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Bacterial Pathogens of Lower Respiratory Tract in University of Ilorin Teaching Hospital, Ilorin, Nigeria

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Abstract: *This study investigated the bacterial pathogens of lower respiratory tract (LRTI), as well as the susceptibility pattern of the bacterial strains isolated in University of Ilorin Teaching Hospital (UITH) Ilorin, Nigeria. The study was carried out between December, 2013 and February 2014. Sputum specimens were collected from patients and cultured on Blood agar, Chocolate agar and Mac Conkey agar media. Bacterial isolates were identified by Gram staining and biochemical tests. Antimicrobial susceptibility test was performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Out of 103 sputum specimens studied, 16 bacterial species were isolated, giving the overall prevalence of 15.53%. This consisted of 62.50% from male patients and 37.50% from female patients. The bacteria isolated include Klebsiella pneumoniae (81.25%), Pseudomonas aeruginosa (12.50%) and Klebsiella oxytoca (6.25%) in order of ranking. Thirteen 13 (81.25%) isolates were 92.31% susceptible to Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine and fifteen 15 (93.75%) isolates were 87.2% susceptible to Gentamycin, Ceftazidine and Piperacillin. Klebsiella pneumoniae was the most susceptible amongst the isolates. Klebsiella oxytoca displayed the highest number of resistance (83.3%) to most of the antibiotics tested except Augumentin in which, it was moderately resistant. Klebsiella pneumoniae was the most commonly recovered organism from patients with lower respiratory tract infection in this centre. Resistance to all tested antibiotics by Klebsiella oxytoca as recorded in this study is of clinical significance, with associated possible treatment failure. On the other hand, Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine remain useful agents in the management of LRTI in this environment if Klebsiella oxytoca is excluded.*

Keywords: *Pathogens, Respiratory tract, Infection, Antimicrobial*

1. INTRODUCTION

Respiratory tract infections are common and perhaps the most frequently reported of all human infections. They are traditionally divided into two: upper respiratory tract infections and lower respiratory tract infections. Most of these infections are mild, transient and sometimes self limiting, while others may be chronic [1]. Lower respiratory tract infections (LRTIs) occur below the level of the larynx, i.e. in the trachea, the bronchi, or in the lung tissue. They include conditions such as tracheitis, bronchitis, bronchiectasis, lung abscess, tuberculosis, pneumonia (World Health Organisation [2].

Lower respiratory tract infections (LRTIs) are among the most common infectious diseases affecting humans worldwide [3] causing 1.6 million deaths annually in adults [4]. In aging adults, the burden of community acquired pneumonia (CAP) is of even greater concern when considering that the number of persons aged >60 years globally is projected to triple, from 673 million in 2005 to 2 billion by 2050. This will be most apparent in developing regions of the world, where this age group is projected to increase from 64% (2005) to 80% (2050) of the total population. The 50 least developed countries will record a more than 200% increase in their populations, from 0.8 billion in 2007 to 1.7 billion by 2050, compared with developed regions, which are projected to remain stable at a population of 1.2 billion [5].

Acute and chronic lower respiratory tract infections pose a considerable health problem worldwide and they are responsible for a substantial clinical and economic burden and the utilization of health care resources. They are also an important cause of morbidity and mortality for all age groups. Each year approximately 7 million people die as a result of direct consequences of acute and chronic LRTI [6]. Acute respiratory infections (ARI) and Tuberculosis were two of the six leading causes of death across all ages [2]. Out of the total acute respiratory diseases, 20–24% of all deaths are accounted for by Lower Respiratory Tract infection [7].

It is notable that, in Nigeria, LRTIs continue to be a major cause of morbidity [8]. Age, gender, and season are factors that have been implicated to affect the prevalence of LRTIs [9]. Respiratory tract infections impose a serious economic burden on society, work places, and frequent prescription of antibiotics by physician to the victims, even when the causative agents of infection are not bacteria [10]. Previous study before now demonstrated that the major bacterial pathogens causing LRTI in Ibadan are: *Klebsiella* species, *Pseudomonas aeruginosa, Haemophilus influenzae* and *Streptococcus pneumoniae* with an estimated prevalence of (38%), (16.7%), (14.7%), (14%) respectively [11]. A study from Benin city showed a prevalence of 52.5% (*Klebsiella pneumoniae)*, 5.1% (*Escherichia coli)*, 6.8% (*Proteus mirabilis)*, 10.2% (*Staphylococcus aureus),* 5.1% (*Pseudomonas aeruginosa)*, 3.4% (*Proteus vulgaris)*, 1.7% (*proteus morgani)* and 1.7% (*Streptococcus pyogenes)* [8]. Another study in Benin City Nigeria also documented that the prevalence of LRTIs increased significantly with age, with the age group of 71 years and older having the highest prevalence of 48.57% [12]. While a study in Abeokuta Ogun state showed the overall prevalence of lower respiratory tract infection of 24.24% [13].

