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Prevalence And Genetic Determinants Of Multiple Antibiotic Resistance In Gram-Negative Bacteria From Clinical Samples In Ile-Ife, Nigeria

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ABSTRACT

Background: The antimicrobial resistance exhibited by gram-negative bacilli is a significant issue in controlling infections in the world but under-reacted in Nigeria. The paper examined the two concepts of clinical Gram-negative infection prevalence and genetics of multiple antibiotic resistance (MAR) in Ile-Ife.

Methods: A cross-sectional laboratory research study was conducted at Obafemi Awolowo University Teaching Hospitals Complex between the years between 2020 to 2021. A discovery of three hundred and fifty Gram-negative, non-duplicated isolates softened on different kinds of specimens using the standard phenotypic procedures and using the Microbact mean probe on GNB 24E kits molecular confirmation of the picked isolates validated the findings. It assumed the measurement of antimicrobial susceptibility, using KirbyBF-diffusion-disk and MAR index with microbial resistance (Capture) bla TEM and aa SHV gene: the presence of 1 -lactamases,/ -lactamases--bla strength response- MAR index and virulence genes via 1 -lactamases induction establishes the presence of multidrug dephosphorylation enzymes= -lactamases- kderryya germicoglliens autoresistance to 1-lact

Results: Escherichia coli (31.4) was the second most common followed by Klebsiella pneumoniae (34.8). Most of the isolates (71.4 percent) were MAR index of greater than 0.2 and 32.3 percent were ESBL producers. The most prevalent were blainstTEM (62%), blainstSHV (55%) and blainstCTX-M (48%), sul1 (45%) and tet39 (38%). The ESBL producing E. coli carried critical virulence genes aggR, stx1 and bfpA.

Conclusion: Multidrug-resistant, ESBL -producing Gram-negative bacilli are highly widespread in the Ile-Ife area, and they bear transferable resistance and virulence factors. Stabilized molecular surveillance and antimicrobial stewardship is desperately required to stop their proliferation.

Keywords: *Gram-negative bacteria; antimicrobial resistance; ESBL; resistance genes; Nigeria*

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1. INTRODUCTION

Global Burden of Antimicrobial Resistance

The problem of antimicrobial resistance (AMR) is included in the scope of the most acute crises in public health of the 21 st century. Otherwise it is expected to lead to up to 10 million deaths per year by 2050 and disastrous economic impact in case it remains uncontrolled (O'Neill, 2016) (Adeyemo et al., 2020; Olowo-Okere et al., 2020). According to the World Health Organization (WHO, 2014) and other international health organizations, AMR is potentially undoing decades of healthcare advancement, contributing to more morbidity and death plus escalating healthcare costs (Prestinaci, Pezzotti & Pantosti, 2015). On top of impacting on human health, AMR also impacts on agriculture, food security, and economic

development (O'Neill, 2014) (Alabi et al., 2017; Wilkie et al., 2025).

Gram-Negative Bacteria as High-Priority Pathogens

Players in the global multidrug resistance arena have included gram-negative bacteria (GNB) like Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, as well (Breijyeh, Jubeh and Karaman, 2020). The microbes belong to the infamous ESKAPE list of pathogens such as microorganisms that evade the impact of antimicrobials and cause severe infections in a healthcare setting (De Oliveira et al., 2020). Enzymatic decomposition of antibiotics, mechanism of antibiotic targets modifications, by-overexpression of efflux pumps, and membrane permeability make them less susceptible (Darby et al., 2023) (Olaniran et al., 2022; Wilkie, Alao, Sotala, et al., 2024). These changes cause an increase in the rates of treatment failure, extension of hospitalization, and mortality (Sharma, Misba & Khan, 2017).

