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Screening for Plasmid Mediated Antibiotic Resistance among Multi Drug Resistant Bacteria Isolated from Patients with Lower Respiratory Tract Infections Attending some Hospitals in Kano Metropolis

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Abstract

Lower Respiratory tract infections (LRTIs) are among the serious infections in humans that have been worsening by the emergence of drug resistant bacteria and aggravated by plasmids transfer. The study aimed to screen Multidrug-resistant (MDR) bacteria isolated from patients with LRTIs for the presence of Plasmid. Sputum samples (400) were collected from patients with LRTIs and processed using standard microbiological procedures to isolate lower respiratory tract bacteria. The identified isolates were subjected to antibacterial susceptibility testing using the disc diffusion method. Multidrug resistant isolates were further subjected to plasmid curing using Acridine orange and the cured isolates were tested for loss of drug resistance. Among the 400 samples, 185 (46.2 %) harbored significant bacterial growth, with 83 (44.9%) Gram-positive and 102 (55.1%) Gram-negative bacteria. Bacteria isolated include Escherichia coli (29), Klebsiella pneumonia (46), Moraxella catarrhalis (13), Pseudomonas aerugonosa (14), Staphylococcus aureus(24) and Streptococcus pneumoniae (59). A higher infection rate was recorded in males (46.99%) and patients aged 41-50 (52.59%). The highest resistance was exhibited by Klebsiella pneumoniae (93%) against cefuroxime, followed by Streptococcus pneumoniae against oxacillin (75%). Of the 185 isolates, 37 (20%) were MDR, out of which 25 (67.6%) isolates had Plasmids. Following curing, 20 (80%) of the isolates were cured, and only 5 (20%) retained their plasmids. Most importantly, Escherichia coli and Klebsiella pneumonia were found to be sensitive to levofloxacin, gentamicin, and imipenem but retained their resistance to Cefuroxime, while Streptococcus pneumoniae was found to be sensitive to levofloxacin only with the highest resistance to oxacillin, doxycycline, and erythromycin. The study establishes a high prevalence of MDR isolates among LRTIs, with some exhibiting plasmid-mediated resistance, and therefore recommends treatment options to be solely based on antibiotic susceptibility testing and continuous plasmid profiling to detect plasmid-mediated resistance to enable appropriate drug administration.

Keywords: Lower Respiratory Tract Infections, MDR Bacteria, Plasmids, Kano

INTRODUCTION

Lower respiratory tract infections (LRTIs) affect the lower airways. LRTIs are the most prevalent infection in humans and a major global source of morbidity and mortality (Troeger *et al.*, 2018). Acute bronchitis and pneumonia are the most prevalent infections of the lower respiratory tract, which account for 4.4% of all hospital admissions and are associated with high morbidity, mortality, and excessive health costs (Nowicki and Murray, 2020). It is estimated that in 2016, lower respiratory infections caused 652 572 deaths in children younger than 5 years, 1 080 958 deaths in adults older than 70 years, and 2 377 697 deaths in people of all ages worldwide (Troeger *et al.*, 2018). The most common bacterial agents of LRTIs are Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus spp*, and Gram-negative bacteria such as *Pseudomonas spp*, *Acinetobacterspp*, *Klebsiella pneumonia*, *Haemophilus influenza* and *Streptococcus pneumonia* (Troeger*et al.*, 2018). Age, gender, season, the kind of population at risk, the distribution of the causative agents, and the frequency of antibiotic resistance are some of the factors that can affect the incidence and associated morbidity of lower respiratory tract infections (LRTIs) (Torres *et al.*, 2021).

Over time, multidrug-resistant (MDR) bacteria have seriously threatened world health (Prestinaci *et al.*, 2015). According to Fair and Tor (2014), MDR is a state in which an organism concurrently uses a variety of strategies to dodge the effects of multiple antimicrobial drugs from various chemical classes or subclasses. A number of strategies are employed by the bacteria for antimicrobial resistance strategies, one of which is the possession of a number of genes, each of which codes for drug resistance within a cell, and this buildup typically takes place on-resistance (R) plasmids (Shahidullah *et al.*, 2012; Munita and Arias, 2016; Peterson and Kaur, 2018; Grohmann *et al.*, 2003). Nikaido (2009) explained that antibiotic-resistant bacteria can transmit copies of the DNA that specify a defense mechanism to other bacteria or to even closely related species, and this leads to the passing of the resistant genes through horizontal gene transfer, thereby creating new generations of antibiotic-resistant bacteria. According to David and Nanette (2013) and Peterson and Kaur (2018), plasmids are extra genetic material found in bacteria and many other cells that typically provide a special characteristic to the cell, such as drug resistance and toxin synthesis. The evolution of antibiotic resistance is mostly attributed to plasmids, which put human health at risk by enabling pathogenic bacteria to acquire several resistance genes in a single transfer event (Peterson and Kaur, 2018). However, if the gene that codes for the antibiotic resistance is chromosomal, the bacteria will retain its resistance after curing as curing does not remove the chromosomal gene (Freifeld, 1983). Multidrug resistance may be advantageous and, as a result, be chosen for and spread further in environments where bacteria are constantly exposed to antibiotics, such as some large production animal farms or hospitals (Woerther *et al.*, 2013).

