

Original Research Article

**SURVEY ~~OFFOR~~ MULTIDRUG RESISTANCE ~~TE~~ BACTERIA IN PATIENTS WITH
LOWER RESPIRATORY TRACT INFECTION ATTENDING SOME HOSPITALS
IN KEBBI STATE, NIGERIA**

Abstract

The rise in multidrug resistance is a growing public health concern among agents of respiratory tract infection, which is responsible for morbidity, mortality and costs in Africa. This study was designed to survey for multidrug resistant bacterial pathogens in patients with lower respiratory tract infection (LRTI) attending some hospitals in Kebbi State, Nigeria. Three hundred and fifty sputum samples were collected from ~~consented~~ patients with the symptoms of LRTI attending six different hospitals in Kebbi State. The samples were all screened for bacterial pathogens using standard microbiological techniques. The bacterial isolates were identified using conventional biochemical tests and then confirmed using commercial biochemical test kit (MICROBACT) according to manufacturer's instruction. Antimicrobial susceptibility ~~test-tests~~ were determined using disc diffusion method to detect resistant isolates as well as multidrug-resistant (MDR) isolates. The overall incidence of multidrug-resistant isolates in this study was 39.8%. High level of MDR was observed among *Staphylococcus aureus* (28.0%) and then *Klebsiella pneumoniae* (21.0%). MDR were also detected amongst *Aeromonas hydrophila* (11.6%), *Pseudomonas aeruginosa* (11.6.0%), *Klebsiella oxytoca* (11.6%), *B. pseudomallei* (7.1%) *Escherichia coli* (4.7%) and *Acinetobacter baumannii* (4.7%). In conclusion, MDR isolates were predominately isolated among *Staphylococcus aureus* and *Klebsiella pneumoniae*, ~~and also~~ Indiscriminate use of antibiotics remain the risk factor for developing multidrug resistance isolates in ~~this-Kebbi~~ state location.

Keywords: Multidrug resistance, Bacteria, Patients, Lower respiratory tract, Infection

Introduction

Antibiotic resistance is considered to be a worldwide problem, and unwise use of antibiotics has been recognized as a key contributor to the increasing rates of resistance (Ventola, 2015). Therefore, clinical practice typically uses empirical antibiotic selection to target the most likely pathogens based on antibiotic sensitivity data (Zhang *et al.*, 2014). Most studies have described patterns in the resistance of bacterial pathogens among adults with respiratory tract

infections, these patterns may occur differently in adults and children (Felmingham, 2004; Adam *et al.*, 2013). According to an analysis of ~~hospital~~ hospital-acquired pneumonia (HAP) in Japan, methicillin resistant *S. aureus* accounts for 17.5%, *P. aeruginosa* 13.9%, and methicillin-sensitive *S. aureus* 6.5%. A similar analysis of nursing and healthcare-associated pneumonia (NHCA) reported *S. pneumoniae* in 16.4% of cases, *Klebsiella pneumoniae* in 9.6%, and methicillin-resistant *S. aureus* in 9.6%. However, it should be noted that in about half of cases, the bacteria responsible for aspiration pneumonia could not be identified (Naoya and Yasuhiro, 2018). The choice of antimicrobial therapy for bacterial LRTIs is relatively straight forward when the etiologic agents and their antibiotic susceptibility patterns are known. However, the clinical presentation is usually not specific enough to make a firm etiologic diagnosis whether in the community or hospital setting (Shah *et al.*, 2010). In almost all cases, eradication of causative agents requires initiation of antimicrobial therapy before obtaining culture report; however, during the last few years, the increase in antibiotic resistance has compromised the selection of empirical treatment (Jonaidi, 2009) and how to choose an effective antimicrobial agent is a new challenge to the clinicians, as the composition and the resistance to antimicrobial agents of infection pathogens was changing frequently. This trend is presumably due to the empirical administration of antibacterial therapy even before the availability of the culture results (Ahmed, 2013). Various other factors also contribute to the emergence of resistance such as irrational use of antibiotics, transmission of resistant bacteria from patient to patient and from healthcare practitioners to patients and vice versa (WHO, 2012; Mahmoud and Balkhy, 2012).

