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Current Trend in the Antibiogram of Bacterial Pathogens of Adult Lower Respiratory Tract Infections in a Nigerian Tertiary Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Lower respiratory tract infections (LRTIs) are among the commonest infectious diseases requiring hospitalization. There is an increasing resistance development of bacterial pathogens of LRTIs to the commonly prescribed antibiotics necessitating regular surveillance for these bacteria and their antibiogram.
Aim: To identify bacterial pathogens of adult LRTIs, determine their antibiotic susceptibility pattern, and suggest the best empirical treatment of adult LRTIs in the setting.
Study Design: Descriptive cross-sectional study.
Methods: A total of 194 respiratory samples from 194 consecutive consenting adult in-patient of a Federal Teaching Hospital were processed. Identification of isolated bacteria and antibiotic susceptibility testing of the isolates were carried out following the standard protocol.
Results: Bacteria isolation was seen in 52.1% of all specimens, highest isolation rate was from sputum (55.2%). Isolation was higher in males (54.9%) than females (48.1%) but no significant

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difference was seen (*P*=0.36). Gram negative bacteria were predominantly isolated (64.4%) and *Klebsilla pneumoniae* was the most common (33.7%). Eight extended-spectrum beta-lactamase (ESBL) producers and 3 methicillin-resistant *Staphylococcus aureus* (MRSA) were also detected. All isolates were sensitive to imipenem and meropenem. All MRSAs were sensitive to vancomycin. There was poor sensitivity pattern seen against most antibiotics tested.

Conclusion: Gram negative bacteria were the predominant bacterial pathogen isolated, and isolates were resistant to most antibiotics tested, though, all were sensitive to carbapenems. Levofloxacin plus gentamicin, and carbapenems were the suggested first and second line empirical treatment of choice respectively for adult LRTIs in this and similar settings.

Keywords: Antibiotic resistance; bacterial isolates; lower respiratory tract infection; respiratory specimen.

1. INTRODUCTION

Infections of the lower respiratory tract, a region from the trachea to the alveoli include pneumonia, emphysema, lung abscess, bronchiolitis, bronchitis, bronchiectasis, lung abscess, and pleural effusion. Acute forms of these infections are among the commonest human infectious diseases globally. Human of all age-groups are affected with associated significant morbidity and mortality [1, 2]. They are a significant contributor to out-patient consultation (6%) and all hospital admission (4.4%). Among adults up to 60 years, lower respiratory tract infections (LRTIs) account for 3%-5% of mortality [3]. Globally, it is estimated that about 4.2 million deaths from acute LRTIs occur among all age groups annually. However, the burden of the diseases is higher in developing countries, where pneumonia is among the most common cause of hospital attendance among adults [4]. The morbidity and mortality arising from these infections varies depending on the underlying etiological agents and their virulence [5]. The incidence and associated mortality due to LRTI can be influenced by several factors including characteristics of the population at risk, standard of the healthcare facilities available, use of immunosuppressive drugs, inappropriate antibiotic therapy, distribution of causative agents, and prevalence of antimicrobial resistance [6].

Each of the different types of LRTIs presents with different epidemiology, clinical presentation, pathogenesis, and outcome [1,4]. Also, the etiology, pathogenesis, clinical presentation and prognosis of each of LRTIs vary with age, sex, season, the type of population at risk and various other factors [7].

The commonly isolated bacteria from various cases of LRTIs include the Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and the Gram-negative bacteria including *Klebsiella* species, *Pseudomonas* species, *Escherichia coli, Acinetobacter* species, and other non-fermentative Gram-negative bacilli (NFGNB) [1,7]. These causative agents of LRTIs vary from one region to another, and also from time to time. Also, the antibiogram of isolated bacteria varies both geographically and from time to time [5].

