Original Research Article

A review of microbiologic isolates of adults with lower respiratory tract infection

ABSTRACT

Aims: This study was designed to determine the isolates from microbial cultures and the antibiotics susceptibility pattern of adults with lower respiratory tract infection.

Study design: A retrospective study

Place and Duration of Study: This study was carried out at the Pulmonology units of Medicine department and Microbiology research laboratory unit, Microbiology and Parasitology department, University of Abuja Teaching Hospital (UATH) Gwagwalada, Federal Capital Territory (F.C.T) from August 2015 to September 2018 (Thirty-six months).

Methodology: This was a retrospective study. patients with LRTI who met the inclusion criteria were reviewed. The data were extracted from patients' case notes using well-structured tools. The quality of clinical and laboratory work up were verified by the contribution of specialist Pulmonologist and Microbiologist in the management of patients.

Results: A total of one hundred and ninety-eight sample were reviewed of which fifty-seven percent (112) were males and the mean age of the study populations was 37± 13.8 years. From the positive cultures 86.9% were bacteremia and 4.0% were fungaemia. *Streptococcus pneumoniae* (30.3%) was the most predominant bacteria recovered from the sputum specimens; closely followed by *Klebsiella pneumoniea* and *Staphylococcus aureus.* imipenem and ofloxacin had good susceptibility activity and bacteria eradication rate with susceptibility rate of 92.8% and 92.5% respectively. Erythromycin, Augmentin and ceftriaxone had susceptibility of 66.2%, 89.4% and 90.0% respectively.

Conclusion: *Streptococcus pneumonia*, *Klebsiella pneumoniea* and *Staphylococcus aureus* are the most common bacteria isolated from sputum of patient who presented with LRTI. Quinolone (ofloxacin) and imipinem are the most sensitive antibiotics and should be considered in initiation of empiric antibiotic treatment.

Keywords: lower respiratory tract infection, sputum isolates, antibiotic susceptibility

1. INTRODUCTION

Lower respiratory tract infection (LRTI) is an umbrella name for a wide range of respiratory diseases such as acute bronchitis, pneumonia, lung abscess, and acute exacerbation of chronic lung diseases such as COPD or bronchiectasis. It is a leading cause of morbidity and mortality in adults worldwide,[1] as it accounts for nearly 2.38 million deaths in 2016 making them the leading infectious cause of death and the fifth leading cause of death overall across all ages; they are the second leading cause of disability adjusted life years (DALYs) [1]. It is particularly an important cause of death in low- and middle-income countries [2]. The burden of lower respiratory tract respiratory disease in Nigeria is equally significant, in addition it is a common cause of hospital admission [3].

Sputum is the most common sample collected from patients from lower respiratory tract infections and it is very important for the etiological identification of these infections [4]. On the other hand, identification of the causative agent plays an important role in reducing the microbial resistance to drugs, unnecessary drug cost and avoidable side effects empirical treatment [5,6]. There seem to be a fairly uniform distribution of the common pathogenic organism associated with LRTI across various part of the country, there is paucity of data in this region of the country.

This study was designed to determine the isolates from microbial cultures associated lower respiratory tract infection. Furthermore, the findings from this preliminary survey will guide clinicians with an evidence base medicine on the choice of initiating empirical antibiotic treatment.

2. MATERIAL AND METHODS

Study background

This study was carried out at the Pulmonology units of Medicine department and Microbiology research laboratory unit, Microbiology and Parasitology department, University of Abuja Teaching Hospital (UATH) Gwagwalada, Federal Capital Territory (F.C.T). The Hospital is located in Gwagwalada whose geographical coordinates are 8° 56' 29" North and 7° 5' 31" East. It has an area of 1,043 km². The Federal Capital Territory had a projected population of 1,406,239 inhabitants in the year 2006, of which 157770 (11.22% approximately) inhabitants reside in Gwagwalada [7]. The hospital provides health care services to the inhabitants of Abuja and neighboring states.

Study population

Two hundred and ten folders containing clinical and laboratory records were retrieved and studied. One hundred and ninety-eight (198) were met the inclusion criteria for the study, twelve folders had incomplete record and non-interpretation of laboratory result.

