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# Isolation and Identification of Bacteria Associated with Lower Respiratory Tract Infection among Patients Attending General Hospital Katsina

***Usman, A.D.[1] and Muhammad Amina[2]**
1. *Department of microbiology, Bayero University, Kano. P.M.B. 3011, Kano, Nigeria.ualiyu@gmail.com
2. Department of Microbiology, Umaru Musa Yar'adua University, Katsina.

**Abstract**
Lower respiratory tract infections (LRTIs) have been one of the major causes of morbidity and mortality worldwide. Routine diagnosis of LRTIs in our hospitals does not include adequate and extensive identification of these organisms. This study was carried out to isolate and identify some of the bacteria associated with LRTIs and their common antibiotic sensitivity pattern. *Streptococcus pneumonia*e was screened by Optochin disc sensitivity testing in all the samples collected. A total of 35 (41.18%) bacterial pathogens were isolated from the 85 samples collected from General Hospital Katsina. *Klebsiella pneumoniae* (34.29%) has the highest percentage of occurrence, followed by *Staphylococcus aureus* (31.43%), *Pseudomonas aeruginosa* (25.71%) and *Escherichia coli* (8.57%). *Streptococcus pneumoniae* was not isolated in this study. Patients in age groups 21 – 30 years and 31 – 40 years had the highest percentage of occurrence. Gentamycin and fluoroquinolones showed higher activity on the bacterial isolates and are therefore essential in the treatment of LRTIs.
**Key Words:** Lower respiratory tract, Infections, Pneumonia, Antibiotics

## INTRODUCTION

Respiratory tract infections are divided into infections of the upper respiratory tract, involving the ears, throat, nasal sinuses and the trachea, and the lower respiratory tract (LRT), where they affect the airways, lungs and pleura (Denyer *et al.*, 2011). Respiratory tract infections are the most frequently reported of all human infections, which are mostly mild, transient lasting and sometimes self-limiting (Taura *et al.,* 2013).Lower respiratory tract infections (LRTIs) are among the most common infectious diseases affecting humans world-wide (Carroll, 2002). They are important causes of morbidity and mortality for all age groups, and each year approximately seven million people die as a direct consequence of acute and chronic respiratory infections (Ozyilmaz *et al.,* 2005). In Africa, lower respiratory tract infection and tuberculosis are ranked second and eighth leading cause of mortality rate respectively where as in Nigeria, lower respiratory tract infections constituted the second leading cause of mortality rate in all age brackets in 2002, a year in which tuberculosis was the seventh leading cause of death, accounting for 4% of all deaths (Umoh *et al.,* 2013).

In African countries, the situation is more complicated and management is often difficult due to the problem associated with the identification of the etiological agents and administration of appropriate treatment in cases requiring antibiotic therapy (Alter *et al.,* 2011).

In the developing world, the term lower respiratory tract infection (LRTI) is widely used instead of pneumonia, because of poor access to x-ray and difficulties in radiological confirmation of diagnosis (Wojsyk-Banaszak and Bręborowicz, 2013).

Current knowledge of the organisms that cause LRTIs and their antibiotic susceptibility profiles are therefore necessary for the prescription of appropriate therapy (Egbe *et al.,* 2011). Changes in the characteristics of the population as it ages and the swelling numbers of patients with immunocompromising conditions have increased the number of individuals at risk (Carroll, 2002). The clinical laboratory plays a vital role in the diagnosis of these infections with lots of challenges due to the complexity of LRTIs, including quality of specimen and diversity; contamination of specimens with oropharyngeal flora; a diverse pathogen population that includes bacteria, viruses and fungi; and the complex pathophysiology of respiratory tract infections (Campbell and Forbes, 2011). There are numerous organisms causing LRTI including bacteria, fungi and viruses.