The emergence of resistance of these bacteria especially the Gram negative isolates to a wide range of commonly prescribed antibiotics has posed a big challenge to the management of LRTIs in our various health facilities with attendant limitation of therapeutic options when faced with such pathogen. This might be connected to the usual initiation of 'inappropriate' antibiotic therapy for suspected cases of LRTIs even before result of culture and antibiotic susceptibility pattern is out. This definitely will increase associated morbidity, duration of treatment, cost of treatment and mortality from these infections.

Bearing this in mind, there is a need for regular surveillance of bacterial pathogen of LRTIs and their antibiotic susceptibility profile to quickly identify such multidrug resistant variants and alert clinicians, suggest empirical antibiotic therapy for LRTIs and help in periodic formulation of antibiotic policy on LRTIs.

There are no antibiotic policies on LRTIs in this center despite regularly caring for patients with these infections, to this end, this study was conducted to identify various bacterial pathogens of LRTIs and their antibiotic susceptibility patterns among adult patients admitted for treatment in our centre with the aim of determining the best empirical treatment for cases of LRTIs in this group.

1.1 Objectives

To identify different bacterial agents associated with different cases of LRTIs at Federal Teaching Hospital, to determine the antibiotic susceptibility pattern of isolated bacteria from cases of LRTIs, and to suggest best empirical antibiotic treatment options for cases of LRTIs among adult patients admitted to the centre.

2. MATERIALS AND METHODS

2.1 Study Design and Hospital Setting

This was a descriptive cross-sectional study conducted at the Department of Medical Microbiology and Parasitology of a tertiary hospital in Southwestern Nigeria. The hospital serves as a referral center to all primary and secondary healthcare facilities in the Southwestern Nigeria. It is a 290 bedded hospital with many modern facilities for healthcare.

2.2 Study Population, Sampling Method

The study was carried out on 194 respiratory tract samples collected from 194 consecutive consenting adult patients with clinically diagnosed cases of LRTIs in all the adult patients' units of the hospital between January 2020 and February 2021. Patients' clinical history and other relevant details were recorded in a predesigned form.

2.3 Specimen Collection and Processing

Three (3) types of respiratory samples; sputum, pleural fluid and endotracheal aspirate (ETA) were collected throughout the study. One sample type was collected from each participant; the sample type depended on the type of patient and the clinical diagnosis. Each sample was aseptically collected in a sterile wide mouth container. The quality of sputum and ETA was assessed based on criteria of American Society for Microbiology (ASM) which asserted that a reliable specimen after gram staining would have more than 25 leucocytes and fewer than 10 epithelial cells per low power field of microscope, only such reliable specimen was expected to yield a 'significant' isolation of pathogen [8]. All sputum and ETA samples that failed to fulfill these criteria were rejected for repeat specimen collection. The undiluted sputum samples were inoculated onto blood agar, chocolate agar and MacConkey agar (Oxoid) plates. Endotracheal aspirates and pleural fluid samples were vortexed for 1 minute and centrifuged at 3000 rpm for 10 minutes, the centrifuged specimens were inoculated onto blood agar (Oxoid), chocolate agar (Oxoid) and MacConkey agar (Oxoid) plates. The inoculated MacConkey and blood agar plates were incubated aerobically while the chocolate agar plates were incubated in the presence of carbon dioxide (CO_2), all at 37°C for 18-24 hours. All isolates from these well assessed and processed specimens were considered as 'significant'. Identification of the isolates was performed by standard microbiological procedures including the study of colony morphology, Gram stain reactions and standard biochemical tests. In addition, *Streptococcus pneumoniae* was further identified by optochin sensitivity test [9].

Antibiotic susceptibility testing for all isolates was performed using modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid) and on blood agar for *Streptococcus pneumoniae*. The results were read and interpreted following the guidelines of Clinical and Laboratory Standard Institute (CLSI) [9]. The following antibiotic discs were tested; in Gram negative bacteria isolates, ampicillin (10µg), amoxycillin-clavulanate (20/10 µg), piperacillin–tazobactam (100/10 µg), gentamicin (10 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), and in Gram positive bacteria; erythromycin (15 µg), penicillin ((10 µg), amoxycillin-clavulanate (20/10 µg), piperacillin-tazobactam (100/10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), cotrimoxazole (25 µg), imipenem (10 µg), merpenem (10 µg), vancomycin ((30 µg). All antibiotic discs were from Oxoid™. Sensitivity of all *S. aureus* including the MRSA isolates to vancomycin was carried out using E-test (BioMeurieux).