The multiple antibiotic resistances present a serious and growing clinical problem regarding bacterial infections in humans globally, as the bacteria may become hard to treat living few or even no treatment options and in some cases led to the use of antibiotics that are more toxic for the patients. It is evident from documented research that antibiotics cannot keep up with the rate at which bacteria-resistant strains evolve. Hence, numerous approaches are required to slow the spread of resistant genes. Identification of plasmid associated with drug resistance in our environment and the curing of the resistant isolates may shed light and guide clinical management in choosing the most suitable antibiotic for appropriate drug treatment of some of the most notorious drug resistant bacteria. This study aimed to screen Multi-drug-resistant bacteria for plasmid-mediated antibiotic resistance among patients with lower respiratory tract infections attending some hospitals in the Kano metropolis.

MATERIAL AND METHODS

Ethical Clearance

Ethical Clearance for this study was obtained from the medical ethics committee Ministry of Health, Kano, before the commencement of the study. Only patients who consented to the study were included.

Inclusion and exclusion criteria

Patients with lower respiratory tract infections were included in the study, and those patients without lower respiratory tract infections were excluded.

Sample Size

Four hundred (400) sputum samples were collected from consented patients presenting with symptoms of lower respiratory tract infections reported to the hospitals mentioned above. The sample size was calculated according to Henderson (1982) and a prevalence of 40% (Kumar, 2021) as follows:

$n= Z^2pq/L^2$
Where n = sample size, Z = standard normal distribution at 95% confidence interval = 1.96,
p = prevalence =40% , q= 1-p, L= allowance error (5% = 0.05)
$n = (1.96)^2 \times 0.4\ (1-0.4)/(0.05)^2$
n = 368
The sample number of 368 was approximated to 400.

Sampling technique

A total of 400 sputum samples were collected from the Microbiology laboratories of the

selected three hospitals as follows: two hundred (200) samples from Murtala Muhammad General Hospital Kano, One hundred (100) samples from Aminu Kano Teaching Hospital, and One hundred (100) samples from Infectious Disease Hospital Kano. Samples were collected from patients using a simple random sampling technique. This was done daily until the required sample size was achieved (Amadi *et al.*, 2013).

Sample Collection

Early morning sputum samples were collected aseptically from patients with lower respiratory tract infections in a sterile container. The samples were subjected to further processing.

Isolation and identification of bacteria from the sputum samples

Samples were inoculated onto MacConkey and Chocolate agar using the streak plate technique, incubated at 37^0C for 24 hours, and identified using cultural characteristics, gram staining, and biochemical characterization as described by Cheesbrough (2006).

Antibiotic Susceptibility Testing

Antibiotic susceptibility of the isolates was determined by the Kirby Bauer disc diffusion method using commercially available antimicrobial discs (Oxoid UK) according to CLSI (2022). Five different antibiotics belonging to different classes were selected. For *Streptococcus pneumoniae*, levofloxacin (5ug), ampicillin (10ug), Erythromycin (15ug), Doxycycline (30ug), and Oxacillin(1ug). For *Moraxella catarrhalis*, Levofloxacin (5ug), Imipenem (10ug), Cefuroxime (30ug), amoxicillin-clavulanic acid (2ug), Erythromycin (15ug) was used. For *Escherichia Coli* and *Klebsiella pneumonia,* Levofloxacin (5ug), Ampicillin (10ug), Gentamicin (10ug), Imipenem (10ug), and Cefuroxime (30ug) were used. For *Pseudomonas aerugonosa*, Levofloxacin (5ug), gentamicin (10ug), Imipenem (10ug), Ceftazidime (30ug) and Peparacillin tazobactam (10ug) were used. For *Staphylococcus aureus*, Levofloxacin (5ug), gentamicin (10ug), Ampicillin (10ug), Cefoxitin (30ug), and Imipenem (10ug) were used. Previously standardized isolates suspension were aseptically subcultured using a sterile cotton swab onto prepared Mueller Hinton agar (MHA). The selected standard antibiotic discs were placed on the inoculated agar plates' surface and then incubated at 37°C for 24 hours. After incubation, inhibition zones were read and interpreted according to CLSI (2022).