The aetiologic agents of LRTIs may vary from area to area [13&14] hence the susceptibility profile may also differ between geographical locations. Knowing the local susceptibility profile is important, as antimicrobial therapies for LRTIs are frequently empirical and presumptive [13]. Current knowledge of the organisms that cause LRTIs and their antibiotic susceptibility profiles are necessary for the prescription of appropriate therapy. However this study focused on some common non-tuberculous bacterial pathogens of lower respiratory tract infections, such as: *Strepococcus pneumoniae, Haemophilus influenzae, Klebsiella species, Staphylococcus aureus and Pseudomonas aeruginosa* excluding *Mycobacteria* and Atypical bacteria due to the inadequate facilities to handle them in this centre.

The essence of this study, therefore, is to identify the bacteria that are associated with Lower Respiratory Tract Infection in patients attending University of Ilorin Teaching Hospital, with a view to determining their susceptibility to available antibiotics in use in the hospital. The results of this study would act as a guide in the empirical management of lower respiratory tract infections in this region

2. MATERIAL AND METHODS

2.1 Study Population

The study population, were both male and female adult patients who attended the General out Patients Department of the University of Ilorin Teaching Hospital, Ilorin, Nigeria, with the clinical evidence of lower respiratory tract infections, as diagnosed by the attending physicians. These patients did not include those who were on antibiotics about two weeks prior to collection of the specimens.

2.2 Specimen collection

Sputum specimens were collected from adult patients attending UITH, at the phlebotomy centre after being diagnosed by the attending physician. The sputum samples were collected using well-labelled sterile, leak proof, wide mouthed container, with tight fitting cover, which was taken to the laboratory for analysis without delay.

2.3 Bacteriological investigation

The purulent or mucopurulent portion of the sputum specimens were aseptically inoculated onto Blood agar plates, Chocolate agar plates and Mac Conkey agar plates. Blood agar plates and Mac Conkey agar plates were incubated aerobically at 37^{o}C for 24 hours while Chocolate agar plates were incubated in an atmosphere containing extra carbon dioxide (in candle jar). All the bacteria were isolated and identified using morphology, microscopy and biochemical tests according to [2&15] recommendations.

2.4 Antibiotic susceptibility testing

Antimicrobial susceptibility was determined using disc diffusion method, the disc diffusion method that was presented in this study, is a modification of the Kirby Bauer technique that has been carefully standardized by CLSI-M100-S12, (2002) as described by [16] in Manual of Antimicrobial Susceptibility Testing. Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. The colonies was suspended in saline, Then, the inoculums was adjusted to a turbidity equivalent to a 0.5 McFarland standard by placing the tubes in front of a white paper with black lines.

After adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed the excess inoculums from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. The discs were distributed evenly so that they are no closer than 24 mm from center to center. The plates was inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. The plates were then incubated. After 16 to 18 hours of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured,

The organisms were reported as susceptible, intermediate or resistant to the agents that have been tested. For this study the following antibiotics were tested against the isolates as described by the procedures stated above: Augumentin (30 µm), Piperacillin (30 µm), Ceftriaxone (30 µm), Ceftazidine (30 µm), Cefuroxime (30 µm), Gentamycin (30 µm).

3. RESULTS

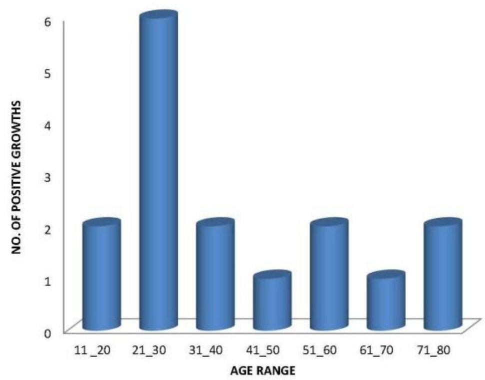

Figure 3.1: *Occurrence of bacterial pathogens in relation to age.*

Table 3.1 Distribution of LRTI by gender

Gender	No. examined (%)	No. with positive growth(%)
Male	54 (52.43%)	10 (62.50%)
Female	49 (47.57%)	6 (37.50)
Total	**103 (100%)**	**16 (100%)**

X^2= 0.3666, p-value=0.4256.