All Gram negative bacteria isolates were tested for ESBL production by double disc synergy test using ceftazidime (30 µg) and ceftazidime/clavulanate (30/10 µg) discs (Oxoid). All *S. aureus* and CONS were tested for methicillin-resistance using cefoxitin disk-diffusion method (Oxoid) [10].

2.4 Inclusion and Exclusion Criteria

All consenting patients with clinical diagnosis of LRTI were included in the study while patients

with history of antibiotic usage within 1 week prior to admission, patients with pulmonary tuberculosis and those receiving immunosuppressive drugs were excluded from the study.

2.5 Data Entry and Analysis

Data entry was done by the researchers onto Microsoft Excel 2017, and analysis was done using the Statistical Package for Social Sciences (SPSS) version 20. Results were presented in tables.

3. RESULTS

Of the total 194 participants included in this study, 113 were males and 81 were females. The age-range 36-55 years had the highest number of participants; 48 (42.5%) males and 32 (39.5%) females, and the mean age of the participants was 50.294+16.621. Table 1.

A total of 194 samples were collected from the 194 participants, including 172 (88.7%) sputum specimens. Significant pathogen isolation was seen in 101 (52.1%) of the total specimen collected, of which sputum specimen had the highest isolation rate of 55.2% Table 2.

Overall isolation of pathogen from specimens was higher in males (54.9%) compared to females (48.1%) but no significant difference was seen between the isolation rates ($\chi 2$=0.85, OR=1.31, *P*=0.36) Table 3.

Only one bacteria specie was isolated from each specimen in 100 out of the 101 specimens with significant isolation, whereas only 1 had dual isolation of a bacterium (*Klebsiella pneumoniae*) and *Candida albicans*. *Klebsiella pneumoniae* was the most commonly isolated bacteria (34=33.7%) followed by *S. aureus* (19.8%) Table 4.

Overall, Gram negative bacilli were the predominantly isolated bacteria (65=64.4%), and a total of 8 (12.3%) of these were ESBL-producer, only 3 (8.3%) of the total 36 (35.6) Gram positive bacterial isolates were methicillin-resistant, and the whole 3 were *S. aureus* (MRSA) Table 5.

Table 1. Age and sex distribution of the participants

Age range	Male n (%)	Female n (%)	Total n (%)
16-25	4 (3.5)	8 (9.9)	12 (6.2)
26-35	20 (17.7)	13 (16.0)	33 (17.0)
36-55	48 (42.5)	32 (39.5)	80 (41.2)
>55	41 (36.3)	28 (34.6)	69 (35.6)
Total	113 (58.2)	81 (41.8)	194 (100.0)

Mean age: 50.294±16.621

Table 2. Frequency of significant pathogen isolation from different specimens

Clinical specimen	Total n (%)	Significant isolation n (%)
Sputum	172 (88.7)	95 (55.2)
Pleural fluid	12 (6.2)	4 (33.3)
Endotracheal aspirate	10 (5.2)	2 (20.0)
Total	194 (100.0)	101 (52.1)

Table 3. Sex distribution of participants in relation to significant pathogen isolation from different specimens

	Male		Female		$\chi 2$ value	*P*-value
Clinical specimen	Total n (%)	Significant isolation n (%)	Total n (%)	Significant isolation n (%)		
Sputum	98 (86.7)	58 (59.2)	74 (91.4)	37 (50.0)		
Pleural fluid	8 (7.1)	2 (25.0))	4 (4.9)	2 (50.0)		
Endotracheal aspirate	7 (6.2)	2 (28.6)	3 (3.7)	0 (0.0)		
Total	113 (58.2)	62 (54.9)	81 (41.8)	39 (48.1))	0.85	0.36

