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# Antibiotic Susceptibility Pattern of Gram-negative Isolates of Lower Respiratory Tract Infection

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## ABSTRACT

**Background:** Lower respiratory tract infection is a common cause of morbidity and mortality worldwide. A cross-sectional study was carried out with an objective to study the antibiogram of Gram-negative isolates of patients with lower respiratory tract infection visiting Kathmandu Model Hospital.

**Methods:** A total of 274 specimens including sputum, endotracheal aspirates, suction tips were cultured as per standard microbiological technique. Antibiotic susceptibility and detection of Extended-spectrum beta- lactamases (ESBLs) were performed following Clinical Laboratory Standard Institute (CLSI 2014) guidelines.

**Results:** Respiratory pathogens were recovered from 24.6% (n=65) cases. *Klebsiella pneumoniae* (40%) was the commonest isolates. The highest prevalence of multidrug-resistance (69.23%) was observed in *Acinetobacter calcoaceticus baumannii* complex. Extended-spectrum beta- lactamases were detected in Escherichia *coli* (n=4), *Klebsiella pneumoniae* (n=4) and *Acinetobacter calcoaceticus baumannii* complex (n=1).

**Conclusions:** High prevalence of multidrug-resistance and extended- spectrum beta- lactamase producers were observed in respiratory isolates. For effective management of lower respiratory tract infections, an ultimate and detailed microbiological diagnosis and susceptibility testing is required.

**Keywords:** Extended -spectrum β-lactamase; multidrug-resistance.

## INTRODUCTION

Lower respiratory tract infection (LRTI) is considered as one of the major public health problems and a leading cause of morbidity and mortality in many developing countries.[1-3] It is a global problem accounting for over 50 million deaths each year and occurs in both community and health care settings.[4] In developing countries, the situation is more complicated, and management is often difficult due to the problem associated with the identification of the etiological agents and the administration of an appropriate treatment in cases requiring antibiotic therapy.[5] Gram-positive bacteria such as, *Staphylococcus aureus*, *Streptococcus pneumoniae* and Gram-negative bacteria such as *Haemophilus influenzae*, *Pseudomonas* spp, *Acinetobacter* spp., *Klebsiella* spp. have been recovered from LRTIs.[6] This study was conducted with the aim of studying the Gram-negative etiological agents causing LRTI in patients of all age groups and their antibiogram with special interest to MDR in Kathmandu Model Hospital.

## METHODS

This was a hospital based cross-sectional study which was conducted at Kathmandu Model Hospital, Kathmandu from the month of January 2016 to June 2016. A total of 274 specimens from the patients of suspected lower respiratory tract infection were included in the study. Lower respiratory tract (LRT) representing specimens, viz., sputum, endotracheal (ET) secretion and bronchial washings received for culture and sensitivity which met the criteria as recommended by American Society for Microbiology (ASM) were included in the study.[7]

The digested samples were cultured on Chocolate agar (CHA), 5% Sheep Blood agar (BA) and MacConkey agar (MA) plates. The CHA and BA plates were incubated in CO2 incubator (10% $CO_2$) at 37$^0$C for 24 hours while MA plates were incubated at 37$^0$C for 24 hours in aerobic atmosphere.

All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures.[7]

All the respiratory isolates were tested for antibiotic susceptibility by modified Kirby Bauer disc diffusion method in compliance with CLSI 2014 guidelines on Mueller Hinton agar plates using amoxicillin (10 ug),

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cephotaxime (30 ug), ceftriaxone (30 ug), ciprofloxacin (5 ug), ofloxacin (5 ug), chloramphenicol (30 ug), cotrimoxazole (25 ug), chloramphenicol (30 ug), gentamycin (10 ug ) and azithromycin (30 ug). Isolates were labeled as MDR if they were resistant to at least two classes of first line agents. The confirmation of ESBL was done by combination disc method in which ceftazidime (CAZ) and cefotaxime (CTX) alone and in combination with clavulanic acid (CA) (10µg) was used. An increased zone of inhibition (ZOI) of ≥ 5 mm for either antimicrobial agent in combination with CA versus its zone when tested alone confirmed ESBL.[8]

## RESULTS

A total of 274 specimens from patients with LRT were processed according to the standard microbiological methods. Specimens processed in this study include Sputum (n=264), ET secretion (n=6) and Suction tip (n=4). Among 264 sputum specimens, 254 were further processed while the remaining 10 specimens were rejected as they were found to be oral contamination. Among the total processed specimen (N=264), 65 (24.6%) showed significant growth (Table 1).

