Microbes, Infection and Chemotherapy

ORIGINAL RESEARCH

https://doi.org/10.54034/mic.e1549

Antibiogram of Bacteria Isolated from Patients with Lower Respiratory Tract Infection at the general outpatient department of some hospitals in Kebbi State, Nigeria

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Abstract

The rise in antibiotic resistance could be a growing public health concern among agents of respiratory tract infection, which is liable for morbidity, mortality, and costs in Africa. This study was designed to determine the antimicrobial susceptibility pattern of bacteria isolated from patients with lower respiratory tract infection (LRTI) attending some Kebbi State, Nigeria hospitals. 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 significant bacterial growth using standard microbiological techniques. The bacterial isolates were identified using conventional biochemical tests and then confirmed using a commercial biochemical test kit (MICROBACT) according to the manufacturer's instructions. Antimicrobial susceptibility tests were identified using the disc diffusion method. Staphylococcus aureus was the foremost predominant bacteria isolated, followed by Klebsiella pneumoniae, with an estimated percentage occurrence of 31.1% and 22.2%, respectively. Other bacteria isolated include 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%). Most of the isolates were ssusceptible to piperacilin (51%), trimethoprim-sulfamethoxazole (61%), Azithromycin (70%), Ciprofloxacin (71%) and Gentamycin (74%), so as of ranking. High resistance was recorded in βeta-lactam antibiotics, erythromycin and vancomycin tested. Finally, it was revealed that Staphylococcus aureus is the most predominant bacteria isolated. Most of the isolates were resistant to the β-lactam antibiotic tested. Azithromycin, Ciprofloxacin, Gentamycin, and piperacillin remain helpful antibiotics for treating LRTIs in these centers.

Keyword: antibiotics, susceptibility, pathogens, lower respiratory tract, resistance.

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Submitted: September 23, 2022
Reviewed : November 22, 2022
Approved: December 05, 2022

How to cite:
Kalgo ZM, Amin BM, Muhammed B, Saka HK. Antibiogram of Bacteria Isolated from Patients with Lower Respiratory Tract Infection at the general outpatient department of some hospitals in Kebbi State, Nigeria. Microbes Infect Chemother. 2022; 2: e1549

Introduction

Antibiotic resistance is considered a global problem, and inappropriate use of antibiotics has been recognized as a pivotal contributor to the increasing rates of resistance (1). The use of antibiotics in developing countries mostly depends on empirical antibiotic selection (2). The resistance patterns of bacterial pathogens of respiratory tract infections may occur differently in adults and children (3)(4). A study on hospital-acquired pneumonia (HAP) in Japan recorded that methicillin-resistant *S. aureus* accounted for 17.5%, *P. aeruginosa* 13.9%, and methicillin-sensitive *S. aureus* 6.5%. An analogous analysis of nursing and healthcare-associated 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 cases, the bacterial cause of bronchial pneumonia could not be identified (5). The selection of antimicrobial therapy for lower respiratory tract infections (LRTIs) caused by bacteria is comparatively easy when the etiologic agents and their antibiotic susceptibility patterns are known. However, the clinical presentation is typically not specific enough to form a firm etiologic diagnosis in the community or hospital setting (6).

Most cases, lower respiratory tract infections are treated empirically before obtaining a culture report. Still, the rise in antibiotic resistance has compromised the choice of empirical treatment in recent years (7). The way decides on an efficient antimicrobial agent could be a new challenge to clinicians because the resistance pattern of pathogens to commonly used antimicrobial agents was frequently changing. Various factors contribute to the emergence of resistance, like the irrational use of antibiotics, the transmission of resistant bacteria from patient to patient, healthcare practitioners to patients, and the other way around (8,9).

The global medical and research community considers antibiotic resistance exerted by microorganisms

against antibiotics a significant issue (8,9). Hence, clinicians and microbiologists worldwide specialize in knowledge and methods to limit antimicrobial resistance. In developing countries, including Nigeria, treatment of LRTI is usually done empirically, during which the etiologic agent is never identified. So, identifying the bacterial pathogens from patients with LRTI and drug resistance profiles would be valuable in reducing morbidity and mortality due to the disease (10). Therefore, this study was conducted to identify the bacteria in patients with lower respiratory tract infections and assess their antimicrobial susceptibility to commonly used antibiotics in Kebbi State, Nigeria.