Table 4. Pattern of isolates from the 101 specimens with significant pathogen isolation

Isolates	Frequency	Percentage isolation (%)
Klebsiella pneumonia	33	32.7
Staphylococcus aureus	20	19.8
Escherichia coli	16	15.8
Pseudomonas aeruginosa	12	11.9
Streptococcus pneumonia	10	9.9
Coagulase-negative *S. aureus*	6	5.9
Citrobacter freundii	2	2.0
Acinetobacter baumanni	1	1.0
Klebsiella pneumonia + Candida albicans	1	1.0
Total	101	100.0

NB: Total Klebsiella pneumonia isolated was 33+1=34 (33.7%)

Table 5. Pattern of multidrug-resistant bacteria from the isolates

Gram negative bacteria	Frequency n%	ESBL-producer n (%)
Klebsiella pneumonia	34 (52.3)	4 (11.8)
Escherichia coli	16 (24.6)	1 (6.3)
Pseudomonas aeruginosa	12 (18.5)	3 (25.0)
Citrobacter freundii	2 (3.1)	0 (0.0)
Acinetobacter baumanni	1 (1.5)	0 (0.0)
Total gram negative bacteria	65 (64.4)	8 (12.3)
Gram positive bacteria	Frequency n%	Methicillin-resistant n(%)
Staphylococcus aureus	20 (55.6)	3 (15.0)
Streptococcus pneumonia	10 (27.8)	0 (0.0)
Coagulase-negative *S. aureus*	6 (16.7)	0 (0.0)
Total gram positive bacteria	36 (35.6)	3 (8.3)
Total bacterial isolates	101 (100.0)	

All (100.0%) Gram negative bacterial isolates including the ESBL-producers were sensitive to imipenem and meropenem tested. A good sensitivity pattern was also seen against levofloxacin (86.2%), ciprofloxacin (81.5%), ceftazidime (81.5%) and gentamicin (76.9%). Table 6.

All (100.0%) Gram positive bacterial isolates including the MRSA, were sensitive to vancomycin, imipenem and meropenem tested. Cefuroxime (77.8%) and levofloxacin (77.8%) demonstrated fair activity against the isolated Gram positive isolates. Table 7.

4. DISCUSSION

Lower respiratory tract infections are among the most common causes of hospital admissions with significant morbidity and mortality [2]. The laboratory diagnosis and treatment of LRTIs pose immense challenge in our environment due to limited access to health facilities and emergence of multidrug resistance pathogen, necessitating the need for formulation of treatment guidelines in treating these infections and preventing further generation of multidrug-resistant pathogens. This study aimed at generating data in supporting formulation of guidelines for managing LRTIs in the setting.

A high pathogen isolation rate of 52.1% seen in this study is similar to some previous studies on lower respiratory tract infection pathogens: Elumalai **(**65.14%), Ullah *et al* (64%), Ravichitra and Subbarayudu (58.9%), but some other studies have reported lower isolation rates; Raakhee *et al* (16.04%), Ahmed *et al* (17.03%), Okesola and Ige (27%), Amarasinghe *et al* (29.4%), Usman and Muhammad (41.18%), Tripathi and Dhote (42.2%), and Tchatchouang *et al* (46.8%) [5, 11-19]. The variability in isolation rates from different studies may be as a result of varying patterns of antibiotic use by the patients prior to sample collection which may affect isolation rates from specimens, and since the agents of LRTIs vary from one region to another, and also from time to time [5], the possibility of viruses as causative agents of some clinically diagnosed LRTIs may be a factor affecting the variability in isolation rate.