Plasmid Detection

Following antibiotic susceptibility testing, isolates identified as Multidrug-resistant (MDR) were further assessed for the presence of Plasmid.

Plasmid DNA Extraction

Plasmid DNA extraction was done according to the modified methods of Livak (1987). DNA extraction was carried out directly from the samples by boiling as follows. 1.5ml of the organisms in broth was centrifuged at 10,000rpm for 5 minutes. The supernatant was discarded, and the pellets were washed twice with sterile water. After this, 200µl of sterile water was added to the pellets. The pellets were vortexed to homogenize and boiled in a dry bath at 100°C for 10 minutes. This was followed by vortexing and centrifugation at 12,000 rpm for 5 minutes. The DNA supernatant was transferred to another tube and stored at -20°C. The concentration and purity of the extracted DNA were estimated using a Nanodrop spectrophotometer.

PCR Amplification

The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 20 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM MgCl2, 200µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 20mM of each primer Ok(Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water were used to make up the reaction mixture. Thermal cycling was conducted in a Pielter thermal cycler (MJ Research Series) for an initial denaturation at 95°C for 5 minutes, followed by 30 amplification cycles of 30 seconds at 95°C,1 minute at 56°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel, and

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electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. The 100bp DNA ladder (Solis Biodyne) was used as a molecular weight marker.

Plasmid Curing

Multidrug resistant isolates with Plasmids were cured by treatment with acridine orange according to the methods of Brown (2000). Nutrient broth was prepared and supplemented with 0.1mg/ml acridine orange. Twenty microliters (20ul) of an overnight culture of the bacteria were subcultured into 5mls of the nutrient broth containing acridine orange. The samples were incubated at 37°C for 72 hours. After 72 hours of incubation, the isolates were subcultured onto Mueller Hinton agar and re-tested for drug resistance loss.

Antibiotic susceptibility testing of the cured isolates

Following curing, the isolates were re-subjected to antibiotic susceptibility testing for the second time by the Kirby Bauer disc diffusion method using commercially available antimicrobial discs (Oxoid UK), according to CLSI (2022). As indicated previously, five different antibiotics belonging to different classes were selected and used.

RESULTS

The result of the study showed that out of the 400 sputum samples studied, 185 (46.2%) samples yielded bacterial growth and that patients aged >90 years had an insignificantly higher occurrence of LRTIs with 62.5%, followed by those aged 41-50 years (52.2%) and 81-90 years (50%) respectively (Table 1) (P= 0.948). The distribution of LRTIs among the patients was also found to be insignificantly associated with sex, although males had a higher occurrence of 46.99% compared to females (44.78%) (Table 1) (P= 0.872).

The result revealed the presence of six (6) organisms: four Gram-negative and two Gram-positive, with *Streptococcus pneumoniae* having the highest percentage of occurrence of 31.9%, followed by *Klebsiella pneumonia* (24.9%), and the last occurrence was recorded against *Moraxella catarrhalis* (7.0%) (Table 2).

The antibiotic susceptibility profile of the Gram-negative bacteria exhibited a high resistance level by *K. pneumonia* against cefuroxime (93%) and *E.coli* against ampicillin (86%) (Table 3). In Gram-positive bacteria, a high resistance was reported in *S. pneumoniae* against oxacillin (75%) and doxycycline (56%) (Table 4).

The result of the study showed that out of 185 positive isolates, 37 (20.0%) were found to be MDR, and among the Gram-negative, *K. pneumonia* had the highest number of MDR isolates of 8 (21.62%), followed by *P. aeruginous* with 5 (13.51%) whereas *M. catarrhalis* had the least 2 (5.4%) (Table 5). S. pneumoniae had the highest number of MDR isolates for the Gram-positive bacteria, 11 (29.73%) (Table 5).

The result showed that out of the 37 MDR bacteria subjected to Plasmid detection, 25 (67.6%) were found to harbor Plasmids, out of which *S. pneumoniae* had the highest number with 8 (32%), followed by *S. aureus* with 6 (24%) and *K. pneumonia* with 5 (20%) (Table 6). Isolates of *Pseudomonas aerugonosa* had no Plasmids, while only 2 (8%) of *M. catarrhalis* had Plasmids (Table 6).

