



Antibiotics resistance and molecular detection of extended spectrum beta-lactamase genes on *Klebsiella pneumoniae* isolated from door handles in selected schools in Amac, Abuja, Nigeria

Iwelumor Clara Isioma¹, Ishaleku David¹ and Ekeleme Ike Kenneth³

¹Department of Microbiology, Faculty of Natural and Applied Science, Nasarawa State University, Keffi, P.M.B 1021 Keffi, Nasarawa State, Nigeria

²Department of Basic sciences, Faculty of Natural and Applied Science, Phoenix University, Agwada Nasarawa State, Nigeria

Corresponding author; Iwelumor Clara Isioma; E-mail: iwelumorci@gmail.com

Abstract

This study investigated the Antibiotics resistance and molecular detection of extended spectrum beta-lactamase genes on *Klebsiella pneumoniae* isolated from door handles in selected schools in Abuja, Nigeria. Swab samples were collected from toilet doors, classroom doors, staffroom doors, and library doors using swab stick. Standard Microbiological techniques were used to isolate and identify *Klebsiella pneumoniae* isolates. Antimicrobial Susceptibility testing of the *Klebsiella pneumoniae* were carried out using disc diffusion method and interpreted in accordance with Clinical and Laboratory Standards Institute guidelines. The antibiotics susceptibility tests and phenotypic detection of ESBL production was carried out using disc diffusion and double disc synergy test method respectively. Out of 284 swab samples from door handles, the overall occurrence of *Klebsiella pneumoniae* was 27.4%. The highest occurrence of *K. pneumonia* isolated was from GssA 35.8% and the lowest was from GssGW 22.4%. *K. pneumoniae* from GssG were more resistant to Co-trimoxazole (89.3%) but less resistant to ciprofloxacin and gentamicin (41.6%). From GssA *K. pneumoniae* were more resistant to amoxicillin/clavulanic acid (47.0%) but less resistant to cefoxitin (28.5%) and gentamicin (21.4%). The *Klebsiella pneumonia* isolated were Multiple antibiotic resistances with the most common being 0.5 (41.6%) from GssG, 0.8 (23.0%) from GssA, 0.7 (35.7%).

From GssJ and 0.7 (27.2%) GssM. out of 33 β -lactam resistant isolates, 7 (21.2%) were ESBL producers while 26(78.7%) were not ESBL producers. The PCR showed different ESBL genes namely *blaSHV*, *blaTEM* and *blaCTX-M*. The occurrence of *blaTEM*, was 6 (85.7%) higher than the occurrence of *blaCTX-M* 4(57.1%) and *blaSHV*, 2 (28.5%) genes. Therefore, it is imperative to educate staff and students on the need for proper use of 33 β -lactam antimicrobial to avoid high rising 33 β -lactam drug resistance of *K. pneumoniae* and need of hand washing after using rest room to avoid spread of resistant *K. pneumoniae*.

Keywords: *Klebsiella pneumonia* Antimicrobial Resistance, ESBL genes, door handles

1. Introduction

Microorganisms are found on different types of surfaces such as floors, tables, door handles, bed rails, carpets, and curtains in health care facilities, food production facilities, schools, and the community (Channabasappa *et al.*, 2016). According to Mensah *et al.*, (2002) transmission of enteric pathogens is assumed to occur predominantly directly from person to person, followed by indirect transmission through contaminated surfaces. Many organisms stay infectious for up to several weeks, which is considered another important factor in environmental transmission (Lindberg *et al.*, 2004). Diseases commonly spread by means of environmental surfaces such as computers, classroom walls, door handles, toilets, chairs, and so on include the common cold, cold sores, conjunctivitis, giardiasis, impetigo, meningitis, pin worm disease, diarrhoea and pneumonia, to mention but a few (WHO, 2011).

Klebsiella pneumoniae, *Escherichia coli*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* as well as *Corynebacterium diphtheriae* cause pneumonia, diarrhoea, dysentery, food poisoning and intoxication as well as whooping cough respectively WHO, (2011). Human hands have been implicated as the major transmitter of microorganisms to environmental surfaces (Yu *et al.*, 2014; Channabasappa *et al.*, 2016). Hands often act as vectors that carry disease causing pathogens including bacteria and viruses from person to person either through direct contact or indirectly via surfaces (Augustino *et al.*, 2014). Mensah *et al.*,(2002). Reviewed that defective personal hygiene can facilitate the transmission of

some of these pathogenic bacteria found in the environment to human hands.

Surfaces that are routinely touched with hands have higher bacteria load as compared to restroom floor and toilet seats. This observation could be due to cumulative contamination of door handles as result of poor sanitary conditions (not washing and cleaning hands with disinfectants after using the toilets) Augustino *et al.*, (2014). Hand washing which is traditional was the first line of defense in preventing the spread of disease; it has been neglected and must be embraced vigorously by families, schools and healthcare professionals.