Study design

This was a retrospective study conducted from August 2015 to September 2018 (Thirty-six months).

METHODS

The data were extracted using well-structured questionnaire. The quality of clinical and laboratory work up were verified by the contribution of Consultant Pulmonologist and Microbiologist in the management of patients. The quality of sensitivity pattern involves the use of the same brand product and McFarland standard in order to eliminate bias.

SPECIMEN COLLECTION, TRANSPORTATION AND PROCESSING

From the case file, sputum specimen was collected in a sterile plain container with screw cap. Each specimen received was examined for quality, in terms of amount, sterility and presence or absence of debris. Therefore, sputum collected were documented as mucoid, mucopurulent and purulent and some had blood fleck.

The BACTEC culture bottles were inspected and the top of the culture bottle was cleansed with a sterile swab containing ethanol alcohol. With vacutainer needle, about 5mls of blood was moved into the culture bottle and scanned into the BACTEC chamber to await positive alert. The sample collected were examined for grams reactions and later cultured on Chocolate, Blood, MacConkey and Sabouraud agar plates (for possible fungal growth). After overnight incubation on these agars, the growth characteristics were noted and pure growth was Gram stained. Colonies that were Gram positive were further characterized using the catalase, coagulase and novobiocin disc tests. Those with Gram negative were further characterized using the API^{R} 20 (Oxoid, 211667 Hampshire, UK). Gram negative identification and iMMVPC test (indole, motility, methyl red, Voges-Proskauer and citrate) [8]. Culture plates with positive results were tested for antimicrobial sensitivity using the Kirby-Bauer Diffusion Susceptibility test protocol and the turbidity of the suspensions (both test and controls) were compare to 0.5 McFarland standards. The zone diameter of each antibiotic of control were compared with the CLSI standards, if within the CLSI acceptable limits for quality control strains, then the zone diameter of each antibiotics of the test were compared with CLSI zone diameter breakpoints and was recorded sensitive, intermediate or resistance. Control strains obtained from the records were: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212).

DATA ANALYSIS

The data obtained was coded on entering and analyzed using Epi Info version 3.5.1 package. Confidence interval was 95% and the p value was 0.05. The data was statistically represented in terms of mean, percentage, range and standard deviation. Comparison between different groups was done using Fisher's exact test, Pearson Chi-Square, Student t-test and test of correlation was performed using Spearman correlation test.

3. RESULTS AND DISCUSSION

RESULTS

Out of the 198 patients investigated with lower respiratory tract infections, 180 patients had positive cultures result representing 90.9%. From the positive cultures yields 86.9% were bacteremia and 4.0% were fungaemia.

There were 39 purulent sputum specimens collected within the period representing 19.7% of the total sputum specimens collected. The predominant sputum specimen produced by patients was mucopurulent with 73.7% of the total specimen collected while sputum specimen with blood was the least accounting for 2.0% of the whole sputum collected.

Gram negative bacteria were the most isolated bacteria from the sputum specimens collected for investigation of LRTI with 89 isolates representing 58.1% while the total Gram positive isolated from the sputum specimens were 83 bacteria representing 41.9%. There were no anaerobes isolated in the study and polymicrobial infection was not documented.

Out of the one hundred and ninety-eight sputum specimen analyzed the following organisms were isolated; *Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E.coli), Pseudomonas aeruginosa (P. aeruginosa) and Proteus mirabilis* as Gram negative bacteria. *Staphylococcus aureus (S. aureus) and Streptococcus pneumoniae* as Gram positive bacteria (Table 1). In terms of the isolates, *Streptococcus pneumoniae* (30.3%) was the most predominant bacteria recovered from the sputum specimens; forty-seven colonies of *Klebsiella pneumoniae (K. pneumoniae)* isolates were recovered from sputum specimens and represented 23.7% of the total sputum isolate. *Escherichia coli, P. aeruginosa* and *Proteus mirabilis* isolation rate were 10.1%, 7.6% and 3.5% respectively (Table 1). There were eight Candida species isolated during the period of review and this represents 4.0% of the total isolates.