Following curing, the study revealed that all the isolates of *K. pneumoniae* and *M. catarrhalis* were 100% cured, *E. coli* and *S. pneumoniae* were 75% cured, and S. aureus was 67% (Table 7).

The antibiotic resistance profile of the cured isolates showed that after Plasmid curing, *E. coli* and *K. pneumonia* were found to be sensitive to levofloxacin, gentamicin, and imipenem. However, *K. pneumonia* retained its resistance to cefuroxime, while *M. catarrhalis* was found to be sensitive to cefuroxime and amoxicillin-clavulanic acid (Table 8). Additionally, *S. aureus* was found to be sensitive to levofloxacin and gentamicin, whereas *S. pneumoniae* was found to be sensitive to levofloxacin only, with the highest resistance to oxacillin, followed by doxycycline, then erythromycin (Table 9).

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Table 1: Distribution of bacteria associated with lower respiratory tract infections among studied patients

Demographic Variables	No examined	No positive with bacterial growth (%)	P. value
Ages (Years)			
11 – 20	26	8 (30.77)	0.948
21 - 30	39	14 (35.90)	
31 - 40	63	29 (46.03)	
41 - 50	116	61 (52.59)	
51 - 60	85	40 (47.06)	
61 - 70	26	11 (42.31)	
71 - 80	21	9 (42.85)	
81 - 90	16	8 (50.0)	
>90	8	5 (62.5)	
Total	**400**	**185 (46.25)**	
Sex			
Male	266	125 (46.99)	0.872
Female	134 (33.5)	60 (44.78)	
Total	**400**	**185 (46.25)**	

Table 2: Morphological appearance, grams staining, biochemical tests, and percentage occurrence of the bacteria associated with Lower Respiratory Tract Infections

Morphological Appearance			Grams Reaction	Biochemical Test									Organism identified	Occurrence (n=185) No (%)
Colony Color	Cho	Mac		Cat	Oxi	Coa	Opt	Ind	MR	VP	Cit	Ure		
Pink	NH	LF	GNB					+	+	-	-	-	*E. coli*	29 (15.7)
Red	NH	LF	GNB					-	-	+	+	+	*K. pneumoniae*	46 (24)
Grayish White	NH	NG	GNDC	+	+								*M. catarrhalis*	13 (7.0)
Colourless	ßH	NLF	GNB	+	+								*P. aeruginosa*	14 (7.6)
Golden Yellow	ßH	NG	GPCcl	+		+							*S. aureus*	24 (13)
Yellow Green	αH	NG	GPCch	-	-		S						*S. pneumoniae*	59 (31.9)

Key: Choc=Chocolate agar, Mac=MacConkey agar, Cat=Catalase, Oxi=Oxidase, Coa=Coagulase, Opt=Optochin, Ind=Indole, MR=Methyl red, VP=Vogespreskaur , Cit=Citrate, Ure=Ureas, NH=No hemolysis, LF=Lactose Fermentation, NG=No Growth, ßH=Beta hemolysis, NLF=No Lactosefermentation, αH=alpha hemolysis, GNB=Gram negative bacilli,GNDC=Gram negative diplococcic, GPCcl=Gram positive cocci in clusters, GPCch=Gram positive cocci in chain.

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Table 3: Antibiotics Susceptibility Profile of the Gram Negative Bacteria

Gram Negative Isolates			*E. coli* (n=29)	*K. pneu* (n=46)	*M.cat* (n=13)	*P.aer* (n=14)	**Total (102)**
LE	**S**	**No (%)**	25 (86)	36 (78)	7 (54)	5 (36)	**73**
	R	**No (%)**	4 (14)	10 (22)	6 (46)	9 (64)	**29**
CN	**S**	**No (%)**	20 (69)	26 (57)	-	14 (100)	**60**
	R	**No (%)**	9 (31)	20 (43)	-	0 (0)	**29**
AMP	**S**	**No (%)**	4 (14)	22 (48)	-	-	**26**
	R	**No (%)**	25 (86)	24 (52)	-	-	**49**
ERY	**S**	**No (%)**	-	-	11 (85)	-	**11**
	R	**No (%)**	-	-	2 (15)	-	**2**
IPM	**S**	**No (%)**	29 (100)	46 (100)	13 (100)	5 (36)	**93**
	R	**No (%)**	0 (0)	0 (0)	0 (0)	9 (64)	**9**
CXM	**S**	**No (%)**	9 (31)	3 (7)	9 (69)	-	**21**
	R	**No (%)**	20 (69)	43 (93)	4 (31)	-	**67**
TZP	**S**	**No (%)**	-	-	-	4 (29)	**4**
	R	**No (%)**	-	-	-	10 (71)	**10**
AMC	**S**	**No (%)**	-	-	7 (54)	-	**7**
	R	**No (%)**	-	-	6 (46)	-	**6**
CAZ	**S**	**No (%)**	-	-	-	10(71)	**10**
	R	**No(%)**	-	-	-	4(29)	**4**