This study therefore focused on determine the antibiotics resistance and molecular detection of extended spectrum beta-lactamase genes on *Klebsiella pneumoniae* isolated from door handles in selected schools in AMAC, Abuja, Nigeria

2. Materials and Methods

2.1 Study Area

The study area was carried out in selected public and private secondary schools within Federal Capital Territory (FCT), Abuja. Abuja falls within latitude 7°45E and at latitude 7°39N at the equator and 850m above the sea level. Federal Capital Territory (FCT) Abuja is approximately 58k from Keffi.

2.2 Sample collection

Two hundred and eight four Swab samples were collected from toilet doors, classroom doors, staffroom doors, and library doors using swab stick. The sterile swabs were remove from it

packet and deep into a sterile normal saline use and swab the surface of the fomites. After swabbing a drop of sterile normal saline was drop into the pack of the swab stick. The swab samples were transported to microbiology laboratory Nasarawa state university Keffi for analysis

2.2 Isolation of Bacteria

The swab Samples collected the cotton wool portion used was cut off using sterile sclerosis and transfer in nutrient broth and incubates for 18hours at temperature of 35°C. Then the broths were streak on prepared MacConkey agar and incubate at 37°C for 24 hours.

2.3 Identification of the *Klebsiella pneumoniae*

2.3.1 Gram Staining

Gram staining of the suspected organism was carrying out as described previously (CLSI, 2018). Briefly, a small portion of cultural organism was transferred to the slide, and emulsified in the drop of distilled water until a thin homogeneous film is obtained, then the wire loop will be re-sterilized and thin homogeneous film was allowed to dry, fixed by passing through the flame, after the smear is made, air dried, heat fixed, it was then flood with crystal violet for 1 minute, and then rinsed with distilled water. The stain was then be flooded with Lugol's iodine for 1 min, and rinsed with distilled water and then decolorized, rapidly with acetone until no more color appeared to flow from preparation and rinsed appropriately with distilled water. The stain was counter stained with neutral red for 1 min, and rinsed with distilled water and allowed to air-dry and viewed microscopically using x100 oil immersion objective. If the organism retained the neutral red which indicate a Gram-negative rod-shaped bacterium

2.4 Biochemical Tests

The following biochemical tests were carried out on the suspected isolates: Catalase test, Indole, Methyl red, Vorges-Proskauer tests, Nitrate reduction, Urease production, Citrate utilization, and glucose fermentation tests.

2.5 KB003HI25™ Identification of *Klebsiella pneumoniae*

The *K. pneumoniae* isolates that was suspected and that was gram negative, rod shaped, indole positive, methyl red positive, citrate and voges proskauer negative were further identified using KB003HI25™ kit. Following the manufacturer's instruction, into 5ml of sterile normal saline, 2 pure colonies of suspected organisms was transferred. The turbidity of the suspension will be then adjusted to the turbidity equivalent to 0.5 McFarland Standard. Aseptically, the kits were opened and 50ul of the adjusted inoculum was inoculated in each well of the kit and the kit was sealed off and incubated at 37°C for 18 h. After incubation, 2 drops of Reagent R036 and 1 drop of Reagent R015 was added to well no.5, 1 drop of Reagent R009 was added to well six. Exactly 2 drops Reagent R029 and 1 drop of Reagent R030 was added to well 9, 1 drop of Reagent R1007 will be added to the well and finally, 1 drop of Reagent R008 was added to the well. The result will be read and interpreted following the standard given in the identification index.

2.6 Antimicrobial Susceptibility Testing

Testing of the antibiotics susceptibility of the isolates was carried out as earlier described by Clinical and Laboratory Standards Institute (CLSI, 2018). The inoculation of (1) discrete colonies of the isolated *K. pneumoniae* from fomites samples of selected schools in Abuja into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) BaCl₂.2H₂O will be added into 99.5 ml of 1% (w/v) H₂SO₄. A sterile swab stick was soaked in the standardized bacteria suspension and streaked on Mueller Hilton agar plates and the antibiotic disc was aseptically place at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility was interpreted in accordance with

the susceptibility break point earlier described by Clinical and Laboratory Standards Institute (CLSI, 2018).

2.6.1 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined using the formula: MAR Index = No. antibiotics isolate is resistant to/No. of antibiotics tested as described previously (Ngwai *et al.*, 2014).

2.6.2 Phenotypic of Extended Spectrum β -Lactamase Production

The phenotypic confirmatory test for ESBL production by isolates resistant to both third generation cephalosporins (ceftazidime and cefotaxime) was carried out using Double-Disc Synergy Test (DDST) method earlier described by CLSI (2018).