Table 3.2: Distribution of Bacterial agents in LRTI

Bacteria	Males	Females	Total
K. pneumonia	8 (50%)	5 (31.25%)	13 (81.25%)
K. oxytoca	_	1 (6.25%)	1 (6.25%)
P. aeruginosa	2 (12.50%)	_	2 (12.50%)
Total	**10 (62.50%)**	**6 (37.50%)**	**16 (100%)**

X^2=0.2462 P-value= 1.000.

Table 3.3: *Antibiotic susceptibility patterns of the isolated pathogens*

Antibiotic pattern (%)								
Isolates	**No**		**CRO**	**GM**	**CXM**	**AUG**	**CAZ**	**PRL**
K. pneumonia	13	S	12(92.31)	12(92.31)	12(92.31)	1(7.69)	12(92.31)	5(38.46)
		I	0(0)	0(0)	0(0)	4(30.77)	0(0)	2(15.38)
		R	1(7.69)	1(7.69)	1(7.69)	8(61.54)	1(7.69)	6(46.15)
P. aeruginosa	2	S	0(0)	2(100)	NT	NT	2(100)	2(100)
		I	1(50)	0(0)	NT	NT	0(0)	0(0)
		R	1(50)	0(0)	NT	NT	0(0)	0(0)
K. oxytoca	1	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		I	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
		R	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)

Key: CRO – Ceftriaxone, GM – Gentamycin, CXM – Cefuroxime, AUG – Augumentin, CAZ – Ceftazidine, PRL – Piperacillin, NA – Not Tested, S – Susceptible, I – Intermediate.

4. DISCUSSION

This study has demonstrated that out of 103 sputum samples analyzed, Sixteen (15.53%) of the specimens yielded growth of various bacteria while 87 (84.47%) yielded no growth. This observation is similar to studies carried out by [8; 11; 12; 13& 17] who also reported negative results of 157 (78.5%), 125 (75.76%), 1248 (81.09%) 406 (73%), and 189 (76.21%) respectively. This negative result may be attributed to viral, protozoan, atypical bacteria or other etiologic agents [13]. This finding disagrees with the work of [18; 19] who reported greater number of positive bacterial growth of 232 (92.8%) and 337 (93.6%) respectively. Prevalence of the infection varied with the geographical locations or even region within the same country [20]. Indeed, the study conducted by [19] in Tamil Nadu, India, and [18] conducted the study in the Specialist Hospital, Yola, Adamawa State, Nigeria, signifying geographical differences.

The occurrence of bacterial pathogens varies with age, in this study; age group ranging from 21-30 years reported the highest number of occurrence 6 (37.5%) followed by 11-20 years, 31-40 years,51-60 years and 71-80 years with the prevalence of 2 (12.5%). The least age group in terms of occurrence were within the ranges between 41-50 years and 61-70 years with the prevalence of 1(6.25%). The highest prevalence recorded for the age group 21-30 years might be probably due to the fact that, most of the people in these age groups in this community are more exposed to agents responsible for causing respiratory tract infections than the elderly ones. This findings is in agreement with the work of [11&17] who reported the highest occurrence of the bacterial pathogens in this age group with the prevalence of 10 (23.5%) and 40 (26.7%). This report disagrees with the studies of [13&17] in which they reported highest occurrence of bacterial pathogens among the age groups ranging from 31-40 years and 60-70 years respectively.

Previous reports had indicated higher prevalence of lower respiratory tract in women than in men [11; 8] where as studies reported by [12&18] demonstrated that lower respiratory tract infections did not differ significantly between men and women. However In this study, 10 (62.50%) male were positive for LRTI, while 6 (37.50%) female had an established bacterial aetiology of LRTI. This is consistent with the findings of other studies conducted by [13;17&21] who's demonstrated that LRTI were more prevalent in males than in females. According to [17] as reported by [13] the reason for the high risk in males of LRTI is attributed to decreased in local immunity in the respiratory tract due to smoking, use of tobacco, alcohol consumption etc.