Nowadays, antibiotic resistance exerted by microorganisms against antibiotics is considered as a serious issue by global medical and research community (WHO, 2012; Mahmoud and Balkhy, 2012). Therefore, the clinicians and microbiologists worldwide are focusing on knowledge and strategies to limit the development of antimicrobial resistance. A study conducted at Kathmandu Model Hospital, Kathmandu, Nepal on Antibiotic Susceptibility Pattern of Gram-negative Isolates of Lower Respiratory Tract Infection revealed that among 6 multidrug resistance (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 (Rakshya *et al.*, 2018). The resistance to cephalosporins, aminoglycosides and carbapenem was remarkable from January 2010 to December 2012 in

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the Microbiology Department of a Teaching Tertiary Care Hospital, Central India (Bajpai *et al.*, 2013). Study of Gram negative Bacterial Isolates from Lower Respiratory Tract Infections (LRTI) and their antibiogram pattern in a Tertiary Care Hospital in South India showed that, most of the Gram negative bacterial isolates were resistance to commonly used cephalosporins. *Klebsiella Spp* showed resistance to most of the drugs like [Amoxycylav amoxicillin-clavulanate](#) (87.8%), cefuroxime (83.3%), ceftazidime (81.1%), ceftriaxone (70.5%). *Pseudomonas aeruginosa* showed relatively lesser resistance pattern. *E. coli* showed resistance to most of the cephalosporins, flouroquinolones and [amoxicillin-clavulanate](#) ~~Amoxycylav~~ (63.5%). *Acinetobacter* showed sensitivity only to higher level antibiotics like Imipenam (4.8%) and Piperacillin/Tazobactam (16.7%) (Arthi *et al.*, 2016).

~~The resistance pattern varied for different organisms. *Staphylococcus aureus* isolates were mostly resistant to amoxicillin and ceftazidime (89.2%), whereas, *Streptococcus pneumoniae* was resistant to ceftazidime, amoxicillin and cotrimoxazole (81.2%). In case of Gram negative isolates, *Klebsiella sp.* was mostly resistant to ceftriaxone, ceftazidime and amoxicillin (100%). *Escherichia coli* was resistant to amoxicillin, cotrimoxazole and vancomycin (100%) (Borkot *et al.*, 2016).~~ The antibiotic resistance associated with CA-LRTIs varies significantly depends on geographical locations and investigated populations (Ibrahim *et al.*, 2014; Felmingham, 2004). Therefore, it is not adequate to simply copy the guidelines from other countries, which may be inappropriate and lead to serious problems in clinical practice. **For example, the incidence of aminoglycosides and quinolones resistant-MRSA is relatively high in the USA (McDougal *et al.*, 2010). Penicillin-resistant *S. pneumoniae* is also relatively high in USA (McDougal, 2008) and Southeast Asia (Felmingham, 2004).**

In developing countries including Nigeria, treatment of LRTI is made usually empirically in which the etiologic agent is rarely identified. So, identifying the most common bacterial pathogens from patients with LRTI, drug resistance profile and assessment of risk factors associated with the infection would be valuable to reduce morbidity and mortality as a result of the disease (Temesgen *et al.*, 2019). Therefore, this study was conducted to survey for multidrug resistant bacterial pathogens in patients with ~~Respiratory Tract infection~~ [LRTI](#) attending some hospitals in Kebbi State, Nigeria.

Material and Methods

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Study area

This study was conducted in Kebbi State, Nigeria, Kebbi State is found on latitude 11.6781° N and longitude 4.0695° E, the state is bounded by Sokoto State to the north and east, Niger State to the south, and Benin Republic to the west. The foremost ethnic groups are Hausa and Fulani, other ethnic groups includes Dakarkari, Zabarmawa, Dukkawa and Kambari. Kebbi State have a complete acreage of 36,129 sq. km. Agriculture is that the main occupation of the people especially in rural areas. Crops produced are mainly grains. Animal rearing and fishing also are common. The state has the full population of 3,256,541 people as projected from the 2006 census. The study site includes: Sir Yahaya Memorial Hospital Birnin Kebbi, Kebbi Medical Centre, Aisha Buhari General Hospital Jega, General Hospital Yauri and General Hospital Argungu.