The Gram negative bacteria were predominantly isolated from different respiratory specimens in our study (64.4%). Other studies have reported similar findings; Ravichitra and Subbarayudu

Table 6. Antibiotic sensitivity pattern of the Gram-negative bacterial isolates

Antibiotics	*Klebsiella pneumoniae*(n/34) %	*Escherichia coli* (n/16) %	*Pseudomonas auriginosa* (n/12) %	*Citrobacter freundii* (n/2) %	*Acinetobacter baumanni* (n/1) %	*Total* (n/65) %
Ampicillin	12 (35.3)	7 (43.8)	2 (16.7)	1 (50.0)	0 (0.0)	22 (33.8)
Amoxycillin-clavulanate	20 (58.8)	11 (68.8)	4 (33.3)	1 (50.0)	0 (0.0)	36 (55.4)
Piperacillin–Tazobactam	24 (70.6)	13 (81.3)	8 (66.7)	2 (100.0)	1(100.0)	48 (73.8)
Gentamicin	27 (79.4)	12 (75.0)	8 (66.7)	2 (100.0)	1 (100.0)	50 (76.9)
Ceftriaxone	24 (70.6)	11 (68.8)	8 (66.7)	2 (100.0)	0 (0.0)	45 (69.2)
Cefepime	25 (73.5)	12 (75.0)	8 (66.7)	2 (100.0)	1 (100.0)	48 (73.8)
Ceftazidime	26 (76.5)	13 (81.3)	11 (91.7)	2 (100.0)	1 (100.0)	53 (81.5)
Ciprofloxacin	28 (82.4)	13 (81.3)	9 (75.0)	2 (100.0)	1 (100.0)	53 (81.5)
Levofloxacin	31 (91.2)	13 (81.3)	9 (75.0)	2 (100.0)	1 (100.0)	56 (86.2)
Imipenem	34 (100.0)	16 (100.0)	12 (100.0)	2 (100.0)	1 (100.0)	65 (100.0)
Meropenem	34 (100.0)	16 (100.0)	12 (100.0)	2 (100.0)	1 (100.0)	65 (100.0)
Cotrimoxazole	14 (41.2	9 (56.3)	4 (33.3)	1 (50.0)	0 (0.0)	28 (43.1)

Table 7. Antibiotic sensitivity pattern of the gram positive bacterial isolates

Antibiotics	*Staphylococcus aureus* (n/20) %	*Streptococcus pneumonia* (n/10) %	Coagulase-negative *S. aureus* (n/6) %	Total (n/36) %
Erythromycin	10 (50.0)	8 (80.0)	5 (83.3)	23 (63.9)
Penicillin	6 (30.0)	8 (80.0)	3 (50.0)	17 (47.2)
Amoxycillin-clavulanate	7 (35.0)	9 (90.0)	4 (66.7)	20 (55.6)
Piperacillin–Tazobactam	12 (60.0)	8 (80.0)	4 (66.7)	24 (66.7)
Cefuroxime	15 (75.0)	8 (80.0)	5 (83.3)	28 (77.8)
Ceftriaxone	12 (60.0)	8 (80.0)	4 (66.7)	24 (66.7)
Ceftazidime	13 (65.0)	8 (80.0)	4 (66.7)	25 (69.4)
Ciprofloxacin	14 (70.0)	8 (80.0)	5 (83.3)	27 (75.0)
Levofloxacin	14 (70.0)	9 (90.0)	5 (83.3)	28 (77.8)
Cotrimoxazole	11 (55.0)	7 (70.0)	3 (50.0)	21 (58.3)
Imipenem	20 (100.0)	10 (100.0)	6 (100.0)	36 (100.0)
Meropenem	20 (100.0)	10 (100.0)	6 (100.0)	36 (100.0)
Vancomycin	20 (100.0)	10 (100.0)	6 (100.0)	36 (100.0)
Cefoxitin	17 (85.0)		6 (100.0)	

reported Gram negative bacterial isolates as constituting 65.5% of the total isolates from cases of LRTIs in an Indian tertiary care hospital. Amarasinghe *et al* reported Gram negative bacterial isolates as constituting more than 80% of all isolates from cases of LRTIs in a Sri Lanka hospital, however, Ullah *et al* has reported higher isolation (82.81%) of Gram positive bacterial isolates from cases of LRTIs in a Banglandesh hospital [11, 12, 16]. The difference in the type of isolates may be related to the type of patients recruited into the study; hospital in-patients are more prone to infections caused by Gram-negative bacteria compared to out-patients due to various instrumentations and procedures carried out on them. In our study and those with similar findings as ours, hospital in-patients were the sole participants.