Material and methods

Study area

This study was conducted in Kebbi State, Nigeria, Kebbi State is found on latitude 11.6781° N and longitude 4.0695° E, Sokoto State bounds the state to the north and east, Niger State to the south, and Benin Republic to the west. the main ethnic groups are Hausa and Fulani, other ethnic groups include Dakarkari, Zabarmawa, Dukkawa, and Kambari. Kebbi State has a complete acreage of 36,129 sq. km. Agriculture is the people's primary occupation, especially in rural areas. Crops produced are mainly grains. Animal rearing and fishing also are common. The state has a total population of 3,256,541, 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.

Study Population

The study population included male and female patients of all ages presenting clinical evidence of lower respiratory tract infections like fever, rigors, fatigue, anorexia, diaphoresis, dyspnea, productive cough, and pleuritic pain (11). The attending physician diagnosed all subjects at the General Outpatient Department (GOPD) of the chosen hospitals.

Study Design

A cross-sectional and hospital-based study was realized.

Sampling Technique

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

Sample Size

The sample size was calculated as 274 sputum specimens as a minimum from patients with LRTI using Fisher's formula N=Z2 pq/d2 for the population above 10,000, a confidence interval at 95%, error at 5% and estimated prevalence of LRTI in 0.2319, resulting in 274 subjects.

Inclusion Criteria

All consented patients with clinical signs and symptoms of LRTI as diagnosed by the attending physician and people who had not taken antibiotics a minimum of two weeks before sample collection were included in this study.

Exclusion Criteria

Patients who did not consent or took antibiotics 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 consent, both oral and written, was obtained from all participants, while ascent was obtained from parents in the case of children. All data were stored anonymously and were handled only by the investigator and authorized personnel.

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 the way to collect the sputum samples aseptically, i.e., they were asked to cough deeply into a well-labelled sterile, leakproof, wide-mouthed container with a 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 (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% CO_2) 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 (12). Colonies were then sub-cultured for purification, preserved on culture medium slants, and stored in an exceeding refrigerator (4°C) for subsequent analysis.

Identification of the isolated bacteria

The bacterial isolates were identified based on colonial morphology, gram staining characteristics, and a series of biochemical tests, which include 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 a commercial biochemical test kit (MICROBACT) in line with the manufacturer's instructions.

Antimicrobial Susceptibility Pattern of the Isolated Bacteria

Antimicrobial susceptibility tests were determined using the 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 (13) as described below

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. Therefore, the resulting mixture was kept in a screw cap tube covered with aluminium foil (13).

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 tube wall 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 ensure an excellent inoculum distribution. The lid was then left slightly open for 3 to 5 minutes to permit any excess surface moisture to be absorbed before applying the drug-impregnated discs (14). The antibiotics discs used for this research include Azithromycin, erythromycin, Ciprofloxacin, Ceftriaxone, Ceftazidime, Cefixime, Cefuroxime, Amoxicillin, Gentamycin, Trimethoprim-sulfamethoxazole, Cefotaxime, Cloxacillin, 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 down to ensure complete contact with the agar surface. The discs were distributed evenly in order that they were no closer than 24 mm from center to center. The plates were inverted and placed in an incubator set to 36C within a quarter-hour after the discs were applied and incubated for 18 hours at 370C.

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 millimeter, employing a ruler, which stayed at the rear of the inverted Petri plate. The organisms were reported as susceptible, intermediate, or resistant to the tested agents (13).

Results

Table 1

Distribution of bacterial pathogens of lower respiratory tract infection in Kebbi State

S/N	Bacterial pathogens isolated	Number of occurrences	% Occurrence
1	*Staphylococcus aureus*	34	31.1
2	*Klebsiella pneumoniae*	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

Antimicrobial Susceptibility Pattern of the bacterial isolates to commonly used antibiotics