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Comment [H6]: Are these all secondary hospitals? If they are it should reflect in your title, study aim and your trend of discussion, this would help the reviewer and reader greatly.

Study Population

The study population included both male and female patients from all age groups that presented the clinical evidence of lower respiratory tract infection (LRTI) like fever, rigors, fatigue, anorexia, diaphoresis, dyspnea, productive cough and pleuritic pain (Ashby and Turkington, 2007) as diagnosed by the attending physician at the General out Patient Department (GOPD) of the chosen hospital(s).

Study Design

This was a cross-sectional and hospital based study.

Sampling Technique

Stratified sampling technique was employed for this study until the sample size was completed.

Sample Size

Sample size was calculated as 274 sputum specimen as minimum from patients with LRTI using Fisher's formula $N = Z^2 pq/d^2$ for the population above 10,000

N= the required sample size

Z= the confidence interval at 95% (1.96)

p= estimated prevalence of LRTI which is put at 0.2319 (Ajobiewe *et al.*, 2018)

q= 1 – p

d= margin of error at 5% (0.05)

$N = 1.96^2 * 0.2319 (1-0.2319) / 0.05^2$

$= 3.8416 * 0.2319 (0.7681) / 0.0025$

$= 0.6843 / 0.0025$

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= 274

Inclusion Criteria

All consented patients with clinical sign and symptoms of LRTI as diagnosed by the attending physician and people who haven't taken antibiotic a minimum of time period of two weeks before sample collection were included into this study.

Exclusion Criteria

Patients who didn't give their consent or people who took antibiotic within two weeks before sample collection were excluded from this study.

Ethical Approval

Ethical approval was obtained from the Ministry of Health ethical review committee in Kebbi State. Informed consents both oral and written were obtained from all the participants while assents were obtained from parents in case of children. All data were stored anonymously and was handled only by the investigator and authorized personnel.

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Sample Collection

Early morning sputum specimens were collected aseptically from patients attending the chosen hospitals in Kebbi State after obtaining ethical approval. All patients were instructed on a way to collect the sputum samples aseptically, i.e. they were asked to cough deeply into a well-labeled sterile, leak proof, wide mouthed container, with tight fitting cover, which was taken to the laboratory for analysis.

Culture of the sputum

The sputum samples were cultured on chocolate agar, blood agar and MacConkey agar plates (oxid). On the Chocolate agar, bacitracin and optochin disks were placed at secondary inoculation to screen *S. pneumoniae*. The chocolate agar plates were incubated in an incubator (5% CO₂) at 37°C for twenty-four hours while blood agar and MacConkey agar plates were incubated in an aerobic atmosphere at 37°C for twenty-four hours (Borkot *et al.*, 2016). Colonies were sub-cultured for purification and thereafter preserved on culture medium slants and stored in an exceedingly refrigerator (4°C) for subsequent analysis.

Identification of the isolated bacteria

The bacterial isolates were identified base on colonial morphology, gram staining characteristics and series of biochemical tests which includes: catalase test, coagulase test, indole test, citrate test, Urease test oxidase test, TSI, Mannitol fermentation, growth on eosine

methylene blue (EMB) agar, The isolates were further confirmed using commercial biochemical test kit (MICROBACT) in line with manufacturer's instructions.