Out of the 16 bacterial isolates, Gram negative bacteria accounted for all the isolates (100%). This finding disagree with the earlier studies by [11] among patients attending Diagnostic Microbiology Laboratory of University College Hospital, Ibadan, Nigeria and [13] among patients attending Federal Medical Center Abeokuta, Ogun State, Nigeria, who reported both Gram positive and Gram negative bacteria, but they recorded a high prevalence of Gram negative bacteria accounting for up to (75.4%) and (82.5%) respectively. Among the bacterial isolates *Klebsiella pneumonia* 13 (81.25%) was the most common isolate followed by *Pseudomonas aeruginosa* 2 (12.50%). The finding in this work correlate with the studies of [12;13&11] who reported *Klebsiella pneumonia* as the commonest single pathogen isolated, with the prevalence of 92 (30.16%), 54 (38%) and 28 (70%) respectively. *Pseudomonas aeruginosa* was found to be the second most recovered organism, accounting for up to 2 (12.5%) of the isolates. This is consistent with the findings of [11] in which *Pseudomonas aeruginosa* was reported to be the second most recovered organism after *Klebsiella pneumoniae*. The lung infection with *Pseudomonas aeruginosa* is associated with cystic fibrosis [22]. *Klebsiella oxytoca* is the least isolated organism with the prevalence of 6.25%.

Lower respiratory tract infections are frequently diagnosed and treated on clinical and radiological findings only [23]. But however, in recent years, the increase in the rates of antibiotic resistance amongst the major pathogens has compromised the selection of empirical treatment for some Lower respiratory tract pathogens with traditional agents and a definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management of LRTI [10]. The increasing frequency of antibiotic resistance has been reported first at sites where penetration of the antimicrobial agent is restricted and the level of therapeutic concentrations is consequently more difficult to be achieved [10].

Bacterial pathogens causing LRTIs have a number of virulence mechanisms including the production of β-lactamases and exchange of resistance markers like plasmids and transposons [10&23]. Recognition of these resistance mechanisms allows them to be targeted, such as with β-lactamase inhibitors. The susceptibility tests in this study demonstrated that the isolates were resistant to one or more antibiotics, although generally a high percentage of the isolates were sensitive to most of the antibiotic tested. *Klebsiella pneumoniae* showed the highest sensitivity to Ceftriaxone, Gentamycin, Cefuroxime, Ceftazidine and Piperacillin, this finding is similar to the results obtained from other studies [8&11]. The sensitivity of *Klebsiella pneumoniae* in this study contradicts the study of [19] in which, they reported resistance to some of this antibiotics (Gentamycin, Ceftriaxone and Cefuroxime), this variation may be attributed to differences in the geographical location, and community saturation of these antibiotics. *Klebsiella pneumoniae* isolates were also reported to be intermediately resistant to Augumentin, this agrees with the study of [17] in which, *Klebsiella pneumoniae* was reported to be intermediately resistance to Augumentin. But this result is not consistent with the other studies [12&13] in which, they reported high sensitivity to Augumentin by *Klebsiella pneumoniae*.

Klebsiella oxytoca was reported to be resistant to almost all the antibiotics tested (Ceftriaxone, Gentamycin, Cefuroxime, Ceftazidine and Piperacillin). *Klebsiella oxytoca* is an opportunistic pathogen that causes primarily hospital-acquired infections, most often involving immunocompromised patients or those requiring intensive care. Reported outbreaks have most frequently involved environmental sources [24&25]. *Pseudomonas aeruginosa* was reported to be sensitive to Gentamycin, Ceftazidine and Piperacillin while it was recorded to be resistance to Cefuroxime.

The sensitivity of *Pseudomonas aeruginosa* in this study is similar to the work of [19] except that in their study they recorded resistance to Piperacillin. Also this report disagree with the findings of [13] in which, *Pseudomonas aeruginosa* was recorded to be sensitive to Cefuroxime

4.1 Conclusion

Klebsiella pneumoniae is the most predominant isolated organism. Generally Ceftriaxone, Gentamycin, Cefuroxime, Ceftazidine and Piperacillin would make an important contribution for the effective management of the organisms responsible in lower respiratory tract infection in this centre. Resistance to Augumentin by the respiratory tract pathogens as well as resistance to almost all the antibiotics by *Klebsiella oxytoca* in this study is of clinical significance. This finding brings to light the need for timely and proper diagnosis of the major microbial causes of LRTI, in order to administer the appropriate therapy based on antibiotic susceptibility test of the causative agent.

4.2 Recommendations

Based on the findings of this study, the followings are recommended:

1. Treatment of LRTI caused by *Klebsiella oxytoca* with the tested antibiotics (Ceftriaxone, Gentamycin, Cefuroxime, Augumentin, Ceftazidine and Piperacillin) in this centre should be discouraged, due to the high level of resistance exhibited by this agent to the tested antimicrobials.
2. Further studies should be carried out to determine the reason for multi drug resistance by *Klebsiella oxytoca* in this environment and how to overcome this, so as to improve treatment outcomes, in patients infected by this organism.
3. Government/ NGOs should sponsor research on molecular characterization of the resistance genes associated with bacterial pathogens isolated from persons with LRTI.

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