Isolates	No.	Pattern	AML	OB	CXM	CAZ	CRO	CFM	CTX	PRL	SXT	AZM	E	VA	CN	CIP
			N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
Staphylococcus aureus	34	S	3(9)	9(26)	8(24)	3(9)	9(26)	5(15)	5(15)	17(50)	25(74)	23(68)	11(32)	8(24)	25(74)	23(68)
		I	0(0)	1(3)	1(3)	2(6)	5(15)	0(0)	2(6)	8(24)	2(6)	3(9)	2(6)	7(21)	1(3)	4(12)
		R	31(91)	24(71)	25(73)	29(85)	20(59)	29(85)	27(79)	9(26)	7(20)	8(23)	21(62)	19(55)	8(23)	7(20)
Klebsiella pneumoniae	24	S	2(8)	1(4)	3(13)	5(21)	4(17)	2(8)	3(13)	14(58)	15(63)	15(63)	4(17)	4(17)	19(79)	19(79)
		I	1(4)	1(4)	3(13)	3(13)	2(8)	1(4)	3(13)	6(25)	0(0)	0(0)	2(8)	1(4)	1(4)	1(4)
		R	21(88)	22(92)	18(74)	16(66)	18(75)	20(83)	18(74)	4(17)	9(37)	9(37)	18(75)	19(79)	4(17)	4(17)
Klebsiella. oxytoca	15	S	0(0)	0(0)	3(20)	2(13)	2(13)	4(27)	3(20)	9(60)	10(67)	11(73)	2(13)	3(20)	13(87)	14(93)
		I	0(0)	0(0)	3(20)	4(27)	3(20)	2(13)	3(20)	5(33)	1(7)	1(7)	1(7)	0(0)	0(0)	1(7)
		R	15(100)	15(100)	9(60)	9(60)	10(67)	9(60)	9(60)	1(7)	4(27)	3(20)	12(80)	12(80)	2(13)	0(0)
Escherichia coli	12	S	0(0)	1(8)	7(58)	5(42)	6(50)	2(17)	4(33)	6(50)	7(59)	11(92)	3(25)	1(8)	9(75)	6(50)
		I	0(0)	0(0)	0(0)	0(0)	3(25)	2(17)	1(8)	1(8)	0(0)	0(0)	0(0)	0(0)	1(8)	3(25)
		R	12(100)	11(92)	5(42)	7(58)	3(25)	8(66)	7(59)	5(42)	5(41)	1(8)	9(75)	11(92)	2(17)	3(25)
Pseudomonas aeruginosa	6	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)	1(20)	5(80)	0(0)	0(0)	4(80)	5(100)
		I	0(0)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	1(0)
		R	6(100)	6(100)	6(100)	6(100)	5(80)	6(100)	6(100)	1(0)	5(80)	1(20)	5(80)	6(100)	2(20)	0(0)

Key: S- Susceptible, I- Intermediate, R- Resistance, AML- Amoxicillin, OB- Cloxacillin, CXM- Cefixime, CAZ- Ceftazidine, CRO- Ceftriaxone, CFM- Cefuroxime, CTX- Cefotaxime, PRL- Piperacillin, SXT- Trimethoprim-sulfamethoxazole, AZM- Azithromycin, E- Erythromycin, VA- Vancomycin, CN- Gentamycin, CIP- Ciprofloxacin

Table 3

Antimicrobial Susceptibility Pattern of the bacterial isolates to commonly used antibiotics

Isolates	NO.	Pattern	AML	OB	CXM	CAZ	CRO	CFM	CTX	PRL	SXT	AZM	E	VA	CN	CIP
Acinetobacter baumannii	5	S	1(20)	1(20)	1(20)	2(40)	2(40)	2(40)	2(40)	2(40)	4(80)	3(60)	1(20)	1(20)	4(80)	5(100)
		I	0(0)	0(0)	1(20)	0(0)	1(200	1(20)	0(0)	2(40)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		R	4(80)	4(80)	3(60)	3(60)	2(40)	2(40)	3(60)	1(20)	1(20)	2(40)	4(80)	4(80)	1(20)	0(0)
Burkholderia pseudomallei	3	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33)	2(67)
		I	0(0)	0(0)	1(33)	0(0)	0(0)	1(33)	0(0)	1(33)	1(33)	0(0)	0(0)	0(0)	1(33)	0(0)
		R	3(100)	3(100)	2(67)	3(100)	3(100)	2(67)	3(100)	2(67)	2(67)	3(100)	3(100)	3(100)	1(33)	1(33)
Aeromonas hydrophila	6	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(17)	0(0)	0(0)	1(17)	4(66)	0(0)	0(0)	2(34)	0(0)
		I	0(0)	0(0)	0(0)	0(0)	1(17)	1(17)	1(17)	2(33)	0(0)	0(0)	0(0)	0(0)	1(17)	2(33)
		R	6(100)	6(100)	6(100)	6(100)	5(83)	4(66)	5(83)	4(67)	5(83)	2(34)	6(100)	6(100)	3(49)	4(67)
Proteus vulgaris	3	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(67)	3(100)	3(100)	2(67)	0(0)	3(100)	3(100)
		I	0(0)	0(0)	1(33)	1(33)	1(33)	1(33)	0(0)	1(33)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		R	3(100)	3(100)	2(67)	2(67)	2(67)	2(67)	3(100)	0(0)	0(0)	0(0)	1(33)	3(100)	0(0)	0(0)
TOTAL	108	S	6(6)	12(11)	23(21)	17(16)	23(21)	16(15)	17(16)	55(51)	66(61)	75(70)	23(21)	17(16)	80(74)	77(71)
		I	1(1)	2(2)	10(9)	10(9)	17(16)	9(8)	10(9)	26(24)	4(4)	4(4)	6(6)	8(7)	5(5)	12(11)
		R	101(93)	94(87)	75(70)	81(75)	68(63)	82(75)	81(71)	27(25)	38(35)	29(26)	79(73)	83(77)	23(21)	19(18)