2.7 Molecular Detection of Extended spectrum β -Lactamase Genes

2.7.1 DNA Extraction

The DNA extraction was performed by boiling method as described by Ashayeri-Panah *et al.* (2014). Following purification on MacConkey agar, bacterial DNA was isolated from a 24-h culture in Lysogeny broth (Luria-Bertani broth/LB broth) prepared according to the manufacturers' protocol. The bacterial cells was harvested by centrifugation at 3200 rpm in a micro-centrifuge for 2 min at room temperature and the supernatant was discarded. The harvested cells was re-suspended in 1 ml of sterile normal saline and the micro-centrifuge tubes was placed in the vortex for 5 sec. Centrifugation was carried out at 3200 rpm for 1 min and the supernatant was discarded. 0.5 ml of sterile normal saline was added to the pellets and the tubes were vortexed for 5 sec after which they was heated in the block heater at 90°C for 10 min. immediately after heating, rapid cooling was done by transferring the tubes into the freezer for 10 min. Cell debris was removed after centrifugation was done at 3200 rpm for 1 min and 300 μ l of the supernatant

will be transferred into a sterile 2 ml Eppendof tube as DNA and stored at -10°C until use.

Estimation of the concentration, purity and yield of the DNA sample was accessed using absorbance method (measurement of absorbance) with the spectrophotometer (Nanodrop 1000). For DNA concentration, absorbance readings was performed at 260 nm (A260) and the readings was observed to be within the instrument's linear range (0.1 – 1.0). DNA purity was estimated by calculating the A260/A280 ratio and this was done by the spectrophotometer's computer software (where A260/A280 ratio ranges from 1.7 – 1.9).

2.7.2 DNA Amplification of Extended Spectrum β -Lactamase Genes

Single plex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes present in the isolates. The presence of *bla*CTX-M, *bla*SHV and *bla*TEM genes was tested for using previously published primer sets and conditions. The primer sequences and expected amplicon size for each gene are listed, *bla*TEM. F: TTTCGTGTCGCCCTTATTCC; R: ATCGTTGTCAGAAGTAAGTT. *bla*SHV; F:CGCCTGTGTATTATCTCCCT; R: CGAGTAGTCCACCAGATCCT, *bla*CTX-M; F:CGCTTTGCGATGTGCAG; R: ACCGCGATATCGTTGGT. The reactions was carried out in 20 μ l reaction volume made up of 10 μ l of Mastermix (Qiagen), 0.32 μ l of primers (0.16 μ l each of forward and reverse primers), 3 μ l of DNA and 6.68 μ l of nuclease free water. The primer concentration stood at 0.2 M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine was closed. Conditions during the reactions was set as: 3 minutes of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 40 sec, initial extension at 72°C for 50 sec, final extension at 72°C for 3 min and a hold at 4°C infinitely.

Statistical Analysis

All statistical analyses was carried out using SPSS for windows version 21.0. Differences by the χ^2 test will be considered significant, if $P < 0.05$. Prevalence data is presented with 95% confidence intervals (CI).

3. Results

3.1 Identified *Klebsiella pneumoniae*

The cultural, morphological, and biochemical characteristics *Klebsiella pneumoniae* from isolated from door handles in selected schools in AMAC, Abuja is as given in 1 Pinkish with mucoid colony on MCA which grew with yellowish on XLD agar, was Gram negative rod

and biochemical reactions namely: Indole negative, methyl red negative, Voges-Proskauer negative, Citrate negative, ONPG- negative indicated *K. pneumoniae*.

3.2 The occurrence *K. pneumoniae* from door handles

The occurrence of *K. pneumoniae* from door handles in selected schools in AMAC, Abuja is as given in Table 2. The overall occurrence was 27.4% while the highest occurrence of *K. pneumoniae* isolated from door handles in the study area was recorded from GssA 35.8% followed by GssM 29.7%, GssK 29.3%, GssG 26.0%, from GssJ 23.6% and the least was from GssGW 22.4% respectively.

Table 1: Cultural, Morphological, Biochemical characteristics of the isolates.

Cultural characteristics	Morphological characteristics		Biochemical Characteristics													Inference
	Gram reaction	Morphology	IND	MR	VP	CT	TDA	ONPG	LYS	ORN	UR	NT	H ₂ S	MAL		
Pink and mucor colony on MCA and yellowish on XLD agar	-	Rod	+	+	-	+	+	-	+	-	-	+	-	-		<i>Klebsiella pneumoniae</i>

+ = Positive, - = negative, IND = Indole; MR = Methyl red; VP= Voges-Proskauer, CT = Citrate, LYS = Lysine, ORN = Ornithine; ONPG = Ortho-Nitrophenyl- β -galactosidase, UR = Urease, NT = Nitrate, H₂S = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminase

Table 2 the occurrence of *Klebsiella pneumoniae* isolated from some selected schools in Abuja