Extended-Spectrum β-Lactamases and Emerging Resistance

Production of extended-spectrum β-lactamases (ESBLs), enzymes with penicillin-cephalosporin inactivation capacity, is a significant culprit in the GNB in AMR (Castanheira, Simner and Bradford, 2021) (Adeleye et al., 2024; Eunice et al., 2021). The spread of ESBL genes including blaTEM, blaSHV and blaCTX-M which is taking place in the world has made treatment difficult and augmented need to depend on carbapenems which are now susceptible to the development of carbon farming resistance (Paterson and Bonomo, 2005; Perez, Endimiani, Hujer and Bonomo, 2007) (Adefarakan et al., 2014; Odetoyin & Adewole, 2021)

Nigerian Context and Knowledge Gaps

In Nigeria, multidrug-resistant and ESBL-producing GNB are also on the rise because of the misuse of broad-spectrum antibiotics and a lack of good antimicrobial surveillance (Adeyankinnu et al., 2014; Olowo-Okere and Ibrahim, 2020) (Adebiyi & Balogun, 2025; Wilkie, Alao, Thonda, et al., 2024). Recent news an instance of increasing ESBL-positive E. coli and K. pneumoniae cases both in hospital and community settings (Raphael, Wong & Riley, 2021). Nonetheless, extensive molecular characterization of resistance and virulence determinants has been done, particularly in the southwestern region of Nigeria (Dada et al., 2023; Odih et al., 2023a).

Study Aim

This paper will examine the incidence and hereditary variances of multidrug resistance within the Gram-negative identifying bacteria in Samples of clinical cases in Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife. In particular, it would like to identify the patterns of antibiotic susceptibility, detection of ESBL-producing bacteria, essential resistance and virulence genes, and the genetic relationship among resistant strains. The results will be applied in infection control policies and also lead antimicrobial stewardship issues in Nigeria.

2. LITERATURE REVIEW

Morphology and Biochemistry of Gram-Negative Bacteria

Gram-negative bacteria (GNB) have a singular cell wall state of cytoplasmic membrane that is inner, a periplasmin space and an outer membrane. The inner membrane helps to control the transport of molecules and energy production (Silhavy, Kahne and Walker, 2010) (Ajayi, 2023). Environ Led by the enzymes and proteins necessary to process nutrients and respond to stress, the periplasmic space is situated between the inner and the outer membrane (Miller and Salama, 2018) (Bebe et al., 2020; Odih et al., 2023b; Ugbo et al., 2025). GNB only has lipopolysaccharides (LPS) in the outer membrane and contains LPS that are important in immune responses and pathogenicity (Simpson & Trent, 2019). The lipid A compartment of LPS is very immunostimulatory enough to activate inflammatory host responses (Skrzypczak-Wiercioch & Sałat, 2022). Despite efforts to control pump influx and efflux through outer membrane porins and antibiotic uptake through efflux pumps and impermeability barriers, respectively, all make outer membranes major contributors to resistance (Reygaert, 2018) (Olowe et al., 2012).

Clinically Important Gram-Negative Bacteria

Escherichia coli

Escherichia coli is an enteric, rod-shaped, and facultative bacterium which is mostly commensal but contains pathologic species like enterohemorrhagic (EHEC), enterotoxigenic (ETEC), and enteroaggregative (EAEC) strains (Mueller & Tainter, 2023) (Ajimuda et al., 2022; Ike et al., 2021). Some of the lineages such as EHEC O157:H7 lead to severe diarrheal disease with the hemolytic uremic syndrome (Siniagina et al., 2021). Mueller and Tainter (2023) state that the treatment is becoming more complicated due to increasing antibiotic resistance in extraintestinal pathogenic E. coli (Tanko et al., 2021).

Klebsiella pneumoniae

Klebsiella pneumoniae is an opportunistic gut commensal, and this bacterium has become an important cause of pneumonia, urinary tract infections (UTIs), systemic infections, and wound infection (Effah, Sun and Liu, 2020) (Agusi et

al., 2024b; Egwuatu et al., 2021). Its thick polysaccharide capsule, the production and secretion of lipopolysaccharide, aerobactin (siderophores), and fimbriae, which facilitate adherence, promote virulence (Opoku-Temeng, Kobayashi & Lampel, 2019; Ballen, Mendoza and Ocampo, 2021). Carbapenem resistant K. pneumoniae (CRKP) has become a crisis in the management (Reyes et al., 2019).

Acinetobacter baumannii

Acinetobacter baumannii is a non-epidemic environmental organism, but it is a scourge of healthcare-associated infections, particularly among intensive care units (Ayoub Moubareck and Hammoudi Halat, 2020). It remains viable on the surfaces, forms biofilms, and possesses an incredible ability to develop multidrug resistance and most importantly carbapenem resistance, which makes it a formidable pathogen (Kyriakidis, Vasileiou and Pana, 2021) (Adeyemo et al., 2025; Olowe et al., 2013).