Key: S- Susceptible, I- Intermediate, R- Resistance, AML- Amoxicillin, OB- Cloxacillin, CXM- Cefixime, CAZ- Ceftazidine, CRO- Ceftriaxone, CFM- Cefuroxime, CTX- Cefotaxime, PRL- Piperacillin, SXT- Trimethoprim-sulfamethoxazole, AZM- Azithromycin, E- Erythromycin, VA- Vancomycin

Discussion

The rise in multidrug resistance is a growing public health concern among agents of the respiratory tract, which is responsible for morbidity, mortality, and costs (15). This makes managing respiratory tract infections a significant challenge in Africa due to the socioeconomic burden, inadequate good healthcare facilities, and inappropriate use of antibiotics (16). The overall incidence of bacterial pathogens of LRTIs recorded in this study was 31%. This finding is slightly higher than those of earlier studies recorded at National Hospital Abuja (14.5%), Ilorin (15.53%), Benin (18.91%), Kano (21.5%), and Nepal (24.6%) (17-21). Higher prevalence was reported in Bangladesh (64%) and some European countries (59%) (12,22), this variation in incidence may be due to differences in geographical location.

The distribution of etiology of the lower respiratory tract as recorded in this study is similar to the previous study at National Hospital Abuja (17), a study in Shanghai, China, from 2013 to 2015 (23), a multicenter Analysis from Turkey (24) and Ethiopia (25) except that, in addition, the current study isolated *Aeromonas hydrophila and B. pseudomallei*. Some studies from neighboring countries such as Yaoundé, Cameroon (16) and other studies in some part of Europe (22) documented *S. pneumoniae* as the leading pathogen of LRTIs, followed by *H. influenzae* which contradict the current findings where Staphylococcus aureus were the most prevalent bacteria isolated followed by *Klebsiella* spp, this is similar to the findings in Bangladesh as reported by (12) and some studies from Southern Ethiopia (26)

The susceptibility pattern of the isolated bacteria in this study supported the findings Yola and Kano, Nigeria (20,27) and also a study in Kathmandu, Nepal (21). High resistance was recorded in almost all the βeta-lactam antibiotics tested. High resistance was also recorded among macrolide (Erythromycin) and Glycopeptide (Vancomycin). These findings correlate with the work carried out by Barkot *et al*. in Bangladesh (12). *Staphylococcus aureus* displayed a wide range of resistance among βeta-lactam antibiotics tested, such as Amoxicillin, cloxacillin, cefuroxime, ceftazidime, ceftriaxone, cefixime, cefotaxime except for piperacillin in which 50% of the isolates were susceptible, 24% were intermediately resistance, and 26% were resistant. Moderately resistant was associated with erythromycin and Vancomycin among *Staphylococcus aureus* isolates in this study. Gentamycin and trimethoprim-sulfamethoxazole remain the drug of choice in treating LRTI caused by *Staphylococcus aureus* in this location, followed by Azithromycin and Ciprofloxacin. The resistance pattern of Staphylococcus aureus is Similar to the work in Zaria among Staphylococcus aureus isolated from different clinical samples (28).