Schools	No. sample	No. isolated (%)
GssG	46	12(26.0)
GssA	39	14(35.8)
GssJ	55	13(23.6)
GssGw	49	11(22.4)
GssK	58	17(29.3)
GssM	37	11(29.7)
Total	284	78(27.4)

GssG: Government secondary school Garki; GssA= Government secondary school Apo; GssJ = Government secondary school Jabi; GssGw Government secondary school Gwarinpa; GssK=Government secondary school Kubwa; GssM=Government secondary school Mpape

3.3 Antibiotic Resistance of *Klebsiella pneumoniae*

Table 3 shows the antibiotic resistance of *Klebsiella pneumoniae* isolated from door handles from selected school in AMCA. The *K. pneumoniae* from GssG were more resistant to Co-trimoxazole (89.3%) followed by Nalidixic acid (75.0%) Ampicillin and Cefotaxime (66.6%), Amoxicillin/clavulanic and Ceftazidime (58.3%), Cefoxitin and Streptomycin (50.0%) but less resistant to Ciprofloxacin and Gentamicin (41.6%). From GssA the *K. pneumoniae* were more resistant to Amoxicillin/clavulanic acid (47.0%) followed by Ampicillin,, Streptomycin and Co-trimoxazole (42.8%) but less resistant to Cefoxitin (28.5%) and Gentamicin (21.4%). From GssJ the isolates were more resistant to Amoxicillin/clavulanic acid (61.5%) followed by Streptomycin (53.8%), Ampicillin and Co-trimoxazole (46.1%) but less resistant to Cefoxitin, Gentamicin, Cefotaxime (30.7%) and Ciprofloxacin (23.0%). Isolates from GssGw were more resistant to Streptomycin and Ceftazidime (72.7%) followed by Ampicillin, Cefotaxim and Co-trimoxazole (63.6%) but less resistant to Cefoxitin and Nalidixic acid (36.3%) and Gentamicin (27.2%). *Klebsiella pneumoniae* isolated from GssK were highly resistant to Streptomycin (82.3%) followed by Co-trimoxazole (76.4%), Amoxicillin/clavulanic acid (64.7%), Cefoxitin (58.8%) and less resistant to Gentamicin and Nalidixic acid (35.2%). From GssM the isolates were more resistant to Co-trimoxazole (81.8%) followed by Amoxicillin/clavulanic acid and Streptomycin (72.7%), Ampicillin and Ceftazidime (63.6%) Cefotaxime (54.5%) but less resistant to Gentamicin (36.3%) as shown in Table 3 respectively

3.4 Antibiotic Resistance of Phenotypes

The most common antibiotic resistant phenotypes were S,SXT,CTX,AMP (16.6%) from GssG,

AMC, CTX, AMP (15.3%) from GssA, S,SXT,CTX,CAZ,FOX,CIP,AMP (21.4%) GssJ, AMC,CAZ,FOX, NA,AMP and AMC,S,SXT,CTX,CAZ,FOX,AMP (18.1%) from GssGw, AMC,S,SXT,CTX,CAZ,FOX,AMP and AMC,CAZ,FOX, NA,AMP(11.7%) from GssK and AMC,S,SXT,CTX,CAZ,FOX,CN, NA,CIP,AMP (18.1%) as given in Table 4 respectively.

3.5 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistances are defined as resistance to more than two antibiotics tested. The MAR indices of the isolates is as shown in Table 5 were > 0.2 with the most common being 0.5 (41.6%) from GssG, 0.8 (23.0%) from GssA, 0.7 (35.7%) from GssJ, 0.7 (27.2%) GssM

3.6 Phenotypic Extended Spectrum β -Lactamase Production

The production of ESBL by β -lactam resistant isolates from door handles from some selected schools in AMCA Abuja, Nigeria is as shown in Table 4.6. Out of 33 β -lactam resistant isolates, 7 (21.2%) were ESBL producers while 26(78.7%) were not ESBL producers

3.7 Occurrence of Extended Spectrum β -Lactamase Resistance Genes

The occurrence of ESBL resistance genes in ESBL producing isolates from door handles is as shown in Plates 1, 2 and 3. The occurrence of *blaTEM*, 6 (85.7%) was high while the occurrence of *blaSHV*, 2 (28.5%) and *blaCTX-M* 4(57.1%) was low. The agarose gel electrophoresis showing amplification of *blaCTX-M*, *blaSHV* and *blaTEM* and their base pair

Table 3: Antibiotic Resistance of *Klebsiella pneumoniae* from door handles from selected school in AMCA