Citrobacter spp.

Such species as Citrobacter freundii and Citrobacter koseri are commonly commensal though may lead to UTIs as well as respiratory infections and bacteremia in immunosuppressed hosts (Ranjan & Ranjan, 2013; Lee, Choi and Kang, 2019). Treatment is complicated by the growing number of reports of multidrug resistant Citrobacter (Pepperell, Kus and Mederski, 2002).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a highly versatile organism and possesses capabilities to live within the hospital settings and develop severe infections in patients with compromised immunity (Reynolds & Kollef, 2021) (Uzairue et al., 2023). It has multifactorial resistance which involves biofilm formation, efflux pumps, and low permeability (Crone, Garvey and McCarthy, 2020).

Gram-Negative Bacteria in Healthcare associated Infections.

GNB are the cause of much of healthcare-associated infections (HAIs) like ventilator-associated pneumonia, catheter-associated UTIs, and bloodstream infections (Oliveira & Reygaert, 2023). These organisms are excellent growers in hospitals, where the treatment of antibiotics is excessive and infection is expected to be hard to manage (Sikora and Zahra, 2023) (Agusi et al., 2024a).

Antibiotic Classes and Mechanisms of Action

Antibiotics show their action via cell wall synthesis inhibition (β-lactams), membrane disruption (polymyxins), protein synthesis inhibition (aminoacid, macrolide, aminoglycoside), and nucleic acid synthesis disruption (Golkar, Bagasra, Pace, 2014) (Ogbolu et al., 2013). This knowledge of such modes of action is essential towards the interpretation of resistance trends.

Mechanisms of Antibiotic Resistance

Insensitivity to GNB has been associated with enzymatic (e.g., β-lactamases) inactivation, target modification, active efflux and decreased resistance of the membrane (Delcour, 2009; Zhang & Cheng, 2022; Gaurav, Malik and Singh, 2023). The resistance gene spread is made easier by the horizontal gene transfer through plasmids, integrons, and transposons (Culyba and Van Tyne, 2021; Crits-Christoph et al., 2022).

Multiple Antibiotic Resistance (MAR)

Multi-antibiotic resistance (MAR) is defined as the ability of bacteria to be resistant to antibiotic classes (at least three) and is closely linked to HAIs (Shallcross and Davies, 2014; van Duin and Paterson, 2020). The causes of MAR include overuse of antibiotics, incomplete use of treatment, and spread of states of clones within healthcare settings (Pulingam et al., 2022).

Extended-Spectrum Beta-Lactamases (ESBLs)

TEM and SHV 3 Likewise, all TEMs and SHVs hydrolyze third-generation cephalosporins and monobactams, providing no therapeutic options (Castanheira, Simner and Bradford, 2021). They are normally plasmid-mediated and therefore spreading very fast (Rawat and Nair, 2010) (Komolafe & Adegoke, 2008). Misuse of cephalosporins and lack of proper infection control have contributed to the dissemination of ESBL-producing EnterobacterIaceae around the globe (Ikeda, Sato and Okazaki, 2012; Majumder et al., 2020). The development of carbapenem resistance is an aspect of alarming tendency in the producers of ESBL (Karaiskos and Giamarellou, 2020).

3. MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional, descriptive, laboratory-based research exploring antibiotic resistance in Gram-negative bacteria (GNB) represented in on a clinical specimen at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria. Microbiological and molecular studies were done at the Department of microbiology, Faculty

of science, Obafemi Awolowo University, Ile-Ife.

Study period: June 2020 to December 2021.

Table 1. Study frame

Item	Details
Design	Descriptive cross-sectional, laboratory-based
Hospital	OAUTHC, Ile-Ife, Nigeria (tertiary referral center)
Laboratory	Dept. of Microbiology, OAU, Ile-Ife
Period	June 2020 – December 2021
Population	In- and out-patients (adults and children) across wards/clinics

Eligibility Criteria

Table 2. Inclusion and exclusion criteria

Category	Criteria
Inclusion	(i) Clinical **Gram-negative bacilli**; (ii) Isolates linked to basic demographic/ward metadata.
Exclusion	(i) Non–Gram-negative bacilli; (ii) Isolates lacking required lab/clinical metadata.