The importance of *Klebsiella pneumoniae* as a bacterial agent of LRTIs was supported by our study where it was the most commonly isolated bacteria. This finding is similar to some previous studies [12-15, 17 20], but other studies reported this bacteria as constituting only minute fraction of bacterial causes of LRTIs; Agmy *et al* [21] reported *K. pneumoniae* as constituting only 14%, 12% and 10% of isolates from cases of hospital-acquired pneumonia (HAP), acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and community-acquired pneumonia (CAP) respectively, while Ullah *et al* [11] reported *K. pneumoniae* as constituting only 4.68% of isolates from cases of LRTIs.

Detection of 8 ESBL-producing Gram-negative bacteria and 3 MRSA (totaling 11 out of 101 [10.9%] bacterial isolates) in this study was a pointer to a significant level of multidrug-resistance bacterial pathogens in our setting. These strains are readily resistant to the most commonly prescribed antibiotics in our institution, thus they constituted a potential threat to the management of diseases associated with them, with the attendant increase morbidity, prolonged hospital stay, limited therapeutic options and in cases of LRTIs, increased mortality. Interestingly however, all ESBL-producing Gram-negative bacteria in this study were sensitive to the carbapenems tested; imipenem and meropenem, also, all the MRSA isolated were sensitive to vancomycin. Antibiotic stewardship needs reinforcement in this setting and antibiotic policies to promote rational antibiotic use must be instituted to reduce the breeding of multidrug-resistant bacterial pathogen in the setting.

All isolated bacterial pathogen in this study, including Gram-positive and Gram-negative, were sensitive to the carbapenems tested. This was a welcome development, in the worst scenario for this setting, imipenem or meropenem may be blindly commenced empirically for all cases of LRTIs. These drugs are to be used with caution however, usually as last resort or second-line, in our environment where resistance to these valuable drugs will definitely connote a disaster. It is important to note the recent reports of emergence of increasing resistance of some Gram-negative bacteria in particular to carbapenems through the production of carbapenemase enzymes [22]. Also, their use in combination with other classes of antibiotics with good sensitivity pattern to the isolated pathogens in this study, such as levofloxacin or gentamicin, is desirable to reduce the rate at which bacteria develop resistance against them. Thus, antibiotic policies to control the use of these drugs in our settings are highly desirable. Based on the findings in this study, levofloxacin in combination with gentamicin is the recommended antibiotic of first choice for empirical treatment of adult LRTIs. An alternative to this is the combination of cefuroxime and gentamicin, while in a confirmed case of LRTI due to MRSA, vancomycin is the recommended antibiotic of choice in this setting.

5. CONCLUSION

Gram negative bacteria were the predominant isolated agents from cases of LRTIs in this study and *K. pneumonia* was the most commonly isolated bacteria specie. Isolates demonstrated poor sensitivity to the commonly prescribed antibiotics, however, all isolates including the ESBL-producers were sensitive to the carbapenems tested. All MRSA isolated were sensitive to vancomycin. Levofloxacin demonstrated good sensitivity pattern against the isolates. There is need for formulation of antibiotic policies on LRTIs using this baseline data generated, to reduce multidrug-resistant pathogen and improve on the management of adult LRTIs patients.

ETHICAL APPROVAL AND CONSENT

Ethical approval (Protocol number: ERC/2020/12/24/470A) for the study was obtained from the Ethics and Research Committee of the hospital. Oral and written informed consent was taken from all participants prior to sample collection.

FINANCE FOR THE STUDY

The research was solely financed by the researcher.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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