No.(%) resistance							
Antibiotics	Disc content (μ/g)	GssG (n=12)	GssA (n=14)	GssJ (n=13)	GssGw (n=11)	GssK (n=17)	GssM (n=11)
Cefoxitin (FOX)	5	6 (50.0)	4(28.5)	4(30.7)	4(36.3)	10(58.8)	5(45.4)
Ampicillin (AMP)	5	8(66.6)	6(42.8)	6(46.1)	7(63.6)	8(47.0)	7(63.6)
Ciprofloxacin (CIP)	5	5(41.6)	5(35.7)	3(23.0)	5(45.4)	9(52.9)	5(45.4)
Amoxicillin/clavulanic acid (AMC)	30	7(58.3)	8(47.0)	8(61.5)	6(54.5)	11(64.7)	8(72.7)
Gentamicin (CN)	10	5 (41.6)	3(21.4)	4(30.7)	3(27.2)	6(35.2)	4(36.3)
Streptomycin (S)	30	6(50.0)	6(42.8)	7(53.8)	8(72.7)	14(82.3)	8(72.7)
Cefotaxime (CTX)	30	8(66.6)	5(35.7)	4(30.7)	7(63.6)	8(47.0)	6(54.5)
Nalidixic acid (NA)	30	9(75.0)	5(35.7)	4(30.7)	4(36.3)	6(35.2)	5(45.4)
Co-trimoxazole (SXT)	25	10(83.3)	6(42.8)	6(46.1)	7(63.6)	13(76.4)	9(81.8)
Ceftazidime (CAT)	30	7(58.3)	3(21.4)	4(30.7)	8(72.7)	9(52.9)	7(63.6)

GssG: Government secondary school Garki; GssA= Government secondary school Apo; GssJ = Government secondary school Jabi; GssGw Government secondary school Gwarinpa; GssK=Government secondary school Kubwa; GssM=Government secondary school Mpape

Table 4.4: Antibiotics resistance phenotypes of *Klebsiella pneumoniae* from door handles from selected school in AMCA Abuja, Nigeria

S/N	Antibiotics Resistance Phenotypes	No. (%) resistant isolates			GssGw (11)		
		GssG (n=12)	GssA (n=13)	GssJ (n=14)		GssK (17)	GssM (11)
1	S, SXT, AMP	1(8.3)	0(0.0)	1(7.1)	1(9.0)	1(5.8)	0(0.0)
2	AMC, CTX, AMP	1(8.3)	2(15.3)	0(0.0)	0(0.0)	1(5.8)	0(0.0)
3	CAZ, CTX, FOX	1(8.3)	0(0.0)	0(0.0)	1(9.0)	0(0.0)	0(0.0)
4	S,FOX,CN	0(0.0)	0(0.0)	1(7.1)	0(0.0)	1(5.8)	1(9.0)
5	S,SXT,CTX,AMP	2(16.6)	1(7.6)	0(0.0)	0(0.0)	1(5.8)	0(0.0)
6	SXT,CTX,CAZ,FOX	0(0.0)	1(5.9)	0(0.0)	1(9.0)	0(0.0)	0(0.0)
7	AMC,S,CTX,AMP	1(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
8	S,CTX,CAZ,FOX	0(0.0)	1(7.6)	1(7.1)	0(0.0)	1(5.8)	1(9.0)
9	S,FOX,CN,CIP,AMP	1(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(9.0)
10	AMC,CAZ,FOX, NA,AMP	1(8.3)	0(0.0)	1(7.1)	2(18.1)	2(11.7)	0(2.2)
11	AMC,SXT,CTX,CAZ,CAZ,AMP	0(0.0)	1(7.6)	0(0.0)	1(9.0)	0(0.0)	1(9.0)
12	S,CTX,CAZ,FOX,CIP,AMP	1(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(9.0)
13	S,SXT,CTX,CAZ,CIP,AMP	0(0.0)	0(0.0)	1(7.1)	0(0.0)	0(0.0)	0(0.0)
14	S,SXT,CTX,CAZ,FOX,CIP,AMP	1(8.3)	0(0.0)	3(21.4)	0(0.0)	1(5.8)	1(9.0)
15	S,SXT,CTX,CAZ,FOX, NA,AMP	1(8.3)	0(0.0)	0(0.0)	1(9.0)	0(0.0)	1(9.0)
16	AMC,S,SXT,FOX,CN,CIP,AMP	0(0.0)	1(7.6)	1(7.1)	0(0.0)	0(0.0)	1(9.0)