**Table 1. Pattern of bacteria isolation in different Lower respiratory tract specimens (N=264).**

| Specimen | Significant growth | No growth |
|---|---|---|
| | **No. %** | **No. %** |
| Sputum(n=254) | 56(22.04) | 198 (77.95) |
| Endotracheal secretion (n=6) | 5(83.33) | 1(16.66) |
| Suction Tip (n=4) | 4(100) | 0(0) |

Out of 65 microbial growth, there was polymicrobial growth (growth of more than one microbe) in 10 specimens (15.36%) while monomicrobial growth was seen in 55 (84.6%). Most of the isolates (63.07%) were obtained from samples of in-patients and 36.92% of isolates were from out-patients.

Eight different bacteria were isolated. Among the 75 bacteria isolates, *K. pneumonia*e (n=30, 40.00%) was found most predominant organism followed by *Pseudomonas* spp (n=16, 21.33%), *Acinetobacter* spp (n=13, 17.33%), *E. coli* (n=11, 14.66%), *C. freundii* (n=2, 2.66%), *C. koseri* (n=1, 1.33%), *Enterobacter* spp (n=1, 1.33%) and *K. oxytoca* (n=1, 1.33%). Distribution of ESBL and MBL in MDR *E .coli* is shown in Table 2.

**Table 2. Distribution of microbial isolates from LRTI (N=75).**

| Organism | Number of cases (n) | % amongst total isolates |
|---|---|---|
| *K. pneumonia* | 30 | 40.00 |
| *P. aeruginosa* | 16 | 21.33 |
| *E. coli* | 11 | 14.66 |
| *ACBC*** | 13 | 17.33 |
| *C. freundii* | 2 | 2.66 |
| *C. koseri* | 1 | 1.33 |
| *K. oxytoca* | 1 | 1.33 |
| *Enterobacter* spp | 1 | 1.33 |

** *Acinetobacter calcoaceticus baumannii* complex

*K. pneumoniae* showed 50% (15/30) sensitivity toward gentamicin, 46.7 % (14/30) sensitivity toward ciprofloxacin, 36.7 % (11/30) toward cefixime. Antibiotic least effective were cotrimoxazole, cefotaxime 33.3% (10/30) each and amoxicillin clavulanic acid 3.3% (1/30). *K. pneumoniae* showed 100% (0/30) resistant toward amoxycillin as shown in the Table 3.

**Table 3. Antibiogram of *K. pneumoniae* (N=30).**

| Antibiotics | Antibiotic susceptibility pattern | | |
|---|---|---|---|
| | Sensitive | Resistant | Sensitivity% |
| Amoxycillin | 0 | 30 | 0% |
| Amoxycillin-clavulanic acid | 1 | 29 | 3.3% |
| Cotrimoxazole | 10 | 20 | 33.3% |
| Cefixime | 11 | 19 | 36.7% |
| Cefotaxime | 10 | 20 | 33.33% |
| Ciprofloxacin | 14 | 16 | 46.7% |
| Gentamicin | 15 | 15 | 50% |
| Azithromycin | 11 | 17 | 43.3% |

*E. coli* are more susceptible to gentamicin, cotrimoxazole and ciprofloxacin 36.4% (4/11) equally followed by cefixime, cefotaxime 27.3% (3/11) and amoxycillin, amoxycillin-clavulanic acid and azithromycin 18.2% (2/11). (Table 4)

*Acinetobacter calcoaceticus baumannii* complex showed more susceptibility toward the cotrimoxazole and gentamicin 30.8% (4/13) followed by azithromycin 23.0% (3/13); whereas amoxycillin-clavulanic acid, cefotaxime, and ciprofloxacin demonstrated 15.4% (2/13) sensitivity each. Amoxycillin and cefixime are least susceptible with 7.7% (1/13) senstivity each. (Table 5)