Sample Size and Sampling

A standard single-proportion formula (95% confidence interval, 5% error, prevalence 30%) was used to calculate the sample size, which was 319 isolates, and 350 isolates were used after adding 10 percent for frequent drop-outs.

There were consecutive Gram-negative clinical isolates of in- and out-patients admitted to male / female medical wards, surgical units, pediatrics, and the general outpatient departments. Isolates were shipped on a cold chain nutrient-agar slant and stored at 4 o C awaiting analysis.

Materials

Glassware, Media, and Reagents

They were tested in conical flasks, test tubes, Petri dishes, and standard microbiology equipment. They consisted of media like MacConkey agar, Nutrient agar, Mueller/Hinton agar and CHROM agar. All reagents used were of an analytical grade.

Microorganisms

Isolates collected were: Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Enterobacter spp., Proteus mirabilis, Providencia spp., Citrobacter spp., Acinetobacter spp., Klebsiella oxytoca, Serratia marcescens, Klebsiella spp., Salmonella spp and Serratia odorifera.

Antibiotic Panel

Table 3. Antibiotics used for disc diffusion

Antibiotic	Disc content
Ciprofloxacin	5 µg
Ofloxacin	5 µg
Gentamicin	10 µg
Amoxicillin	10 µg
Ceftazidime	30 µg
Cefuroxime	30 µg
Cefixime	5 µg

Nitrofurantoin	300 µg
Imipenem	10 µg

Collection of Bacterial Isolates

The OAUTHC Medical Microbiology Laboratory collected GNB cultured using clinical specimens (blood, urine, swabs, sputum, aspirates, stool), in in- and out-patients, which were transferred to the university laboratory to be characterized. Secure logs were used to get the demographic/clinical metadata (age, sex, ward/clinic, provisional diagnosis, lab number).

Identification and Confirmation of Isolates

Preliminary Identification

Colonial morphology (size, color, texture, elevation and margin, pigmentation) was examined in colony populated on MacConkey agar at 37 o C taken as 24 hour colonies. Routine biochemical measurements were Gram stain, citrate reduction, of indole, of methyl red(MR), of VogesProskauer, (VP), oxidase, TSI and fermentation of carbohydrates.

Gram staining (summary): Heat-fixed smear results vie arrow violet (1ms) iodine of Gram (1ms) decolorization 95% ethanol safranin (1min) oil-immersion microscopy. Purple color = Gram-positive, pink/red color = Gram-negative.

Confirmatory Identification (Commercial System)

Enterobacterales and non-fermenting GNB indicated through a Microbact GNB 24E micro-substrate panel according to the criteria of the manufacturer. Incubation at 37 o C produced color reactions to give octal codes reading at the species level using the Microbact software (v2.04) with a 24-hour (arginine) incubation maximum.

Antimicrobial Susceptibility Testing (AST)

The Kirby-Bauer disc diffusion was used in Mueller-Hinton agar by AST. Standardized samples The inocula were inoculated (0.5 McFarland, or approximately 10 8 CFU/mL), and plates incubated at 37 C, 1824 h and incubation zones determined (mm) using a calibrated ruler. Findings were classified into Resistant / Intermediate / Susceptible based on the existing interpretive critiques.

Table 4. Quality-control strains

Purpose	ATCC strain
Routine susceptibility QC (Enterobacterales)	*Escherichia coli* ATCC 25922
ESBL/non-fermenter QC	*Pseudomonas aeruginosa* ATCC 700603
ESBL-positive control	*Klebsiella pneumoniae* ATCC 700603
Negative control (Gram-positive)	*Staphylococcus aureus* (lab control)

Multiple Antibiotic Resistance (MAR) Index

The MAR index in the case of each individual isolate was given by: a / b where a is the number of antibiotics to which the given isolate was considered resistant and b the total number of antibiotics the isolate was tested in the case of that particular isolate. Over 0.2 was regarded as exposing to the high-risk threshold and applied to defining multiple resistance.

