae* (23.73%). A study in India by Choudhuri et al.¹⁹ reported that among the culture positive samples 27% were *K. pneumoniae* which is closer to the present study.

The isolation rate of *K. pneumoniae* was highest from wound swabs (37.33%), followed by sputum samples (26.67%). A study in India reported that highest *K. pneumoniae* (46.70%) were obtained from sputum and other lower respiratory tract secretions followed by blood (31.30%) and wound swab (24.20%)⁵. A study by Chakraborty et al.²⁰ reported that highest prevalence of *K. pneumoniae* was observed in urine sample followed by wound swab. The variation in frequency of isolation of *K. pneumoniae* from different samples may be due to variation in the sample size.

In the present study, higher rate (90.67%) of resistance was exhibited by *K. pneumoniae* against sulphamethoxazole-trimethoprim, which is similar to observations of other authors ranging from 76% to as high as 95.24% resistance^{21,22}. Resistance of *K. pneumoniae* to ciprofloxacin was 88% in the present study. This finding was in agreement with the study by Babakhani et al.²³ who reported 82.50% ciprofloxacin resistance to *K. pneumoniae*. High ciprofloxacin resistance had been reported in other studies

ranging from 63% to as high as 76.9%^{24,25}. High resistance rate to sulphamethoxazole-trimethoprim and ciprofloxacin in the present study might be due to uncontrolled consumption of these antibiotics in Bangladesh²⁶.

In the present study, among the isolated *K. pneumoniae*, 90.67% were resistant to ceftriaxone. This observation is nearly closer to other studies reporting 92.5%, 97.43% and as high as 100% ceftriaxone resistance^{23,27,21}.

In the present study, among the isolated *K. pneumoniae*, 37.33% were imipenem resistant, identified by disc diffusion test. A study in India reported that *K. pneumoniae* isolated from different clinical samples were 32% resistant to imipenem²⁸. Previous studies in DMCH reported 26.32% and 34.61% imipenem resistance among *K. pneumoniae*^{29,30}. The frequency of imipenem resistant *K. pneumoniae* is increasing in Bangladesh which is reflected by these studies. Dissemination of *K. pneumoniae* carbapenemase (KPC) has led to an increase in the prevalence of carbapenem resistant Enterobacteriaceae (CRE)¹².

In the present study, most of the isolates of *K. pneumoniae* were found susceptible to tigecycline (85.33 %), which is similar to observation of Shawky et al.³¹ who reported 86.2% susceptibility to tigecycline.

Conclusion

K. pneumoniae was found to be the second most common organism causing various infections. Our results suggest that there is a high antibiotic resistance among clinical *K. pneumoniae* isolates towards commonly prescribed antibiotics, which is alarming for developing countries like Bangladesh. Continuous monitoring and strict antimicrobial policy will have a great impact on reducing bacterial resistance towards antibiotics and development of proper treatment options against *K. pneumoniae* infections. As antimicrobial susceptibility vary from time to time and in different geographical areas, more studies should be conducted at intervals at different geographical areas.

Conflicts of Interest: None.

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