Key: LE = Levofloxacin CN = Gentamycin, AMP = Ampicillin, ERY = Erythromycin, IPM = Imipenem, CXM = Cefuroxime, TZP =PeparacillinTazobactam, AMC = Amoxicillin clavulanic acid, CAZ = Ceftazidime

Table 4: Antibiotics Susceptibility Profile of the Gram Positive Bacteria

Gram Positive Isolates			*S. aureus*(n=24)	*S. pneumoniae(n=59)*	**Total (83)**
LE	**S**	**No(%)**	24(100)	40(68)	**64**
	R	**No(%)**	0(0)	19(32)	**19**
CN	**S**	**No(%)**	24(100)	-	**24**
	R	**No(%)**	0(0)	-	**0**
AMP	**S**	**No(%)**	7(29)	33(56)	**40**
	R	**No(%)**	17(71)	26(44)	**43**
ERY	**S**	**No(%)**	10(42)	50(85)	**60**
	R	**No(%)**	14(58)	9(15)	**23**
DO	**S**	**No(%)**	-	19(32)	**19**
	R	**No(%)**	-	33(56)	**33**
OX	**S**	**No(%)**	-	15(25)	**15**
	R	**No(%)**	-	44(75)	**44**
FOX	**S**	**No(%)**	11(46)	-	**11**
	R	**No(%)**	13(54)	-	**13**

Key: LE = Levofloxacin, CN = Gentamicin, AMP = Ampicillin, ERY = Erythromycin, DO = Doxycyclin, OX = Oxacillin, FOX = Cefoxitin

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Table 5: Multidrug resistant status of bacteria isolated from patient with lower respiratory tract infections

Isolates	Number screened	Multidrug Resistance Status		P. value
		Positive	Negative	
		No (%)	No (%)	
E. coli	29	4 (10.8)	25 (16.89)	0.472
K. pneumoniae	46	8 (21.62)	38 (25.68)	
M. catarrhalis	13	2 (5.4)	11 (7.43)	
P. aeruginosa	14	5 (13.51)	9 (6.08)	
S. aureus	24	7 (18.92)	17 (11.49)	
S. pneumoniae	59	11 (29.73)	48 (32.43)	
Total	**185**	**37 (20)**	**148 (80)**	

Table 6: Plasmid Detected from Multidrug Resistant (MDR) Bacteria

Isolates	MDR Bacteria examined	No positive (%)	P. value
E. coli	4	4 (100)	0.012
K. pneumoniae	8	5 (62.5)	
M. catarrhalis	2	2 (100)	
P. aeruginosa	5	0 (0.0)	
S. aureus	7	6 (85.7)	
S. pneumoniae	11	8 (72.7)	
Total	**37**	**25 (67.6)**	

Table 7: Plasmid Curing Profile of the Cured Isolates

Isolates	MDR Bacteria Studied	Presence of Plasmid	
		Before Curing	After Curing
		No (%)	No (%)
E. coli	4	4	3 (75)
K. pneumoniae	8	5	5 (100)
M. catarrhalis	2	2	2 (100)
S. aureus	7	6	4 (67)
S. pneumoniae	11	8	6 (75)
Total	**37**	**25**	**20 (80)**

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Table 8: Antibiotics Resistance Profile of the Isolated Gram Negative Multidrug Resistant Bacteria Before and After Plasmid Treatment