Phenotypic Detection of ESBL (Double-Disk Synergy Test)

ESBL screening/confirmation The Double-Disk Synergy Test (DDST) was used: on a lawn culture standardized to 0.5 McFarland, neutral discs of cefotaxime (30 µg) and ceftazidime (30 µg) were placed 25 mm (center-to-center) apart, amongst amoxicillin/clavulanate (20/10 µg). Oxygenase secluded zoning of the clavulanate disc ≥ 5 mm and oxyimino-cephalosporin zone, at 1824 h at 37oC, suggested produces ESBL.

Molecular Detection of Resistance and Virulence Genes

Isolate Selection

ESBL, and other resistance genes were screened in all the phenotypically confirmed ESBL producers. A collection of 30 ESBL-positive E. coli culture was further profiled in terms of major virulence genes.

PCR Workflow

Templates were lysates of boiled options and obtained DNA. Gene-specific primers (below) were used in PCR reactions.

The cycling was performed using standard denaturation/ Annealing /extension conditions that were optimized according to target. Where necessary Multiplex PCR was employed in order to augment throughput.

Table 5. 16S rRNA primer set (species confirmation for selected isolates)

Target	Forward (5′→3′)	Reverse (5′→3′)	Amplicon (bp)
16S rRNA (27F/534R)	AGAGTTTGATYMTGGCTCAG	ATTACCGCGGCTGCTGG	520

Table 6. ESBL and other resistance gene primer sets

Gene	Forward (5′→3′)	Reverse (5′→3′)	Amplicon (bp)
tem	GCATCTTACGGATGGCATGA	GTCCTCCGATCGTTGTCAGAA	150
shv	TCCCATGATGAGCACCTTTAAA	TCCTGCTGGCGATAGTGGAT	250
CTX-M	CGGGCRATGGCGCARAC	GCRCCGGTSGTATTGCC	300
sul1	CGGCGTGGGCTACCTGAACG	GCCGATCGCGTGAAGTTCCG	443
tet39	CTCCTTCTCTATTGTGGCTA	CACTAATACCTCTGGACATCA	701

Table 7. Virulence gene primer sets for *E. coli*

Subtype	Target gene	Forward (5′→3′)	Reverse (5′→3′)	Amplicon (bp)
EPEC	**bfpA**	AATGGTGCTTGCGCTTGCTGC	TATTAACACCGTAGCCTTTCGCTGAAGTACCT	410
ETEC	**ST1 (sta)**	GCTAATGTTGGCAATTTTTATTTCTGTA	AGGATTACAACAAAGTTCACAGCAGTAA	190
EAEC	**aggR**	GTATACACAAAAGAAGGAAGC	ACAGAATCGTCAGCATCAGC	254
EHEC	**stx1**	ACACTGGATGATCTCAGTGG	CTGAATCCCCCTCCATTATG	614

Gel Electrophoresis and Visualization

Products of PCR were separated in 2.5% agarose in 1x TBE and ethidium bromide. Ampicillin. Ampicillin was being sized using a 2000bpr DNA ladder. Gels were stained at 100 V over a period of about 25 min and exposed in UV transilluminated condition and photographed. There was a comparison of band sizes and the expected lengths of products as targets.

Sequencing and Phylogenetic Analysis (Selected Isolates)

Sanger sequencing of the representative amplicons was done. Sequences were clipped, aligned (e.g. MUSCLE/ClustalW) and were then compared to databases to ensure they were the same. MEGA was used to construct phylogenetic trees (e.g. Neighbor-Joining/Maximum Likelihood/UPGMA), with model selection being selected accordingly and bootstrap resampling being used to determine node support. Trees were plotted and noted on (e.g. with FigTree).

Data Management and Statistical Analysis

The demographic and laboratory variables were inputted to Microsoft Excel and processed within SPSS software v21.0. Frequencies, proportions, means, and the standard deviations were summarized using the descriptive statistics. Comparison of ESBL to non-ESBL groups on specific antibiotics was done using independent-samples t-test. ANOVA basic was used to determine variation in ESBL/ non-ESBL and crude ratios between species. The level of statistical significance was 0.05 (two tailed); where a statistical significance is indicated 95 percent confidence interval has been provided.