17	AMC,S,SXT,CTX,CAZ,FOX,AMP	0(0.0)	1(7.6)	1(7.1)	2(18.1)	2(11.7)	0(0.0)
18	S,SXT,CTX,CAZ,CN, NA,AMP	0(0.0)	0(0.0)	1(7.1)	0(0.0)	1(5.8)	0(0.0)
19	AMC,S,SXT,CTX,CAZ,FOX,CIP,AMP	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(5.8)	0(0.0)
20	AMC,S,SXT,CTX,CAZ,FOX,CN,AMP	0(0.0)	1(7.6)	0(0.0)	0(0.0)	1(5.8)	1(9.0)
21	AMC,S,SXT,CTX,CAZ,FOX, NA,AMP	0(0.0)	1(7.6)	2(14.2)	1(9.0)	1(5.8)	0(0.0)
22	S,SXT,CTX,CAZ,FOX,CN,CIP,AMP	0(0.0)	1(7.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
23	AMC,S,SXT,CTX,CAZ,FOX, NA,AMP	1(8.3)	0(0.0)	2(14.2)	0(0.0)	0(0.0)	1(9.0)
24	AMC,S,CTX,CAZ,FOX,CN, NA,CIP,AMP	1(8.3)	1(7.6)	0(0.0)	0(0.0)	1(5.8)	0(0.0)
25	AMC,S,SXT,CTX,CAZ,FOX,CN, NA,CIP,AMP	0(0.0)	1(7.6)	1(7.1)	1(9.0)	2(11.7)	2(18.1)

AMC-Amoxicillin/Clavulanic Acid; AMP-Ampicillin; CTX- Cefotaxime; CAZ-Ceftazidime; CIP-Ciprofloxacin; FOX-Cefoxitin; CN-Gentamicin; NA - Nalidixic acid; S-Streptomycin; SXT-Sulphomethoxazole/Trimethoprim. GssG: Government secondary school Garki; GssA= Government secondary school Apo; GssJ = Government secondary school Jabi; GssGw Government secondary school Gwarinpa; GssK=Government secondary school Kubwa; GssM=Government secondary school Mpape

Table 5: Multiple antibiotic resistance (MAR) index of *Klebsiella pneumoniae* from doorhandles from selected school in AMCA

No. of antibiotics resistance (a)	No. of antibiotics tested (b)	MAR Index (a/b)	No. (%) MAR index					GssW (n=11)
			GssG (n=12)	GssA (n=13)	GssJ (n=14)	GssK (n=11)	GssGW (n=17)	
10	10	1.0	0(0.0)	1(7.6)	1(7.1)	1(9.0)	2(11.7)	2(18.1)
9	10	0.9	1(8.3)	1(7.6)	0(0.0)	0(0.0)	1(5.8)	1(9.0)
8	10	0.8	1(8.3)	3(23.0)	1(7.1)	1(9.0)	3(17.6)	1(9.0)
7	10	0.7	2(16.6)	3(23.0)	5(35.7)	3(27.2)	3(17.6)	3(27.2)
6	10	0.6	1(8.3)	0(0.0)	1(23.1)	1(9.0)	1(5.8)	1(9.0)
5	10	0.5	5(41.6)	0(0.0)	1(7.1)	2(18.1)	2(11.7)	1(9.0)
4	10	0.4	4(33.3)	3(23.0)	1(7.1)	1(9.0)	2(11.7)	1(9.0)
3	10	0.3	3(25.0)	2(23.0)	2(14.2)	2(18.1)	3(17.6)	1(9.0)
2	10	0.2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

GssG: Government secondary school Garki; GssA= Government secondary school Apo; GssJ = Government secondary school Jabi; GssGw Government secondary school Gwarinpa; GssK=Government secondary school Kubwa; GssM=Government secondary school Mpape

Table 6: Occurrence of Extended Spectrum β -Lactamase Producing *Klebsiella pneumoniae* from door handles from selected school in AMCA

Phenotypic ESBL	GssG (n=5)	No. (%) isolated					Total (n=33)
		GssA (n=7)	GssJ (n=4)	GssK (n=5)	GssGW (n=8)	GssM (4)	
Producers	2	2	0	1	2	0	7(21.2)
Non-producers	3	5	4	4	6	4	26(78.7)

GssG: Government secondary school Garki; GssA= Government secondary school Apo; GssJ = Government secondary school Jabi; GssGw Government secondary school Gwarinpa; GssK=Government secondary school Kubwa; GssM=Government secondary school Mpape