The rate of susceptibility of bacteria isolates were represented in table 1, imipenem and ofloxacin had good susceptibility activity and bacteria eradication rate with susceptibility rate of 92.8% and 92.5% respectively. Erythromycin, augmentin and ceftriaxone had susceptibility of 66.2%, 89.4% and 90.0% respectively. Six (12.8%) *K. pneumoniae* and five (25.0%) *E. coli* isolates produce extended spectrum beta Lactamases (ESBL) enzymes.

Table 1: general distribution of sputum isolates and antibiogram

Variables	Frequency	Percent (%)
SPECIMEN		
Mucopurulent	146	73.7
Purulent	39	19.7
Mucosalivary	9	4.5
Purulent +Blood	4	2.0
TOTAL	**198**	**100**
ISOLATES		
S. aureus	60	30.3
K. pneumonia	47	23.7
Streptococcus	23	11.6
E. coli	20	10.1
P. aeroginosa	15	7.6
Candida	8	4.0
P. mirabilis	7	3.5
No Pathogen	18	9.1
TOTAL	**198**	**100**
ANTIBIOGRAM		
Ceftriaxone		90.0
Gentamycin		65.5
Ceftazidime		72.8
Augmentin		89.4
Impenem		92.8
Ofloxacine		92.5
Erythromicin		66.2

In terms of age distribution of the organism isolated; In the younger adult's with age range of (10-40years) streptococcus was the predominant organism, closely followed by s. aureus and klebsiella. streptococcus species and staphylococcus

aureus were the major pathogenic organism isolated from the sputum specimen collected for individuals of older age (61-70 years). Seven cases of p. mirabilis (3.5%) were isolated mostly in young age group of 10-40years with men were more affected than women. Results are shown in table 2 below

TABLE 2: Age distribution of the microbiologic sputum isolates

Age	Freq (%)	Strept. pneu(%)	K. pneu (%)	S. aureu (%)	E coli (%)	P aerug. (%)	Candida (%)	P mira (%)	No pathogen (%)
10-20	23(11.6)	4(6.7)	4(8.5)	5(21.7)	2 (10)	1 (6.7)	0 (0)	2	5
21-30	48 (24.2)	16(26.7)	10(21.3)	3(13.0)	4 (20)	6 (40)	2 (25)	2	5
31-40	60 (30.3)	20(33.3)	14(29.8)	8(34.8)	5 (25)	2 (13.3)	3 (37.5)	2	6
41-50	31 (15.7)	8(13.3)	8(17.0)	4(17.4)	3 (15)	5 (33.3)	1 (12.5)	1	1
51-60	27 (13.6)	9(15)	9(19.2)	3(13.1)	3 (15)	1 (6.7)	1 (12.5)	0	1
61-70	9 (4.5)	3(5)	2(4.3)	0(0)	3 (15)	0 (0)	1 (12.5)	0	0
Total	198(100)	60(100)	47(100)	23 (100)	20(100)	15(100)	8(100)	7 (100)	18 (100)

K. pneu= Klebsiella pneumoniae, Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeru), Proteus mirabilis (P. mira). Staphylococcus aureus (S.) and Streptococcus pneumoniae (Strep. pneu)

DISCUSSION

The empirical choice of antibiotics in LRTI depends on the susceptibility pattern in the environment which in turn depends on the bacteria profile of the environment [w]. Sputum infection rate among subjects in this study was 90.9%. This figure varied with findings in Africa [9,10,11] and other parts of the world [12,13,14]. In Africa, the finding of 90.9% in this study was higher than rates of 84.9%% in Ghana [9]. and 61.4% in Cameroon [10]. In the same geographic area, where this study took place, the isolation rate was higher than 47.2% and 18.91% reported in Benin, south-south Nigeria [15,16], 15.53% from Ilorin, north-central Nigeria [17], 24.24% reported in Abeokuta [18], 6.6% and 27% in Ibadan as documented by Okesola and Oni [19] and Okesola and Ige [20] south-west, Nigeria, 21.5% by Taura in Kano, northwest, Nigeria [21] and 81.5% by Iroezindu in south east, Nigeria [11]. Previous study had indicated higher prevalence of

sputum isolation rate [22,23]. These differences may be due to the methodology employed in our study couple with lower sample size, strict adherence to routine laboratory protocol and accessibility to medical services. This study was a retrospective data and there was no clear strict adherence to research methods but for routine laboratory manipulation, although, the quality of specimen was well documented as part of standard operating procedure in processing sputum specimen. 73.7% of the total sputum specimens collected for analysis was mucopurulent. Moreover, data were derived from hospital record which includes community acquired infections and hospital acquired infections. In one of the previous studies conducted in Ibadan 54% of the subjects were outpatients and 46% were hospitalized patients [19]. The use of NALC contributed to the increased isolation rate in our study, although the use of NALC was not stated in other studies.

