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Survey of Multidrug Resistanct Bacteria in Patients with Lower Respiratory Tract Infection Attending Some Hospitals in Kebbi State, Nigeria

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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

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 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 tests were determined using disc diffusion method to detect

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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%), *Pseudomanas aeruginosa* (11.6.0%), *Klebsiella oxytoca* (11.6%), *Burkholderia 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*. Indiscriminate use of antibiotics remains the risk factor for developing multidrug resistance isolates.

Keywords: Multidrug resistance; bacteria; patients; lower respiratory tract; infection.

1. 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 [1]. Therefore, clinical practice typically uses empirical antibiotic selection to target the most likely pathogens based on antibiotic sensitivity data [2]. 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 [3,4]. According to an analysis of 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 healthcareassociated pneumonia (NHCAP) 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 [5]. The choice of antimicrobial therapy for bacterial LRTIs is relatively straight forward when etiologic agents and their 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 [6]. 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 [7] 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 [8]. 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 [9], (Mahmoud and Balkhy, 2012).

Nowadays, antibiotic resistance exerted by microorganisms against antibiotics is considered as a serious issue by global medical and research community [9], (Mahmoud and Balkhy, 2012). Therefore, the clinicians microbiologists worldwide are focusina 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 [10]. The resistance aminoglycosides cephalosporins, to carbapenem was remarkable from January 2010 December 2012 in the Microbiology Department of a Teaching Tertiary Care Hospital, Central India [11]. 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 resistance to commonly Klebsiella cephalosporins. Spp showed resistance to most of the drugs like Amoxicllinclavulanate (87.8%),cefuroxime (83.3%),ceftazidime (81.1%), ceftriaxone (70.5%). Pseudomonas aeruginosa showed relatively resistance pattern. Escherichia showed resistance to most the cephalosporins, flouroquinolones and Amoxicllinclavulanate (63.5%). Acinetobacter showed sensitivity only to higher level antibiotics like Imipenam (4.8%) and Piperacillin/Tazobactam (16.7%) [12].

The antibiotic resistance associated with CA-**LRTIs** varies significantly depends geographical locations and investigated populations [13,3]. 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 [14]. Penicillin-resistant S. pneumoniae is also relatively high in USA (McDougal, 2008) and Southeast Asia [3]. 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 [15]. Therefore, this study was conducted to survey for multidrug resistant bacterial pathogens in patients with lower respiratory tract infection attending some hospitals in Kebbi State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Kebbi State, Nigeria, Kebbi State is found on latitude 10° N and longitude 60° 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 common ethnic groups are Hausa and Fulani, groups other ethnic includes Dakarkari. Zabarmawa, Dukkawa and Kambari. Kebbi State have a complete acreage of 37,699 sq. Km [16]. 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 [17]. 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.

2.2 Study Population

The study population included both male and female patients from all age groups that presented the clinical evidence of LRTI like fever,

rigors, fatigue, anorexia, diaphoresis, dyspnea, productive cough and pleuritic pain [18] as diagnosed by the attending physician at the General out Patient Department (GOPD) of the chosen hospitals.

2.3 Study Design

This was a cross-sectional and hospital based study.

2.4 Sampling Technique

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

2.5 Sample Size

Sample size was calculated as 274 sputum specimen as minimum from patients with LRTI using Fisher's formula $N=Z^2$ pq/d² 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 [19]

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

= 274

2.6 Inclusion Criteria

All consenting 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.

2.7 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.

2.8 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.

2.9 Culture of the Sputum

The sputum samples were cultured on chocolate agar, blood agar and MacConkey agar plates (oxoid). 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 bood agar and MacConkey agar plates were incubated in an aerobic atmosphere at 37°C for twenty-four hours [20]. Colonies were sub-cultured for purification and thereafter preserved on culture medium slants and stored in refrigerator (4°C) for subsequent analysis.

2.10 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.