Antibiotics Resistance Before and After Plasmid Treatment	Gram Negative Isolates		*E. coli* (n=4)	*K. pneu*(n=5)	*M. cat* (n=2)	**Total (11)**
	LE	**Before**	**No (%)**	4 (100)	5 (100)	2 (100)	**11 (100)**
		After	**No (%)**	0 (0)	0 (0)	2 (100)	**2 (18)**
	CN	**Before**	**No (%)**	4 (100)	5 (100)	-	**9 (82)**
		After	**No (%)**	0 (0)	0 (0)	-	**0 (0)**
	AMP	**Before**	**No (%)**	4 (100)	5 (100)	-	**9 (82)**
		After	**No (%)**	3 (75)	2 (40)	-	**5 (45)**
	IPM	**Before**	**No (%)**	4 (100)	5 (100)	2 (100)	**11 (100)**
		After	**No (%)**	0 (0)	0 (0)	1 (50)	**1 (9)**
	CXM	**Before**	**No (%)**	4 (100)	5 (100)	2 (100)	**11 (100)**
		After	**No (%)**	2 (50)	5 (100)	0 (0)	**7 (64)**
	ERY	**Before**	**No (%)**	-	-	2 (100)	**2 (18)**
		After	**No (%)**	-	-	2 (100)	**2 (18)**
	AMC	**Before**	**No (%)**	-	-	2 (100)	**2 (18)**
		After	**No(%)**	-	-	0 (0)	**0 (0)**

Key: LE = Levofloxacin CN = Gentamicin, AMP = Ampicillin, ERY = Erythromycin, IPM = Imipenem, CXM = Cefuroxime, AMC = Amoxicillin clavulanic acid, *Kpneu* = *Klebsiellapneumonia*, *Mcat* = *Moraxellacatarrhalis*.

Table 9: Antibiotics Resistance Profile of the Isolated Gram Positive Multidrug Resistant Bacteria Before and After Plasmid Treatment

Antibiotics Resistance Before and After Plasmid Treatment	Gram Positive Isolates		*S. aureus*(n=6)	*S. pneumoniae*(n=8)	**Total (14)**
	LE	Before No (%)	6(100)	8(100)	14(100)
		After No (%)	0(0)	0(0)	0(0)
	CN	Before No (%)	6(100)	-	6(43)
		After No (%)	0(0)	-	0(0)
	AMP	Before No (%)	6(100)	8(100)	14(100)
		After No (%)	1(16.7)	1(12.5)	2(14)
	ERY	Before No (%)	6(100)	8(100)	14(100)
		After No (%)	2(33.3)	3(37.5)	5(36)
	DO	Before No (%)	-	8(100)	8(57)
		After No (%)	-	6(75)	6(43)
	OX	Before No (%)	-	8(100)	8(57)
		After No (%)	-	8(100)	8(57)
	FOX	Before No (%)	6(100)	-	6(43)
		After No (%)	4(66.7)	-	4(29)

Key: LE =Levofloxacin, CN = Gentamicin, AMP = Ampicillin, ERY = Erythromycin, DO = Doxycyclin, OX = Oxacillin, FOX = Cefoxitin

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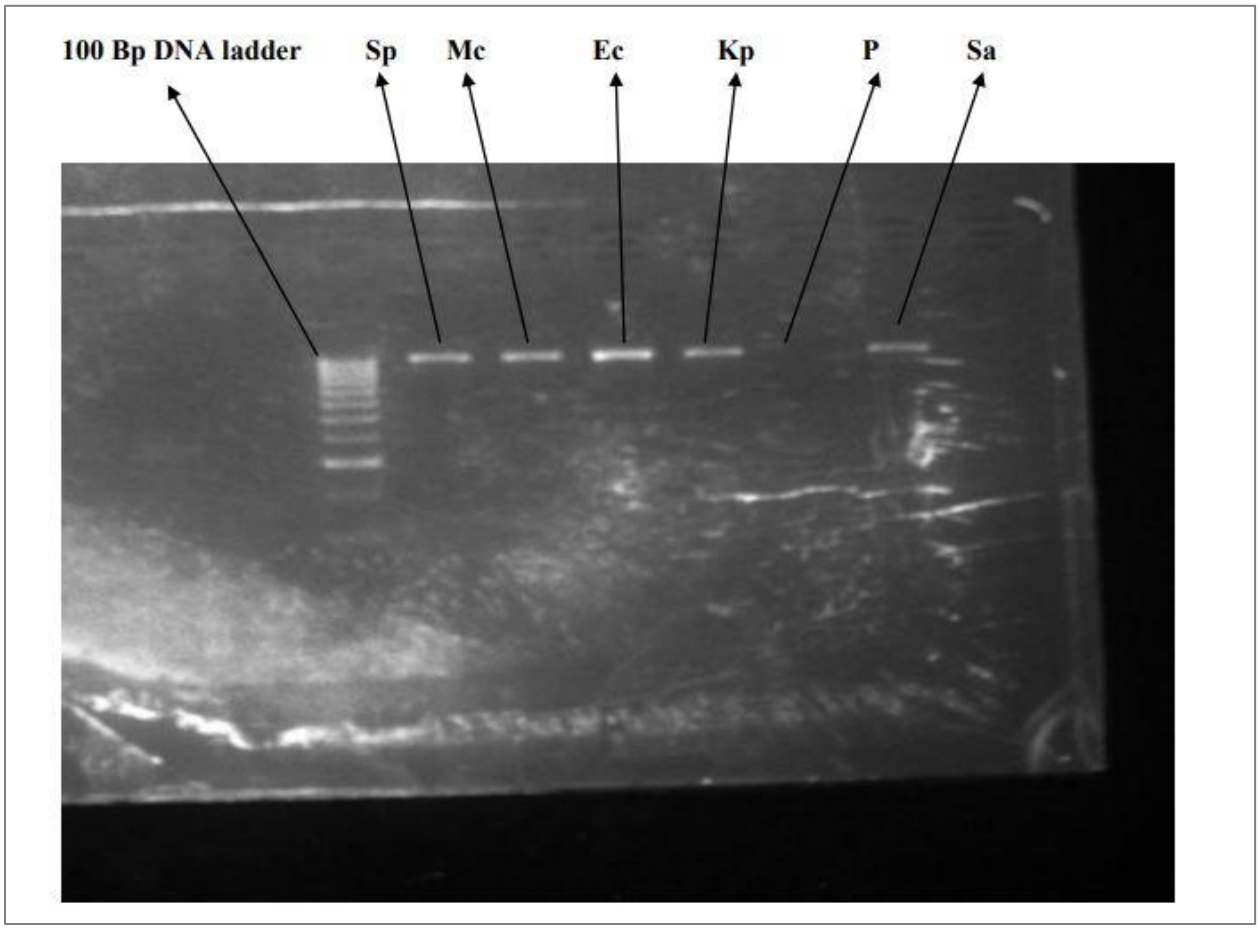