**Table 4. Antibiogram of *E. coli* (N=11).**

| Antibiotics | Antibiotic susceptibility pattern | | |
|---|---|---|---|
| | Sensitive | Resistant | Sensitivity% |
| Amoxycillin | 2 | 9 | 18.2% |

| | | | |
|---|---|---|---|
| Amoxycillin -clavulanic acid | 2 | 9 | 18.2% |
| Cotrimoxazole | 4 | 7 | 36.4% |
| Cefixime | 3 | 8 | 27.3% |
| Cefotaxime | 3 | 8 | 27.3% |
| Ciprofloxacin | 4 | 7 | 36.4% |
| Gentamicin | 4 | 7 | 36.4% |
| Azithromycin | 2 | 9 | 18.2% |

*Pseudomonas aeruginosa* showed 93.7% (15/16) sensitivity toward amikacin followed by gentamicin 87.5% (14/16), ofloxacin 75% (12/16), piperacillin-tazobactam 81.25%(13/16), ciprofloxacin 75% (12/16) and levofloxacin 75%(14/16). Ceftazidime 37.5%(6/16) and piperacillin 25% (4/16) were the least effective to *Pseudomonas aeruginosa*. (Table 6)

**Table 5. Antibiogram of Acinetobacter calcoaceticus baumannii complex (N=13).**

| Name of antibiotic used | Antibiotic susceptibility pattern | | |
|---|---|---|---|
| | Sensitive | Resistant | Sensitivity% |
| Amoxycillin | 1 | 12 | 7.7% |
| Amoxycillin -clavulanic acid | 2 | 11 | 15.4% |
| Cotrimoxazole | 4 | 9 | 30.8% |
| Cefixime | 1 | 12 | 7.69% |
| Cefotaxime | 2 | 11 | 15.4% |
| Ciprofloxacin | 2 | 11 | 15.4% |
| Gentamicin | 4 | 9 | 30.8% |
| Azithromycin | 3 | 10 | 23.0% |

**Table 6. Antibiotic sensitivity pattern of *P. aeruginosa* (N=16).**

| Antibiotics | Antibiotic susceptibility pattern | | |
|---|---|---|---|
| | Sensitive | Resistant | Sensitivity% |
| Piperacillin | 4 | 12 | 25% |
| Ceftazidime | 6 | 10 | 37.5% |
| Ciprofloxacin | 12 | 4 | 75% |
| Gentamicin | 14 | 2 | 87.5% |
| Cefipime | 11 | 5 | 68.75% |
| Amikacin | 14 | 1 | 93.75% |
| Piperacillin-tazobactem | 13 | 3 | 81.25% |
| Ofloxacin | 12 | 4 | 75.0% |
| Levofloxacin | 11 | 4 | 68.75% |

The data showed that *Acinetobacter calcoaceticus baumannii* complex 69.23% (9/13), *E. coli* 54.54% (6/11), *K. pneumoniae* 50% (15/30) and *Pseudomonas aeruginosa* 12.5% (2/16) were found to be MDR. (Table 7)

**Table 7. Distribution of MDR isolates.**

| S.No | Pathogens | No.(%)of bacteria | No.(% ) of MDR |
|---|---|---|---|
| 1 | *K. pneumoniae* | 30(40%) | 15(50%) |
| 2 | *EE. coli* | 11(14.66%) | 6(54.54%) |
| 3 | *ACBC*** | 13(17.33%) | 9(69.23%) |
| 4 | *Pseudomonas aeruginosa* | 16(21.33%) | 2(12.5%) |
| 5 | *Citrobacter freundii* | 2(2.66%) | 0(0%) |
| 6 | *K. oxytoca* | 1(1.33%) | 0(0%) |
| 7 | *Citrobacter koseri* | 1(1.33%) | 0(0%) |
| 8 | *Enterobacter* species | 1(1.33%) | 0(0%) |

** *Acinetobacter calcoaceticus baumannii* complex

Among 6 MDR isolates of *E. coli*, ESBL was detected in 4 (66.67%), among 15 MDR isolates of *K. pneumoniae*, ESBL was detected in 4 (26.66%) and of 9 MDR isolates of *Acinetobacter calcoaceticus baumannii* complex, ESBL was detected in 1(11.11%). All ESBL producing isolates are MDR. The result is shown in Table 8.