In this study, bacteria isolates predominate fungi isolates (86.9% Vs 4.0%) This was consistent with previous study [9,10,12,17,19]. There was no isolation of polymicrobial organisms in any patients and this was in agreement with other previous study in Ibadan, Abeokuta and Ilorin but in a review of hospitalized patients in Ghana, candida species were found in higher rate (13.3%) [9] mostly as polymicrobial and in the study in Benin by Egbe and colleagues, candida species were found as mixed infection with klebsiella pneumoniae [16]. The high monomicrobial isolation rate might be due to meticulous processing mechanism and the use of laboratory standard operating procedure in the research laboratory with adequate attention to details which is considered as usual routine laboratory practice.

All the fungal isolates obtained from chest infection were unicellular yeast cell of *C. albicans*. From our study, there were predominant gram-negative bacteria (58.1%) than gram positive bacteria (41.9%), and no anaerobic bacteria was isolated. This finding was similar to previous study in Cameroon, Ghana and Nigeria (Benin, Ilorin and Kano) [9,10,15,17,21] and contrary to reports from Greece and Nicaragua [13,14]. Gram negative bacteria have potent lipid which has the capacity to induce endotoxic sepsis and consequently increased interleukin 11 and tumor necrotic factor. This study has more hospitalized patients which makes the patients susceptible to nosocomial infections. The routine laboratory analysis of sputum using the early morning sample and educating the patients on modalities of collection of quality sputum samples increases the quality of isolation of both gram negative and gram-positive bacteria.

Streptococcus pneumoniae was the most frequently isolated from the sputum specimens with 30.3% of the total isolates. This is in agreement with previous studies, 38.9% in Cameroon, 19.3% in Greece, 17% in Nicaraqua, 47.6% in south east, Nigeria [10,11,13,14]. However, other previous studies reported *klebsiella pneumonia* as the most prevalent isolate, 30.16% and 52.5% reported in Benin [15,16] and 81.25% reported in Ilorin [17]. In this study, *Haemophilus influenzae* was not documented, this was similar to previous study [9,15]. Studies in Benin (17.05%), Greece (9.0%) and Cameroon

(17.6%) isolated *Haemophilus influenzae* [10,14,16]. This difference in the isolation of *H. infuenzae* might be due to need to apply both the X and V factor to the culture media in order to maximizes yield of the bacteria which is not routinely done in the research laboratory. E. coli and *Pseudomonas aeroginosa* were isolated and documented to cause chest infection (10.1% and 7.6%). These bacteria have been documented as etiology for positive sputum culture [9,11,13,15,16,17,18,21]. The respiratory tract is lined with epithelium that promotes colonization of bacteria most especially the virulent forms.

The antibiotics susceptibility of the isolates obtained showed that ceftriaxone, imipenem and Augmentin were very active against most of the isolate. There is increased antibiotics resistance against common antibiotics that were hitherto, used to cure chest infections, antibiotics such as gentamicin which causes nephrotoxicity. Good sensitivity profile to ofloxacin is consistent with previous studies [13,15,17,19,20,21]. Indiscrimate use of antibiotics, irrational administration of antibiotics and poor administration of drugs may be responsible for this increasing antibiotic resistance [22,23].

CONCLUSION

Streptococcus pneumonia, *Klebsiella pneumoniea* and *Staphylococcus aureus* are the most common bacteria isolated from sputum of patient with LRTI. The Quinolone (ofloxacin) and Imipinem are the most sensitive antibiotics and should be considered in the initial phase of antibiotic treatment. However, Standard microscopy, culture and sensitivity remains the golden diagnostic tools for LRTI and empiric antibiotic treatment should be recommended on the case of patient with non-productive sputum sample collection.

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