Common aetiological agents for LRTI's include *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilusin fluenzae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydia pneumoniae* that are opportunistic bacterial pathogens (Ramana *et al.,* 2013). To ensure appropriate therapy in LRTI, up to date knowledge of the organisms that cause LRTI and their antibiotic susceptibility patterns is necessary.

Age, gender, and season are factors that have been implicated to affect the prevalence of LRTIs. However, there are few studies reported in literature regarding the aetiology and antimicrobial susceptibility patterns of the LRTI's from this particular geographical region. Routine diagnosis of lower respiratory tract infections in our hospitals does not include adequate and extensive identification of these organisms. It is often missed or misdiagnosed due to the procedures involved. The aim of this study was to establish cases of bacteria associated with LRTI in General Hospital Katsina with the view to identify the bacteriaas well as to assess their antibiotic susceptibility pattern.

## MATERIALS AND METHODS

### Study Area

This study was conducted in General Hospital, Katsina state between September to November, 2016. The population studied, was a heterogeneous population of different age groups, with the minimum age of 10 years. Patients with pulmonary tuberculosis and HIV/AIDs were excluded in this study.

### Sample Collection and processing

Early morning sputum specimens were aseptically collected in to appropriate sterile containers from 85 patients attending General Hospital Katsina. Sample size was attained using OpenEpi statistical software,version 2.3, using a prevalence of 4% (Usman, 2013). Each container was clearly labelled with sex and age of the patient and transported to the laboratory.

### Microbiological analysis

The samples were cultured on blood agar and MacConkey agar plates. The plates were then incubated at 35$^{o}$C for 24hours. Colonies from blood agar were subcultured on Mannitol Salt Agar for detection of *Staphylococcusaureus*. Gram-staining was carried out in order to differentiate the bacteria into gram – positive and gram – negative (Cheesbrough, 2006)

### Identification

Organisms isolated were identified through various biochemical tests. These include optochin test for detection of *Streptococcus pneumoniae*, catalase test, coagulase, oxidase, citrate, urease, indole, methyl-red and Voges-Proskauer test.

### Antibiotic susceptibility testing

The confirmed pathogens were then screened for antibiotic susceptibility using the Kirby-Bauer modified disc diffusion technique, using standard antibiotic discs (Cheesbrough, 2006).

## Results

Out of the 85 patients screened, a total of 35 (41.18%) yielded clinically significant pathogens. Table 1 summarises the number and percentage of occurrence of the bacteria isolated. *Klebsiella pneumoniae* (34.29%) was the predominant isolate detected, followed by *Staphylococcus aureus* (31.43%), *Pseudomonas aeruginosa* (25.71%) and *Escherichia coli* (8.57%). There was no *Streptococcus pneumoniae* isolated.

Out of the 85 patients screened, 53 (62.35%) were females and 32 (37.65%) were males. The occurrence in females (60%) is higher than the occurrence in males (40%). This is summarized in Table 2.

Patients in age group 21-30 years have the highest percentage of occurrence (34.29%) followed by 31-40 years (22.86%). Patients within the age group 51-60 have the least percentage of occurrences. Age groups 11-20, 41-50, and 61 above has 8.57%, 17.14% and 11.43% of occurrence respectively (Table 3).

The susceptibility profile of gram-negative bacterial isolates is shown in Table 4. The fluoroquinolones (pefloxacin, sparfloxacin, ofloxacin) and gentamycin showed very high activity on all the bacterial isolates. Ciprofloxacin showed a relatively high activity. Chloramphenicol showed moderate susceptibility. Amoxicillin showed moderate activity on *K. pneumoniae*, low on *P. aeruginosa* and no activity on *E. coli*. Streptomycin and septrinshowed low susceptibility while Augmentin showed very little or no activity against the bacterial isolates.

Table 5 summarizes the susceptibility profile of gram-positive isolate. Gentamycin, fluoroqinolones (ciprofloxacin and pefloxacin) and streptomcin showed high susceptibility. *S. aureus* is moderately susceptible to rocephine. Erythromycin, amoxicillin, Zinnacef and septrin showed low antimicrobial activity. *S. aureus* is resistance to Ampiclox.