Klebsiella pneumoniae is the second most predominant bacteria isolated from patients with clinical evidence of LRTI in this location, followed by *Klebsiella oxytoca*. They demonstrated an alarming resistance among βeta-lactam antibiotics such as cefuroxime, ceftazidime, ceftriaxone, cefixime, and cefotaxime, except piperacillin. While ciprofloxacin and gentamycin demonstrated high activity among *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates, followed by Azithromycin and trimethoprim-sulfamethoxazole, this finding is similar to the work in Nepal (21) and Ogbomoso, Nigeria (29). The activity of βeta-lactam antibiotics on *Escherichia coli* was moderate, except that high resistance was recorded on cefuroxime. This finding correlates with the work in Ogbomoso, Nigeria (29), where most of the *Escherichia coli* isolates were susceptible to gentamycin and trimethoprim-sulfamethoxazole followed by Azithromycin and Ciprofloxacin.

Pseudomonas aeruginosa demonstrated 100% resistance to all βeta-lactam antibiotics tested except piperacillin, and good activity was also documented on

Ciprofloxacin, Gentamycin, and Azithromycin. As presented in this study, the resistance to all antibiotics tested by *Pseudomonas aeruginosa* is of public health concern with associated treatment failure. *P. aeruginosa* is a common pathogen in the lungs of those with cystic fibrosis (CF) and is associated with frequent pulmonary exacerbations and high morbidity and mortality (30). The lungs of patients with CF can harbor this organism for decades. With increasing levels of P. aeruginosa drug resistance, treating pulmonary exacerbations can be increasingly complex over time (31). *Burkholderia pseudomallei* also demonstrated 100% resistance to all the antibiotics tested except that one of the isolates was susceptible to gentamycin and two isolates were susceptible to ciprofloxacin. *Burkholderia pseudomallei* is the causative agent for melioidosis, an often-fatal disease with a predicted global burden of 165,000 cases per year and 89,000 deaths worldwide (32). Regions of melioidosis endemicity, including Southeast Asia and northern Australia, account for up to 40% (33) and 10% (34) case fatality rates, respectively. Transmission routes include percutaneous inoculation, inhalation, and ingesting contaminated soil and water (35). *B. pseudomallei* is intrinsically resistant to many antibiotics, including penicillin, ampicillin, and first- and second-generation cephalosporins (36).

Aeromona hydrophila also shows resistance to almost all the antibiotics tested except Gentamycin and Azithromycin, consistent with the findings in Taiwan (37). This study also found that approximately 67% of the patients with Aeromonas infection had various underlying diseases, such as diabetes mellitus and hypertension. Similar findings have been reported in several case reports. Nagata et al. described a case of A. hydrophila pneumonia in a 75-year-old woman with colon cancer who died of the disease (38), Ye *et al.* reported on a patient with severe pneumonia due to drug-resistant *A. caviae* (39), and Murata et al. reported on a case of fulminant *A. hydrophila* pneumonia in a patient with chronic renal failure and liver cirrhosis (40). The morbidity and mortality rates associated with Aeromonas pneumonia were relatively high (37). Therefore, physicians should be aware that immunocompromised patients of advanced age are at risk of developing Aeromonas pneumonia.

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-sulfamethoxazole (61%), Azithromycin (70%), Ciprofloxacin (71%), and Gentamycin (74%). High resistance was recorded in almost all the βeta-lactam antibiotics tested. Treatment of lower respiratory tract infection with β-lactam antibiotics in this center should be discouraged due to the high level of resistance exhibited by the isolated bacteria.

Acknowledgments

To my supervisors (Dr. Binta Mohammed Amin and Dr. Bashir Mohammed) for their guidance and support throughout this research work, no words can express how I truly learned from their expertise. This is also to acknowledge the Federal University Birnin Kebbi and Tertiary Education Trust Funds (TETFUND) for their financial support during my Ph.D. program at the Department of Microbiology, Bayero University Kano. This, in turn, gave rise to this research article.

Funding

This research work was funded by Tertiary Education Trust Funds through Federal University Birnin Kebbi, Nigeria.

Use of animal/ tissue data

Not applicable.

Competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author's contribution

The authors confirm their contribution to the paper as follows: Study conception and design: Zaharaddin M. Kalgo, Binta M. Amin, and Bashir Muhammed; Data collection: Zaharaddin M. Kalgo and Habeeb K. Saka; Interpretation of results: Zaharaddin M. Kalgo, Binta M. Amin and Bashir Muhammed; Draft manuscript preparation: Zaharaddin M. Kalgo and Habeeb K. Saka. All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

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