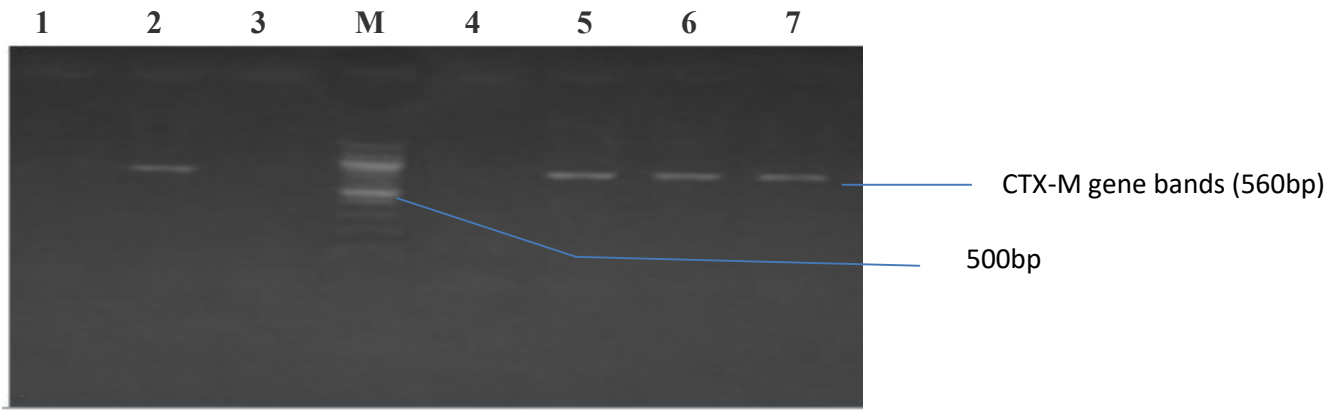


Plate 1: Agarose gel electrophoresis of *CTX-M* (560bp) gene of the *Klebsiella pneumoniae* isolates. Lane M represents a 1000bp molecular ladder

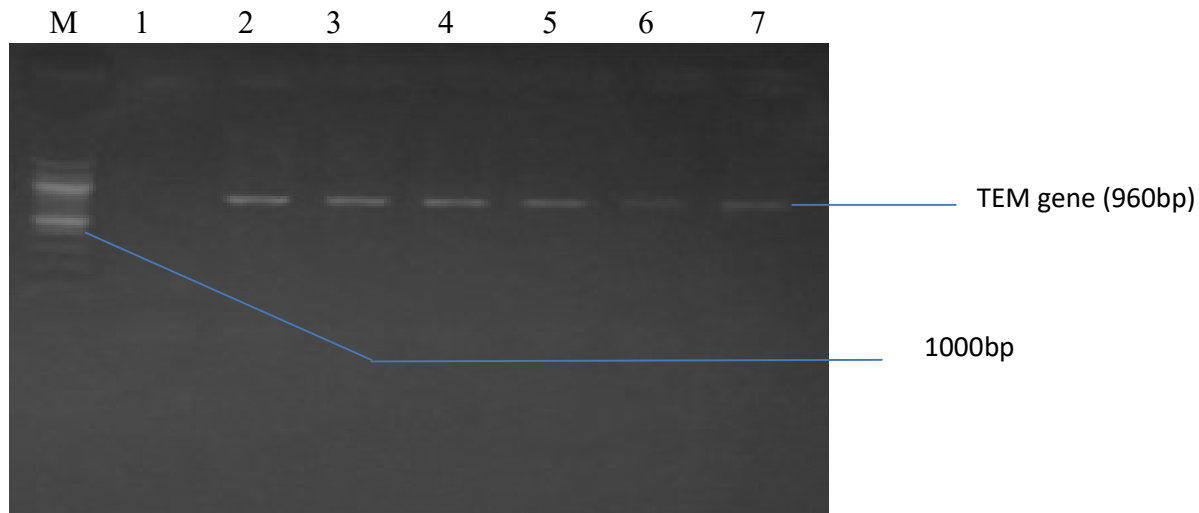


Plate 2: Agarose gel electrophoresis of *TEM* (960bp) gene of the *Klebsiella pneumoniae* isolates. Lanes 2, 3, 4-7 represent TEM gene bands Lane L represents a 100bp molecular ladder