**Table 8. Distribution of ESBL isolates.**

| SN | Pathogens | Total Isolates No. | MDR No. | ESBL Isolates No (%) |
|---|---|---|---|---|
| 1 | *K. pneumoniae* | 30 | 15 | 4(26.66%) |
| 2 | *E. coli* | 11 | 6 | 4(66.67%) |
| 3 | *ACBC*** | 13 | 9 | 1(11.11%) |
| 4 | *P. aeruginosa* | 16 | 2 | 0(0%) |
| 5 | Total | 70 | 32 | 9(28.125%) |

** Acinetobacter calcoaceticus baumannii complex

## DISCUSSION

This study was undertaken to find out the bacteriological profile and the sensitivity pattern of the isolates. Growth of pathogens was obtained in 65/264 (24.62%). Culture positivity depends on the nature of specimen, transportation time and the number of organism present in the sample. In comparison to other studies,[9,10] the yield of sputum culture in this study is low (24.66%) but similar to studies done by Egbe et al and Ahmed et al.[11,12] The lower isolation of pathogens in the present study might be attributed to several potential factors in comparison to other studies. The natural infection in patients may have already been changed by the use of antibiotics by healthcare providers at different level of care before patients reach to hospitals. Major cause of culture negativity in lower respiratory tract infections

might be the prior use of antibiotics.[13] Viruses like adenovirus, respiratory syncytial virus, parainfluenza virus and rhino virus, which are significant contributors of LRTI, were not looked for in our study due to limitation of resources. Likewise, common pulmonary pathogens such as *Mycobacterium tuberculosis*, Mycoplasma, Chlamydia, Pneumocystis, Fungi, Legionella, and anaerobes could not be cultured by routine methods.

Eight different bacteria were isolated, giving the growth rate of 24.62%. The bacteria isolated from the samples included *K. pneumonia* (40%), *P. aeruginosa* (21.33%), *E. coli* (14.66%), *Acinetobacter calcoaceticus baumannii* complex (17.33%), *C. freundii* (2.66%), *K. oxytoca* (1.33%), *C. koseri* (1.33%) and *Enterobacter spp* (1.33%). Pathogens were obtained from 63.07% of samples in case of in-patients and 36.92% in out-patients. *K. pneumoniae* (40.82%) was the most common pathogen isolated from in-patients, whereas *P. aeruginosa* (26.54%) was the second predominant organism in hospitalized patients. This study was very much related to the similar study carried by Ahmed et al.[12] The isolation of *K. pneumoniae* as predominant organism also agrees with other studies carried out elsewhere.[14,15]

The increasing resistance to antibiotics by respiratory pathogens has complicated the use of empirical treatment with traditional agents and a definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management of LRTI.

Quinolones were found to be the most effective antibiotic against the Gram-negative bacteria in this study; most of the isolates were sensitive to the quinolones (ciprofloxacin, ofloxacin), but resistant to amoxycillin, cotrimoxazole and cefotaxime. The data showed that *Acinetobacter calcoaceticus baumannii* complex (69.23%), *E. coli* (54.54%), *K. pneumoniae* (50%) and *P. aeruginosa* (12.5%) were frequent MDR isolates. The pattern of antibiotic resistance recorded in this study among *P. aeruginosa*, *K. pneumoniae* and *E. coli* isolates is correlated well with the results obtained from Kumari et al.[16] and Gauchan et al.[17] The decreased susceptibility of Gram-negative isolates towards third generation cephalosporins (5-40%) could be attributed to ESBL production. ESBL production was the most common among *E. coli* (36.36%) followed by *Klebsiella pneumoniae* (13.33%). But in a study by Pokhrel et al , *K. pneumoniae* (65%) and *E. coli* (70%) were ESBL producers.[18] This is probably because their study was based on infections in intensive care unit, whereas the present study included both community acquired and nosocomial infections. The importance of ESBL producing strains lies in the fact that they are difficult to treat because they carry plasmids that confer resistance to many other antibiotics.

## CONCLUSIONS

High prevalence of multidrug-resistance and extended-spectrum beta- lactamase producers were observed in respiratory isolates. For effective management of lower respiratory tract infections, an ultimate and detailed microbiological diagnosis and susceptibility testing is required. Thus, longitudinal surveillance program, institution of infection control practices and rational use of antibiotics are highly recommended to reduce the infection rate and limit the spread of resistance.

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