**Table 1: Bacterial Pathogens Isolated from the Patients**

| Pathogens Isolated | No. of isolates | % of Occurrence |
|---|---|---|
| *Klebsiella pneumoniae* | 12 | 34.29 |
| *Staphylococcus aureus* | 11 | 31.43 |
| *Pseudomonas aeruginosa* | 9 | 25.71 |
| *Escherichia coli* | 3 | 8.57 |
| *Streptococcus pneumoniae* | 0 | 0 |
| **Total** | **35** | **100** |

**Table 2: Occurrence of LRTIs Based on Gender**

| Gender | No. of patients examined (%) | No. of patients with pathogens (%) |
|---|---|---|
| Males | 32 (37.65) | 14 (40.0) |
| Females | 53 (62.35) | 21 (60.0) |
| **Total** | **85 (100)** | **35 (100)** |

**Table 3: Occurrence of LRTIs Based on Age**

| Age group | No. of patients examined | No. of patients with pathogens (%) |
|---|---|---|
| 11-20 | 13 | 3 (8.57) |
| 21-30 | 28 | 12 (34.29) |
| 31-40 | 15 | 8 (22.86) |
| 41-50 | 11 | 6 (17.14) |
| 51-60 | 7 | 2 (5.71) |
| ≥ 61 | 11 | 4 (11.43) |
| **Total** | **85** | **35 (100)** |

**Table 4: Susceptibility Profiles of Gram-negative Bacterial Isolates from Lower Respiratory Tract Infections.**

| | Bacterial isolates | | |
|---|---|---|---|
| Antibacterial Agents | *Klebsiella pneumoniae* (n=12) (%) | *Pseudomonas aeruginosa*(n=9) (%) | *Escherichia coli* (n=3) (%) |
| AU | 0 (0) | 1 (11.1) | 0 (0.0) |
| CN | 12 (100) | 7 (77.8) | 3 (100) |
| PEF | 12 (100) | 9 (100) | 3 (100) |
| OFX | 12 (100) | 9 (100) | 3 (100) |
| S | 3 (25.0) | 3 (33.3) | 1 (33.3) |
| SXT | 2 (16.7) | 3 (33.3) | 2 (66.7) |
| CH | 6 (50.0) | 5 (55.6) | 1 (33.3) |
| SP | 12 (100) | 9 (100) | 3 (100) |
| CPX | 9 (75.0) | 7 (77.8) | 2 (66.7) |
| AM | 6 (50.0) | 3 (33.3) | 0 (0.0) |

Data are expressed in number of susceptible isolates (percentage susceptibility).
P > 0.05 – No significant difference between the various antibiotics used and the test organisms ( $X^2$= 6.232, P = 0.9952)
Key: AU = Augmentin, CN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin,S =Streptomycin, SXT = Septrin, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, AM = Amoxicillin

**Table 5: Susceptibility Profiles of Gram-positive Bacterial Isolatefrom Lower Respiratory Tract Infection.**

| Organism | Antimicrobial Agents | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus (n=11)* | CN | S | SXT | CPX | AM | E | APX | Z | R | PEF |
| | 9 (83.3) | 9 (83.3) | 2 (18.2) | 11 (100) | 4 (36.4) | 5 (45.5) | 0 (0.0) | 4 (36.4) | 7 (63.6) | 9 (83.3) |

Key: CN = Gentamycin, S = Streptomycin, SXT = Septrin, CPX = Ciproflaxacin, AM = Amoxicillin, E = Erythromycin, APX = Ampiclox, Z = Zinnacef, R = Rocephine, PEF = Pefloxacin.