Antimicrobial Susceptibility Pattern of the Isolated Bacteria

Antimicrobial susceptibility test were determined using disc diffusion method, the disc diffusion method that was presented during this study, was a modification of the Kirby Bauer technique that has been carefully standardized by CLSI, 2017 as described below

Inoculum Preparation

The colonies were suspended in saline, so the inoculums were adjusted to a turbidity equivalent to 0.5 McFarland standards. The prepared solution was mixed well to create a turbid suspension equivalent to 0.5 McFarland standards and therefore the resulting mixture were kept in a very screw cap tube covered with the aluminium foil (CLSI, 2017).

Inoculation of Test Plates

After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was then rotated several times and pressed firmly on the within wall of the tube above the fluid level. This removed the surplus inoculum from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by swabbing the swab over the whole sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° to make sure an excellent distribution of inoculums. The lid was then left slightly open for 3 to 5 minutes, to permit for any excess surface moisture to be absorbed before applying the drug impregnated discs (Lalitha, 2006). The antibiotics discs used for this research includes: Azithromycin, erythromycin, Ciprofloxacin, Ceftriaxone, Ceftazidime, Cefixime, Cefuroxime, Amoxicilin, Gentamycin, Cotrimoxazol, Cefotaxime, Cloxacilin, Vancomycin and Piperacillin.

Application of Discs to Inoculated Agar Plates

The predetermined batteries of antimicrobial discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed all the way down to ensure complete contact with the agar surface. The discs were distributed evenly in order that they're no closer than 24 mm from center to center. The plates were inverted and placed in an incubator set to 36°C within quarter-hour after the discs were applied and incubated for 18 hours at 37°C.

Interpretation of Results

The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the closest whole millimetre, employing a ruler, which was stayed the rear of the inverted Petri-plate. The

Comment [H9]: Indicate the discs used for Gram positive organisms and Gram negative organisms in line with CLSI guidelines. This is the heart of your work in defining MDR and so it has to be done properly.

organisms were reported as susceptible, intermediate or resistant to the agents that were tested (CLSI, 2017).

Determination of Multi-drug Resistance Isolates

Any bacteria that is resistance to at least 1 agent from 3 or more antimicrobial classes were considered as multi-drug resistant isolate (Framow and Nahra, 2013)

Results

Table 1: Distribution of bacterial pathogens of lower respiratory tract infection in Kebbi State

S/N	Bacterial pathogens isolated	Number of occurrence	% Occurrence
1	<i>Staphylococcus aureus</i>	34	31.1
2	<i>Klebsiella pneumoniae</i>	24	22.2
3	<i>Klebsiella oxytoca</i>	15	13.9
4	<i>Escherichia coli</i>	12	11.1
5	<i>Aeromonas hydrophila</i>	6	5.6
6	<i>Acinetobacter baumannii</i>	5	4.6
7	<i>Pseudomonas aeruginosa</i>	6	5.6
8	<i>B. pseudomallei</i>	3	2.8
9	<i>Proteus spp</i>	3	2.8
	TOTAL	108	100

Table 2: Incidence of Multi-drug resistance bacteria in patients with LRTIs in Kebbi State

S/N	Bacterial isolates	No. of Isolated bacteria	MDR	Resistance Pattern
1	<i>Staphylococcus aureus</i>	34(31.5)	12(28.0)	B-lactam antibiotics (AML, OB, CXM, CAZ, CRO, CFM, CTX), Macrolide (E), Glycopeptide (VA)
2	<i>Klebsiella pneumoniae</i>	24(22.2)	9(21.0)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Macrolide (AZM)
3	<i>Klebsiella oxytoca</i>	15(13.9)	5(11.6)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Macrolide (AZM)
4	<i>Escherichia coli</i>	12(11.1)	2(4.7)	B-lactam antibiotics (CRO, CFM, CTX, PRL), Sulfonamides (SXT), Aminoglycoside (CN), fluoroquinolones (CIP)
5	<i>Aeromonas hydrophila</i>	6(5.6)	5(11.6)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Aminoglycoside (CN)
6	<i>Acinetobacter baumannii</i>	5(4.6)	2(4.7)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Macrolide (AZM), Aminoglycoside (CN)
7	<i>Pseudomonas aeruginosa</i>	6(5.6)	5(11.6)	B-lactam antibiotics (CAZ, CRO, CTX), Macrolide (AZM), Sulfonamides (SXT)
8	<i>B. pseudomallei</i>	3(2.8)	3(7.1)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX, PRL), Sulfonamides (SXT), Aminoglycoside (CN), fluoroquinolones (CIP)
TOTAL		108(100)	43(100)	