Ethical Approval

The Health Research Ethics Committee of the Obafemi Awolowo University Teaching Hospitals Complex granted us ethical clearance (HREC No.: [enter number in case there is one]). No patient identifiers were garnered, instead anonymized

isolate data were utilized. Since no direct contact with the patient took place, there was no need of informed consent.

Results

Patient Demographics

There were 350 (not duplicated) Gram-negative isolates which were tested on 178 females (50.9) and on 172 males (49.1). The mean age was 42.6 (SD 19.9) or range (1,98) or 3140 (25.1) or >60 (24.6).

Table A. Demographic summary (n = 350)

Variable	n (%)
Female	178 (50.9)
Male	172 (49.1)
≤10 y	9 (2.6)
11–20 y	37 (10.6)
21–30 y	66 (18.9)
31–40 y	88 (25.1)
41–50 y	35 (10.0)
51–60 y	29 (8.3)
>60 y	86 (24.6)
Mean age ± SD	42.6 ± 19.9

Species Composition and Specimen Sources

We obtained 350 Gram-negative bacilli based on various types of specimens, but most urine (n=156) and wound swabs (n=71). The most common were Klebsiella pneumoniae (122) with 34.8 per cent, Escherichia coli (110), Pseudomonas aeruginosa (41) and Enterobacter spp. (22).

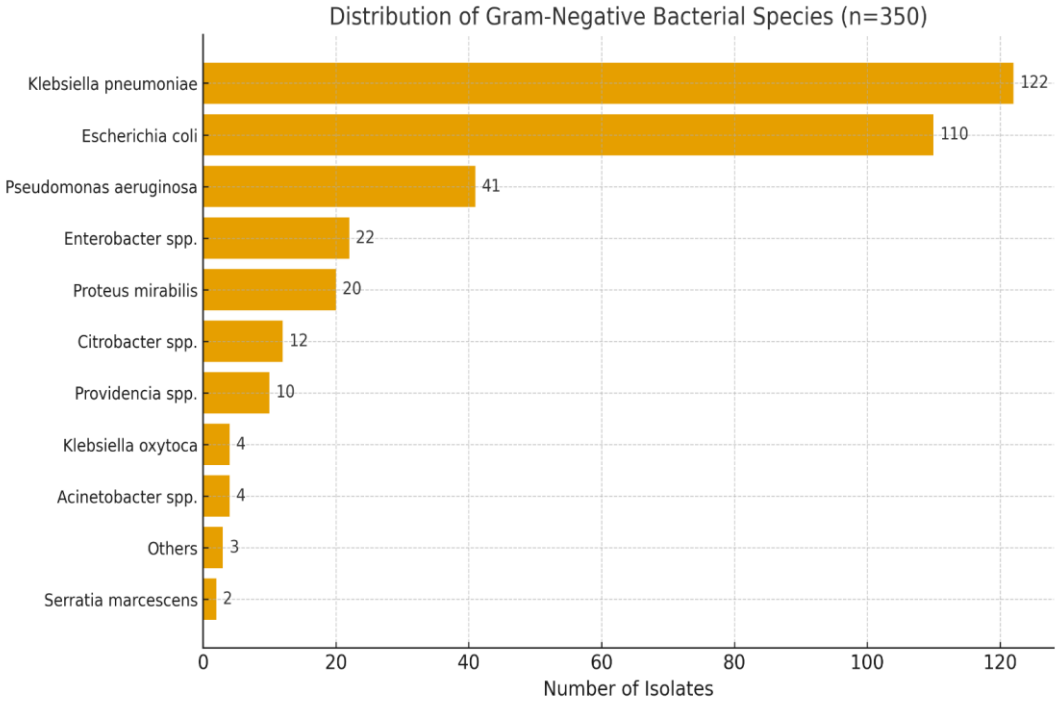

Figure 1. Distribution of major Gram-negative species (bar chart).