**DISCUSSION**

Out of the 85 patients screened, a total of 35 (41.18%) yielded clinically significant pathogens. This occurrence is higher than the figures in previous studies in Nigeria: 21.5% in Kano (Taura *et al.,* 2013) and 18.91% in Benin City (Egbe *et al.,* 2011). It is however similar with the findings of Egbagbe and Mordi (2006) in Benin City which is 47.2%. The bacterial pathogens isolated are similar to that of the work of Vijay and Dalela (2016) in Jhalawar, India. There was no single isolate of *Streptococcus pneumoniae* in this study. It is difficult to offer a reasonable explanation for the inability to isolate *Streptococcus pneumoniae* in this study. This may partly be due to the inability to incubate the blood agar plate in 10% $CO_2$.*K.pneumoniae* was the most predominant pathogen isolated from patients with LRTIs. This is in agreement with previous studies (Egbagbe and Mordi, 2006; Egbe *et al.,* 2011; Vijay and Dalela, 2016). However, the study of Taura *et al*. (2013) reported *S.pneumoniae* as the most predominant pathogen (25.6%), followed by *Klebsiella pneumoniae* (20.9%). Also, the study of Egbagbe and Mordi (2006) reported the absence of *Streptococcus pneumoniae*, with *Staphylococcus aureus* as the second most leading pathogen. The study of Egbe *et al*. (2011) in Benin City, Nigeria, also showed a very low prevalence of *S.pneumoniae* (1.97%).

Previous reports had also indicated higher prevalence of LRTIs in men than in women (Taura *et al.,* 2013; Vijay and Dalela, 2016). However, in this study women have higher prevalence of LRTIs (60%) than men (40%). This is in accordance with the work of El-Mahmoud *et al*. (2010) in Yola and Egbagbe and Mordi (2006).

Patients in the age group ranging from 21-30 years reported the highest number of occurrence 12(34.29%) followed by 31-40 years 8(22.86%). The high occurrence of pathogens reported among patients ranging 21-30 and 31-40 years was probably due to constant exposure to agents responsible for LRTIs as they are the working group individuals, as well as excessive stress which may weaken the immune system. The work of Taura *et al*. (2013) conforms to this study with high occurrence within the age group 20-29 and 30-39 respectively. This study however did not agree with the findings of Panda *et al*. (2012), in which they recorded higher occurrence of *K. pneumoniae* among patients ranging from 51-60 and 60-70 years.

In this study, most of the isolates are highly susceptible to the Sparfloxacin, Ofloxacin, Pefloxacin, Ciprofloxacin and Gentamycin. However, *K. pneumoniae* and *P. aeruginosa* are moderately sensitive to Chloramphenicol (55.6%).Indeed Augmentin is one of the commonly administered antibiotics in Nigeria, which possibly lead to the increased transfer of the antibiotic resistance gene among majority of the organisms.

The gram-positive isolate in table 5 is also highly susceptible to the fluoroquinolones and gentamycin.Ampiclox showed 100% resistance. This indeed may be due to extensive use and misuse of the drug. In a similar study in Kano, some *S.aureus*were resistant to at least one antibiotic (Taura *et al*., 2013).

**Conclusion**

An overall prevalence of 41.18% of LRTIs was observed in this study. *Klebsiella pneumoniae* is the leading causes of LRTIs in this study area. Females are more subjected to lower respiratory tract infections than males. Patients of 21 to 30 years have higher occurrence of LRTI compared to other age groups. Gentamycin and especially the third and fourth generation fluoroquinolones were the most active antibacterial agents. This will be helpful for empiric therapy in our setting, and especially in some notoriously resistance pathogens such as *Pseudomonas aeruginosa*.

**Recommendations**

This study is only limited to some bacterial agents of LRTIs. Studies on other aetiologic agents such as viruses should be conducted. More research on LRTIs should be performed as there are very few studies conducted in Nigeria. Hospitals should employ the methods of thorough identification of aetiologic agents of LRTIs and not limited to only culture and morphological identification. Rampant and extensive administration of antibiotics should be stopped. This will help in tackling the danger of drug resistance.

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