2.11 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:

2.11.1 Inoculums 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 [21].

2.11.2 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 [22]. The antibiotics discs used for this research includes: Azithromycin, erythromycin, Ciprofloxacin. Ceftriaxone. Ceftazidime. Cefixime, Cefuroxime, Amoxicilin, Gentamycin, Cotrimoxazol, Cefotaxime, Cloxacilin, Vancomycin and Piperacillin.

2.12 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.

2.13 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 organisms were reported as susceptible, intermediate or resistance to the agents that were tested [21].

2.14 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 (Fraimow and Nahra, 2013).

3. 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	Staphylococcus aureus	34	31.1
2	Klebsiella pneumonia	24	22.2
3	Klebsiella oxytoca	15	13.9
4	Escherichia coli	12	11.1
5	Aeromonas hydrophila	6	5.6
6	Acinetobacter baumannii	5	4.6
7	Pseudomonas aeruginosa	6	5.6
8	B. pseudomallei	3	2.8
9	Proteus spp	3	2.8
	Total	108	100

Table 2. Distribution of Multi-drug resistance bacteria in patients with LRTIs among the isolates in Kebbi State

S/N	Bacterial isolates	No. of Isolated bacteria	MDR (%)	Resistance Pattern
1	Staphylococcus aureus	34	12(28.0)	B-lactam antibiotics (AML, OB, CXM, CAZ, CRO, CFM, CTX), Macrolide (E), Glycopeptide (VA)
2	Klebsiella pneumoniae	24	9(21.0)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Co-trimoxazole (SXT), Macrolide (AZM)
3	Klebsiella oxytoca	15	5(11.6)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Co-trimoxazole (SXT), Macrolide (AZM)
4	Escherichia coli	12	2(4.7)	B-lactam antibiotics (CRO, CFM, CTX, PRL), Co-trimoxazole (SXT), Aminoglycoside (CN), fluoroquinolones (CIP)
5	Aeromonas hydrophila	6	5(11.6)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Co-trimoxazole (SXT), Aminoglycoside (CN)
6	Acinetobacter baumannii	5	2(4.7)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Macrolide (AZM), Aminoglycoside (CN)
7	Pseudomonas aeruginosa	6	5(11.6)	B-lactam antibiotics (CAZ, CRO, CTX), Macrolide (AZM), Co-trimoxazole (SXT)
8	Burkholderia pseudomallei	3	3(7.1)	B-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX, PRL), Co- trimoxazole (SXT), Aminoglycoside (CN), fluoroquinolones (CIP)
	Total	108	43(39.8)	

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

Table 3. Distribtion 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	Staphylococcus aureus	12	7(58.3)	0(0)	1(8.3)	0(0)	3(25.0)	1(8.3)
2	Klebsiella pneumonia	9	3(33.3)	1(11.1)	2(22.2)	0(0)	0(0)	3(33.3)
3	Klebsiella oxytoca	5	2(40.0)	0(0)	2(40.0)	1(20.0)	0(0)	0(0)

S/N	MDR isolates	No. of Isolates	No co- infection (%)	HIV (%)	TB (%)	Hypertension (%)	Diabetes (%)	Others (%)
4	Escherichia coli	2	1(50.0)	0(0)	0(0)	1(50.0)	0(0)	0(0)
5	Aeromonas hydrophila	5	1(20.0)	0(0)	0(0)	1(20.0)	0(0)	3(60.0)
6	Acinetobacter baumannii	2	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)
7	Pseudomonas aeruginosa	5	2(40.0)	2(40.0)	0(0)	0(0)	0(0)	1(20.0)
8	B. pseudomallei	3	2(66.7)	1(33.3)	0(0)	0(0)	0(0)	0(0)
	Total	43	18(41.9)	4(9.3)	5(11.6)	3(7.0)	3(7.0)	10(23.2)