Figure 1: Gel electrophoresis indicating Plasmid among bacteria isolated from patients with lower respiratory tract infections
Sp = *Streptococcus pneumoniae*
Mc = *Moraxella catarrhalis*
Ec = *Escherichia coli*
Kp = *Klebsiella pneumoniae*
P = *Pseudomonas aeruginosa*
Sa = *Staphylococcus aureus*
bp = Base pair

DISCUSSION

The study findings revealed a high prevalence of bacteria (46.2%) associated with lower respiratory tract infections among the studied patients. This observation is of great public health importance as patients harbouring such bacteria can easily transmit them to the population via the airborne route. The most common bacteria isolated from the patients were *S. pneumoniae* and *K. pneumoniae*. This is unsurprising as the two bacteria have been documented to be the most common causative agents of LRTIs. A recent study by Kalgo *et al.* (2023) in Kebbi state, Nigeria, revealed that *S. aureus* (31.1%) was the most predominant bacteria associated with LRTIs, including *K. pneumoniae* (22.2%), *K. oxytoca* (13.9%) and *E. coli* (11.1%). A study by Salman Khan *et al.* (2015) revealed that *S. pneumoniae* had the highest percentage of occurrence (51.70%) among cases with LRTIs.

The findings of this study revealed that patients aged >90 years had higher bacteria associated with LRTIs than other age groups. This may not be unconnected with their immune status as earlier studies by Akingbade *et al.* (2012) and Panda *et al.* (2012) found that because of waning immunity, the pulmonary defense system underpinning chronic disorders, and silent aspiration, older age groups are more vulnerable to pneumonia due to the presence of Gram-negative bacteria. Patients aged 41-50 also reported a high bacterial presence in this study. Workplace exposure could be a reason for the high bacterial occurrence in this group, as people aged 41-50 years are economically

productive and involved in various types of work for earnings.

The study observed that there was no significant difference in the distribution of bacteria among patients with LRTIs with regard to their gender. However, males had a higher percentage compared to females. Although the difference is not much yet, the high percentage seen in men may not be unconnected with workplace exposure, as males are the predominant breadwinners in the area where this study was carried out. As such, the males may be engaged in workplaces that might expose them to the acquisition of the bacteria more than their female counterparts. According to a related study by Taura *et al*. (2013), men had a greater prevalence of bacteria (50.5%) than females (49.5%). According to Panda *et al*. (2012), males have more bacteria than females.