MDR- Multidrug-resistance, AML- Amoxicillin, OB- Cloxacillin, CXM- Cefixime, CAZ- Cefazidime, CRO- Ceftriaxone, CFM- Cefuroxime, CTX- Cefotaxime, PRL- Piperacillin, SXT- Trimethoprim Sulphametaxazole, AZM- Azithromycin, E- Erythromycin, VA- Vancomycin, CN- Gentamycin, CIP- Ciprofloxacin

Comment [H10]: There are no cut-off values for these antibiotics FOR *S. aureus* in CLSI 2017 as susceptibility to cefoxitin is used to infer same for the cephalosporins generally.

Comment [H11]: How was susceptibility testing performed for vancomycin? You'll have false resistance if performed just like other discs. Broth microdilution methods are what's recommended by CLSI. According to CLSI 2017, "The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition." There's hardly any mention of vancomycin disc in more recent versions of CLSI.

Comment [H12]: This drug (sulfonamide) is not included in the materials and methods section

Comment [H13]: There are no cut-off values for AZM against *K. pneumoniae* in CLSI interpretative criteria. Its used mostly for Gram positive organisms

Comment [H14]: There are also no cut-off values for AZM against *P. aeruginosa* using CLSI cut-off as it's not a drug of choice due to its natural resistance to this antibiotic.

Table 3: Distribution of MDR pathogens of LRTI in relation to co-infection/conditions

S/N	MDR isolates	No. of Isolates	No co-infection	HIV	TB	Hypertension	Diabetes	Others
1	<i>Staphylococcus aureus</i>	12(28.0)	7(38.9)	-	1(20.0)	-	3(100)	1(10.0)
2	<i>Klebsiella pneumoniae</i>	9(21.0)	3(16.7)	1(25.0)	2(40.0)	-	-	3(30.0)
3	<i>Klebsiella oxytoca</i>	5(11.6)	2(11.1)	-	2(40.0)	1(33.3)	-	-
4	<i>Escherichia coli</i>	2(4.7)	1(5.6)	-	-	1(33.3)	-	-
5	<i>Aeromonas hydrophila</i>	5(11.6)	1(5.6)	-	-	1(33.3)	-	3(30.0)
6	<i>Acinetobacter baumannii</i>	2(4.7)	-	-	-	-	-	2(20.0)
7	<i>Pseudomonas aeruginosa</i>	5(11.6)	2(11.1)	2(50.0)	-	-	-	1(10.0)
8	<i>B. pseudomallei</i>	3(7.1)	2(11.1)	1(25.0)	-	-	-	-
	TOTAL	43(100)	18(100)	4(100)	5(100)	3(100)	3(100)	10

MDR: Multi-drug resistance, HIV: Human immunodeficiency virus, TB: Tuberculosis

Table 4: Distribution of Multi-drug resistance bacteria in patients with LRTIs in selected hospitals in Kebbi State

S/N	Bacterial Isolates	No. of MDR	SYM ^H No.(%)	KMC No.(%)	ABGHJ No.(%)	GHA No.(%)	GHY No.(%)	MBGHZ No.(%)
1	<i>S. aureus</i>	12	6(50.0)	4(33.3)	1(8.3)	1(8.3)	-	-
2	<i>K. pneumoniae</i>	9	1(11.1)	2(22.2)	-	2(22.2)	2(22.2)	2(22.2)
3	<i>K. oxytoca</i>	5	-	-	3(60)	-	-	2(40)
4	<i>P. aeruginosa</i>	5	-	-	-	-	1(20)	4(80)
5	<i>E. coli</i>	2	1(50)	-	-	-	-	1(50)
6	<i>A. baumannii</i>	2	-	-	1(50)	-	1(50)	-
7	<i>A. hydrophila</i>	5	-	-	-	4(66.7)	1(16.7)	-
8	<i>B. pseudomallei</i>	3	-	-	-	1(33.3)	1(33.3)	1(33.3)
	TOTAL	43	8	6	5	8	6	10