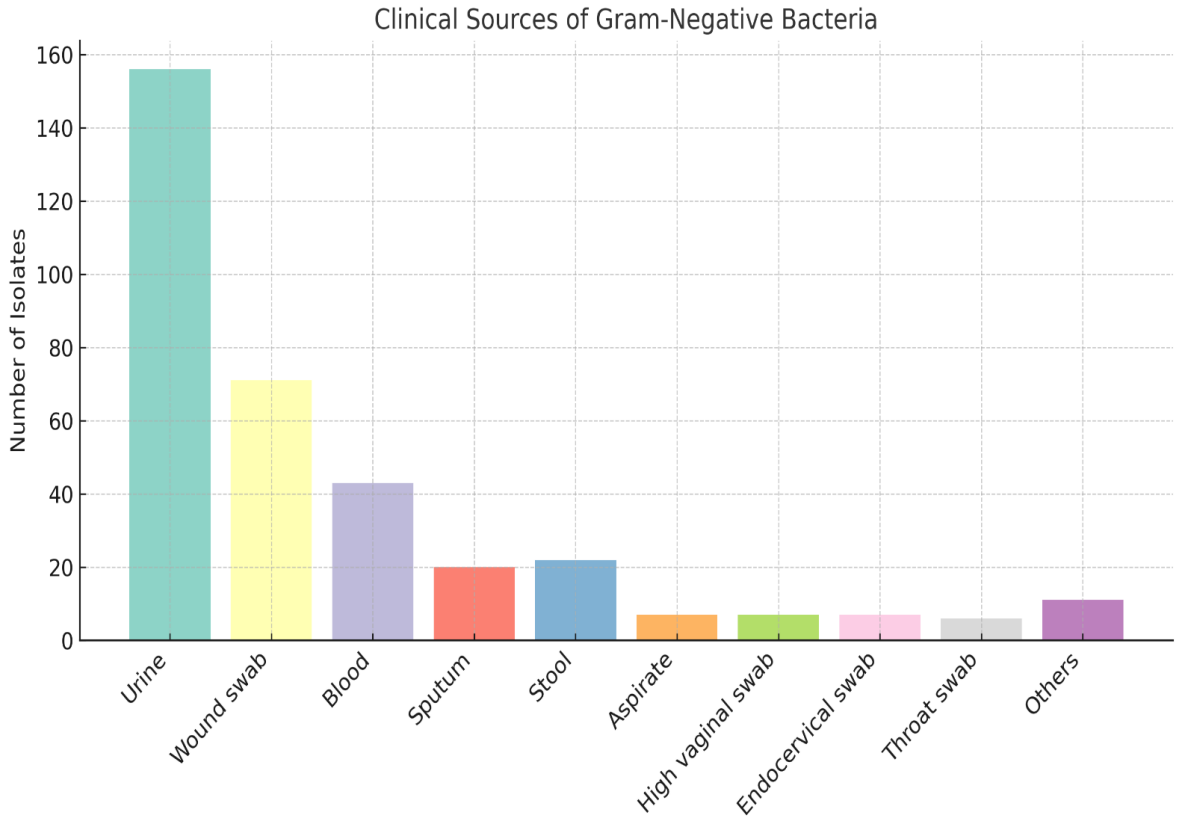

Figure 2. Specimen sources of isolates.

Table B. Top species by frequency

Species	n	%
K. pneumoniae	122	34.8
E. coli	110	31.4
P. aeruginosa	41	11.7
***Enterobacter* spp.**	22	6.3
Others (combined)	55	15.7
Total	**350**	**100**

Antimicrobial Susceptibility (Key Findings)

Among the most predominant pathogens, oral β-lactams and older cephalosporins had the highest rates of resistance, and the imipenem exhibited the highest activity.

K. pneumoniae (n=122): resistance- cefixime 76.2, cefuroxime 75.4, amoxicillin 68.9 imipenem- sensitive 77.1.

H. coli (n=110): resistance-cefixime 69.1, cefuroxime 68.2, ceftazidim64.5amoxicillin 63.6, imipenem susceptible 90.0.

P. aeruginosa (n=41): it was resistant to ceftroxazine methylate (82.9), cefuroxime (82.9), amoxicillin (78.0) and gentamicin (58.5); imipenem sensitive (70.7).