The study findings reported a high resistance level of *K. pneumonia* against cefuroxime, *Escherichia coli* against ampicillin, and *Streptococcus pneumoniae* against oxacillin. This high resistance exhibited by both Gram-negative and Gram-positive bacteria isolated in the study implies that drug-resistant isolates are in circulation in the study area among patients with lower respiratory tract infections and may present challenges to clinicians in the course of treatment and prescription, as well as serve as a source of transmission in the population. Interestingly, S. aureus exhibited 100% susceptibility to levofloxacin and gentamicin, and *E.coli* and *K. pneumonia* also reported 100% susceptibility to levofloxacin, gentamicin, and imipenem. This implies that these antibiotics could still be used as drugs of choice in treating LRTIs against the bacteria mentioned above in the study area.

The study establishes a high rate (20%) of MDR associated with lower respiratory tract infections in the study area. This finding implies that treatment options may be limited for patients with LRTIs in the study area and that there is a possibility of increased transmission of MDR bacteria in the population, posing a major public health issue. Additionally, the majority (67.5%) of the MDR isolates had Plasmids, and *S. pneumoniae* had the highest number of isolates with Plasmids. Interestingly, all five (5) isolates of *P.aerugonosa* had no Plasmid, indicating that its earlier resistance against levofloxacin, Imipenem, Peparacillin, and tazobactam could be chromosomal, not Plasmid-mediated.

Following plasmid curing, 80% of the 25 plasmid isolates were cured and became susceptible again, indicating that the earlier resistance could be Plasmid-mediated. Of these isolates, *E.coli* and *K. pneumonia* were found to be sensitive to levofloxacin, gentamicin, and Imipenem but retained their resistance to cefuroxime. *Moraxella catarrhalis* was found to be sensitive to Cefuroxime and amoxicillin-clavulanic acid. *Staphylococcus aureus* was found to be sensitive to levofloxacin and gentamicin. *Streptococcus pneumoniae* was found to be sensitive to levofloxacin only, with the highest resistance to oxacillin, followed by doxycycline, then erythromycin. All isolates and tested antibiotic classes exhibited resistance before plasmid curing, according to the study's overall results. Following Plasmid curing, some isolates showed a rise in antimicrobial susceptibility, indicating Plasmid-mediated resistance, albeit to varying degrees of susceptibility across the isolates. However, only two isolates of *Streptococcus pneumoniae* were found to have retained their resistance to the tested antibiotics. This result could be due to various contributing factors. Numerous earlier investigations have demonstrated that some isolates maintained resistance even after plasmids were cured, suggesting that resistance was chromosomally mediated (Reboucas *et al*., 2011; Ineta *et al*., 2018). While all of the multidrug-resistant isolates showed plasmid bands during electrophoresis, other findings suggested that some did not (Akindele and Afdidolayan, 2017). Numerous studies have reported bacterial resistance mediated by plasmids (Winokur *et al*., 2000; Lukundi and Zhang, 2018; Ayobola *et al*., 2021).

According to this study, several isolates resistant to multiple drugs carried resistance plasmids, which were most likely obtained by acquisition. According to Fair and Tor (2014) and Agbawa *et al*. (2012), selective pressure from the rising usage and abuse of antimicrobial medications may also contribute to forming drug-resistant plasmids. Nevertheless, other studies revealed that chromosomal mutations or plasmids that can spread from one strain of an organism to another across species can contribute to the establishment of resistance in addition to environmental variables (Yang *et al*., 2008; Oleghe *et al*., 2011). Furthermore, because many plasmids can integrate into the bacterial host genome, it has been observed that in certain bacteria, the lack of a plasmid may not be sufficient evidence to show that the feature is plasmid-encoded (Patwardhan *et al*., 2018).

Moreover, it should be emphasized that even while many plasmids are refractory or challenging to cure, this does not necessarily imply that the plasmid does not encode the resistance characteristics. However, studies have shown that because post-plasmid curing is more susceptible than pre-plasmid curing (Ahmad et al., 1993), plasmid curing can increase antibiotic efficacy. The results of this study support this theory.

CONCLUSION

The study identified bacteria associated with LRTIs in the study area, namely *Escherichiacoli*, *Klebsiella pneumoniae*, *Pseudomonas aerugonosa*, *Moraxella catarrhalis*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The study establishes a high prevalence of multidrug-resistant lower respiratory tract bacterial isolates, with most exhibiting plasmid-mediated resistance. The study recommends regular surveillance and monitoring to provide physicians with knowledge on the updated and most effective empirical treatment of Lower Respiratory tract infections. Plasmid profiling should be continuous research to identify bacteria whose resistance is Plasmid-mediated to enable appropriate drug administration.

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