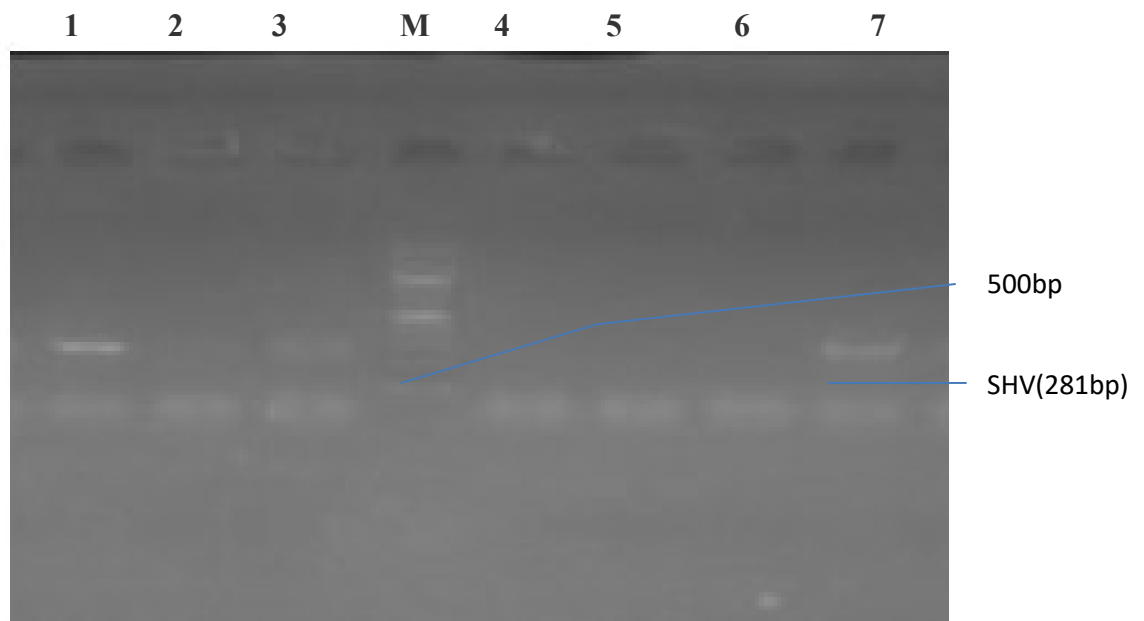


Plate 3: Agarose gel electrophoresis of *SHV* gene of the bacterial isolates. Lanes 1 and 7 showed *SHV* (281bp) bands. Lane M represents a 100bp molecular ladder

4. Discussion

The isolation of *K. pneumoniae* in the study area recorded in this study was similar with study conducted by Azimi *et al.* (2014), Akbariqomi *et al.* (2015) and Feizabadi *et al.* (2019) who reported the occurrence of *K. pneumoniae* isolates from door handles. . The percentage occurrence of the *K. pneumoniae* isolates (27.4%) from selected schools door handles in our study was lower than 30.0% reported by Alonge *et al.* (2019) but higher than 10.6% in the study reported by Aghanya *et al.* (2021). The isolation of *K. pneumoniae* from door handles is probably due to faecal, wound and sputum contaminant as a result of improper hand washing with different disinfectants after use of toilets, holding of wound sites and through contacts with the sputum. The occurrence of *K. pneumoniae* isolates from the door handles is also an indication that the isolates can survive within the environments in which they were isolated (Worku *et al.*, 2018).

The isolation of *K. pneumoniae* isolates from door handles within our study centre's may also have public health implications since this organisms are known to be responsible for infections such as urinary tract infections, *pneumoniae* and many others (Belete and Saravanan 2020).

The resistance of *K. pneumoniae* isolates to all the antibiotics tested in this study was not surprising and this may be due to reasons like; inability of the antibiotics to get to the target sites, decrease uptake as a result of efflux pump, abuse and inappropriate prescription (Sun *et al.*; 2014). The resistance of *K. pneumoniae* isolates to Amoxicillin/clavulanic acid and Ceftazidime observed in our studies contradicts the study by Fody *et al.* (2017) who reported 12.3% and 20.0% susceptibility of *K. pneumoniae* to Amoxicillin/clavulanic acid and Ceftazidime. Also, the percentage resistance of the isolates to Amoxicillin/clavulanic (72.7%) was higher than 29.0% reported by Worku *et al.* (2018). The resistance of *K. pneumoniae* isolates to Ampicillin observed in our study was higher than 3.7% reported by Worku *et al.* (2018) and this may be

due to the indiscriminate use of the Ampicillin for treatment of bacterial infection.

The findings in these studies also show that most of the β -lactam resistant *K. pneumoniae* isolates were ESBL producers. The occurrence of ESBL producers in these studies is in agreement with the study earlier described by Thenmozhi *et al.* (2014) who reported the occurrence of ESBL resistant Enterobacteriaceae in non-human sources like fomites. The occurrence of ESBL producing *K. pneumoniae* isolates from door handles in our study may have implication this is because ESBL resistant *enterobacteria* has evolved as a global threat to human health and can easily be disseminated in settings via inanimate sources (formites) (Thenmozhi *et al.* (2014). The production of ESBL by some β -lactam resistant *K. pneumoniae* isolates is an indication that the enzymes may be responsible for the inactivation of the β -lactam antibiotics by non-ESBL producers may be due to other mechanism like modification of the target site of the β -lactam antibiotics.

The occurrence of ESBL resistance genes is namely; *blaCTX-M*, *blaSHV* and *blaTEM* in ESBL producing isolate an indication that the gene may be responsible for the expression of the ESBL enzymes responsible for β -lactam resistance. The occurrence of ESBL resistance genes in *K. pneumoniae* isolates observed in these studies appears to be first of its kind in the study centres to the best of our knowledge though similar studies on *E. coli* from inanimate objects have been reported by Thenmozhi *et al.* (2014).

Conclusion

The occurrence of *K. pneumoniae* from door handles in the study center's was low and some of the most commonly used antibiotics tested were not effective against the isolates and most of the β -lactam resistant isolates were ESBL producers and the pre-dominant ESBL gene detected was *blaTEM*

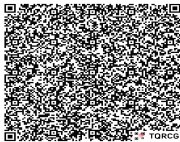
Conflict of Interest statement

The authors declare no conflicts of interest

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