SYM^H- Sir Yahaya Memorial hospital Birnin Kebbi, KMC- Kalgo Medical Centre ABGHJ- Aisha Buhari General Hospital Jega, GHA- General Hospital Argungu MBGHZ- Martha Bamaiyi General Hospital Zuru

Discussion

Staphylococcus aureus (31.1%) is the most predominant bacteria isolated in this location followed by *Klebsiella pneumoniae* (22.2%), *Klebsiella oxytoca* (13.9%), *Escherichia coli* (11.1%), *Pseudomonas aeruginosa* (5.6%), *Aeromonas hydrophila* (5.6%), *Acinetobacter baumannii* (4.6%), *B. pseudomallei* (2.8%) and *Proteus spp* (2.8%) in order of ranking. The distribution of aetiology of lower respiratory tract LRTI as recorded in this study is similar to the previous study at National Hospital Abuja (Abdullahi and Ireghu, 2018), a study in Shanghai, China from 2013 to 2015 (Pengcheng *et al.*, 2018), a multicenter analysis from Turkey (Guclu *et al.*, 2021) and Ethiopia (Dessie *et al.*, 2021) except that, in addition, the current study isolated *Aeromonas hydrophila* and *B. pseudomallei*. Some studies from neighbouring countries such as Yaoundé, Cameroon (Tchatchouang *et al.*, 2019) and other studies in some part of Europe (Leven *et al.*, 2018) documented *S. pneumoniae* as the leading pathogen of LRTIs followed by *H. influenzae* which contradict the current findings where *Staphylococcus aureus* were the most prevalence bacteria isolated followed by *Klebsiella spp.* This is similar to the findings in Bangladesh as reported by Borkot *et al.*, (2016) and some studies from southern Ethiopia (Gebre *et al.*, 2021)

Antimicrobial susceptibility test performed on 108 bacterial isolates in the present study showed that, most of the isolates were susceptible to piperacilin (51%), trimethoprim-sulphamethoxazole (61%), Azithromycin (70%), Ciprofloxacin (71%) and Gentamycin (74%), in order of ranking, these are supported by the findings of

El-Mahmood *et al.*, (2010), Taura *et al.*, (2013) and a study in Kathmandu, Nepal (Rakshya *et al.*, 2018). High resistance were recorded in almost all the beta-lactam antibiotics tested such as Ceftriaxone (63%), Cefuroxime (70%), Cefotaxime (71%), Ceftazidime (75%), Oxacillin (87%) and Amoxicillin (93%). High resistance were also recorded among macrolide (Erythromycin) and Glycopeptide (Vancomycin). This findings correlate with the work carried out by Barkot *et al.*, (2016) in Bangladesh.

The overall prevalence of multidrug-resistant (MDR) isolates in this study was 39.8% which was similar to the findings reported from China by Luan *et al.*, (24.5%) but lower as compared to the studies conducted in Ethiopia by Regasa *et al.*, (2015) and Regasa, (2014) where the prevalence was at 56.7% and 54.8% respectively. Multidrug resistance (MDR) to at least one agent in three or more classes of antibiotics were observed in 108 isolates, and high level of MDR were observed among *Staphylococcus aureus* (28.0%) and *Klebsiella pneumoniae* (21.0%), this finding is in agreement with the work of Dessie *et al.*, (2021) on multiresistant bacterial pathogens causing bacterial pneumonia and analyses of potential risk factors from northeast Ethiopia. MDR-Multi-drug resistance were also detected amongst *Klebsiella oxytoca* (10%), *Escherichia coli* (5%), *Acinetobacter baumannii* (15%) and *Pseudomonas aeruginosa* (12%), the distribution of MDR isolates in this study is in line with the findings in Nepal and Ethiopia (Rakshya *et al.*, 2018; Dessie *et al.*, 2021). MDR in resource constraint settings is highly contributed by the widespread misuse of antimicrobials by patients due to lack of access to appropriate treatment and under use of drugs due to inadequate dosing or incomplete treatment courses. The other factor contributing to MDR might be over use of drugs while the infectious pathogen is not well characterized due to the absence of well-organized bacteriology laboratory in the study area. In Nigeria, it is very common to buy and use antimicrobials and other drugs from private pharmacies without prescription. These all could play a role in the increasing trend of antimicrobial resistance in the different health settings of Nigeria in general and our study site in particular.