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	SYMH	KMC	ABGHJ	GHA	GHY	MBGHZ
		MDR	No. (%)					
1	S. aureus	12	6(50.0)	4(33.3)	1(8.3)	1(8.3)	0(0)	0(0)
2	K. pneumoniae	9	1(11.1)	2(22.2)	0(0)	2(22.2)	2(22.2)	2(22.2)
3	K. oxytoca	5	0(0)	0(0)	3(60)	0(0)	0(0)	2(40)
4	P. aeruginosa	5	0(0)	0(0)	0(0)	0(0)	1(20)	4(80)
5	E. coli	2	1(50)	0(0)	0(0)	0(0)	0(0)	1(50)
6	A. baumannii	2	0(0)	0(0)	1(50)	0(0)	1(50)	0(0)
7	A. hydrophila	5	0(0)	0(0)	0(0)	4(66.7)	1(16.7)	0(0)
8	B. pseudomallei	3	0(0)	0(0)	0(0)	1(33.3)	1(33.3)	1(33.3)
	Total	43	8(18.6)	6(14.0)	5(11.6)	8(18.6)	6(14.0)	10(23.2)

SYMH- 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

4. 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%), Pseudomanas 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 infeection as recorded in this study is similar to the previous study at National Hospital Abuja [23], study in Shanghai, China from 2013 to 2015 [24], a multicenter Analysis from Turkey [25] and Ethiopia [26] except that, in addition, the current study isolated Aeromonas hydrophila and B. pseudomallei. Some studies from neighbouring countries such as Yaoundé. Cameroon [27] and other studies in some part of Europe [28] 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. [20] and some studies from southern Ethiopia [29].

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 [10]. High resistance were recorded in almost all the Beta-lactam antibiotics tested such as Ceftriaxone (63%),Cefuroxime (70%),Cefotaxime (71%), Ceftazidine (75%), Oxacillin (87%) and Amoxicillin (93%). High resistance recorded also 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. [30] and Regasa, (2014)where the prevalence 56.7% and 54.8% was at 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. Multi-drug resistance were also detected amongst Klebsiella oxytoca (10%), Escherichia coli (5%), Acinetobacter baumannii (15%) Pseudomanas aeruginosa (12%), the distribution of MDR isolates in this study is in line with the findings in Nepal and Ethiopia [10,26]. 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 [31,32]. 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 private pharmacies druas from 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(11.1%), Pseudomonas aeruginosa 2(40%), B. pseudomallei 1(11.1%). 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 Staphylocoocus aureus 1(8.3%), Klebsiella pneumoniae 2(22.2%) 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., co-infection with tuberculosis (TB) and bacteria has not been widely reported. Although superadded bacterial infection can occur in TB patients, 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(25%) in diabetes patients, this tallies with the findings of Rajesh et (2017)where they also isolated Staphylococcus aureus in patients with LRTIs except in addition to this study they also isolated Streptococcus pneumonia (4%), Klebsiella pneumonia (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(8.3%), Klebsiella pneumoniae 3(33.3.0%), Aeromonas hydrophila 3(60%), Acinetobacter baumannii 2(100%) and Pseudomonas aeruginosa 1(10.0%). distribution of MDR bacteria among agents of LRTI in selected hospitals revealed that, MBGHZ has the highest number of MDR bacteria 10(23.2) followed by SYMH 8(18.6), GHA 8(18.6), KMC 6(14.0), GHY 6(14.0) and ABGHG 5(11.6). The differences in the distribution of MDR bacteria in the selected hospitals may be attributed to variation in geographical location as well as the differences in lifestyle of individuals from various regions within Kebbi State. Kebbi State comprises of different population with diverse tribes and culture which may affect the distribution of agents of infectious diseases.

5. CONCLUSION

The overall prevalence of multidrug-resistant (MDR) isolates in this study was 39.8%. High MDR were observed among levels of Klebsiella Staphylococcus aureus and pneumoniae. 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, five isolates in tuberculosis suspected patients and three isolates in diabetes patients. Also a significant number of up to ten isolates of MDR were recorded in patients with LRTIs co-infected unspecified diseases/conditions. distribution of MDR bacteria among agents of LRTI in selected hospitals revealed that, MBGHZ has the highest number of MDR bacteria and the least were recorded in ABGHG.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Ministry of Health ethical review committee (approval no. 104:6/2019) in Kebbi State. Informed written consents 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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