Distribution of MDR pathogens of LRTI in relation to co-infection/conditions in the present study revealed that, MDR bacteria were isolated in four HIV infected patients with LRTI, this comprise of *Klebsiella pneumoniae* 1(25.0%), *Pseudomonas aeruginosa* 2(50%), *B. pseudomallei* 1(25%). The distribution of MDR pathogens of LRTIs among HIV infected patients contradict the findings of other study by Ojha *et al.*, (2015) in Nepal, where they

isolated *K. pneumoniae* as the most predominant bacterial pathogens, followed by *E. coli* and *S. pneumoniae*. In tuberculosis suspected patients, the current study isolated *Staphylococcus aureus* 1(20.0%), *Klebsiella pneumoniae* 2(40.0%) and *Klebsilla oxytoca* 2(40.0%). This is similar to the finding on Tuberculosis and other bacterial co-infection in Cambodia as reported by Attia *et al.*, (2019). According to Arora *et al.*, *Eco*-infection with tuberculosis (TB) and bacteria has not been widely reported. Although superadded bacterial infection can occur in TB patients, the simultaneous occurrence of both infections leads to delayed diagnosis and inadequate treatment. Tubercular-bacterial co-infection needs to be considered, especially if TB occurs in atypical pulmonary or extrapulmonary locations (Arora *et al.*, 2015). The current study also isolated three *Staphylococcus aureus* 3(100%) in diabetes patients, this tallies with the findings of Rajesh *et al.*, (2017) where they also isolated *Staphylococcus aureus* in patients with LRTIs except in addition to this study they also isolated *Streptococcus pneumoniae* (4%), *Klebsiella pneumoniae*(4%), *Pseudomonas aeruginosa* (6%), *Escherichia coli* (2%), and *Influenza A (H1N1)* (6%). A significant number of MDR were recorded in patients with LRTIs co-infected with unspecified diseases/conditions which comprise of *Staphylococcus aureus* 1(10.0%), *Klebsiella pneumoniae* 3(30.0%), *Aeromonas hydrophila* 3(30.0%), *Acinetobacter baumannii* 2(20.0%) and *Pseudomonas aeruginosa* 1(10.0%)

Conclusion

Staphylococcus aureus is the most predominant bacteria isolated in this location followed by *Klebsiella pneumoniae* with an estimated percentage occurrence of 31.1% and 22.2% respectively. Most of the isolates were susceptible to piperacillin ((51%), ~~trimethoprim-n~~ sulphamethoxazole (61%), ~~A~~azithromycin (70%), ~~C~~iprofloxacin (71%) and ~~G~~entamycin (74%), in order of ranking. High resistance were recorded in almost all the beta-lactam antibiotics tested. High level of MDR were observed among *Klebsiella pneumoniae* (22.5%) and *Staphylococcus aureus* (22.5%). Also present study found that prior antibiotic treatment was a significant risk factor for MDR bacterial infection.

References

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Comment [H15]: Your materials and methods did not indicate that you issued questionnaires. How did you determine this? I suggest you delete this. Rather include a sentence summarizing your recommendation

Comment [H16]: Read the journal guidelines. Your references did not follow the defined style. Also your citations in the text of the manuscript ought to be numbered accordingly. You need to attend to these.

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