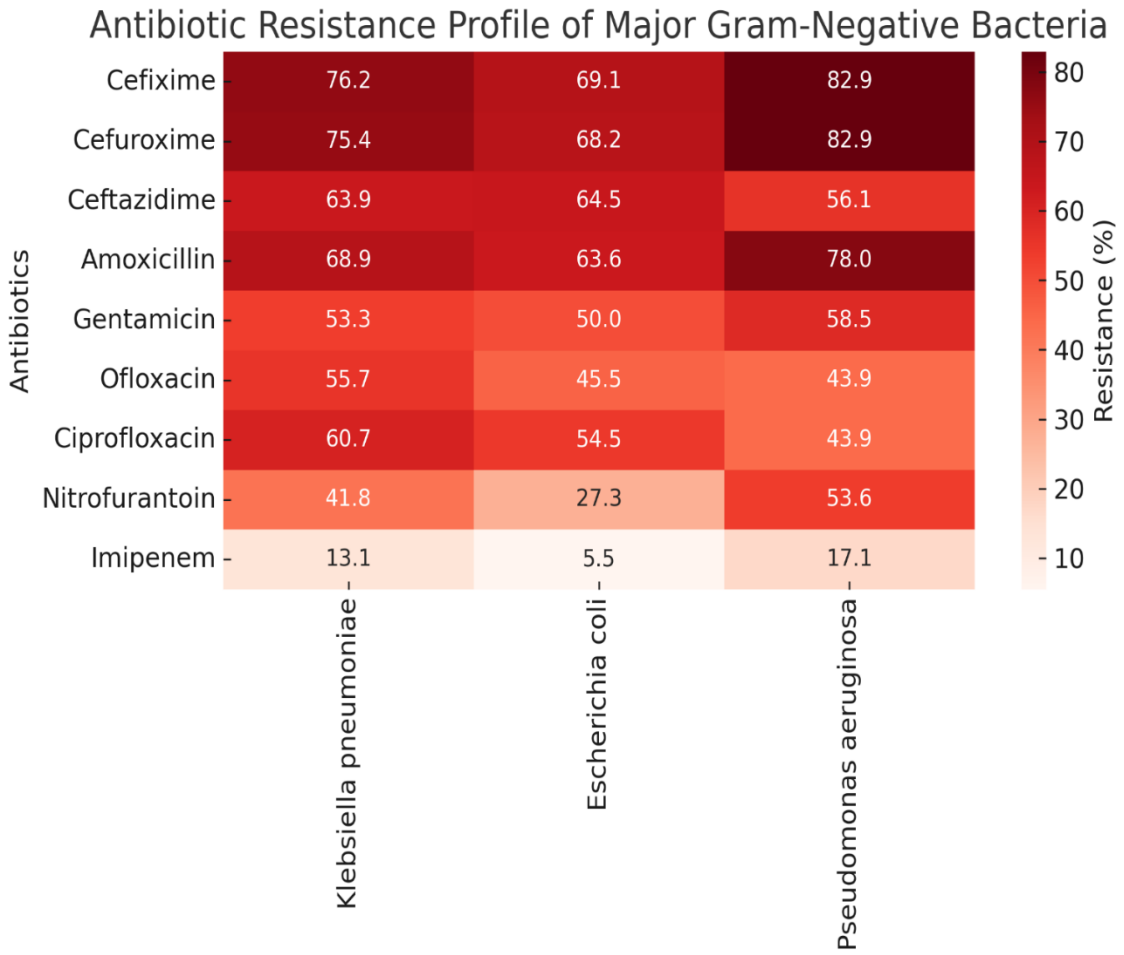

Figure 3. Heatmap of S/I/R profiles for *K. pneumoniae*, *E. coli*, and *P. aeruginosa*.

Table C. Snapshot of susceptibility to key agents

Species	Ciprofloxacin S%	Ceftazidime S%	Cefuroxime S%	Gentamicin S%	Cefixime S%	Amoxicillin S%	Nitrofurantoin S%	Imipenem S%
***K. pneumoniae* (n=122)**	31.9	26.2	18.9	33.6	20.5	15.6	44.3	**77.1**
***E. coli* (n=110)**	40.0	30.9	27.3	38.2	21.8	20.9	61.8	**90.0**
***P. aeruginosa* (n=41)**	43.9	36.6	17.1	31.7	17.1	12.2	31.7	**70.7**

Multiple Antibiotic Resistance (MAR) Phenotypes and Index

Diverse MAR phenotypes were recorded across species. Overall, **261/350 (74.6%)** isolates had **MARI > 0.2**; **34 isolates** had **MARI = 1.0**.

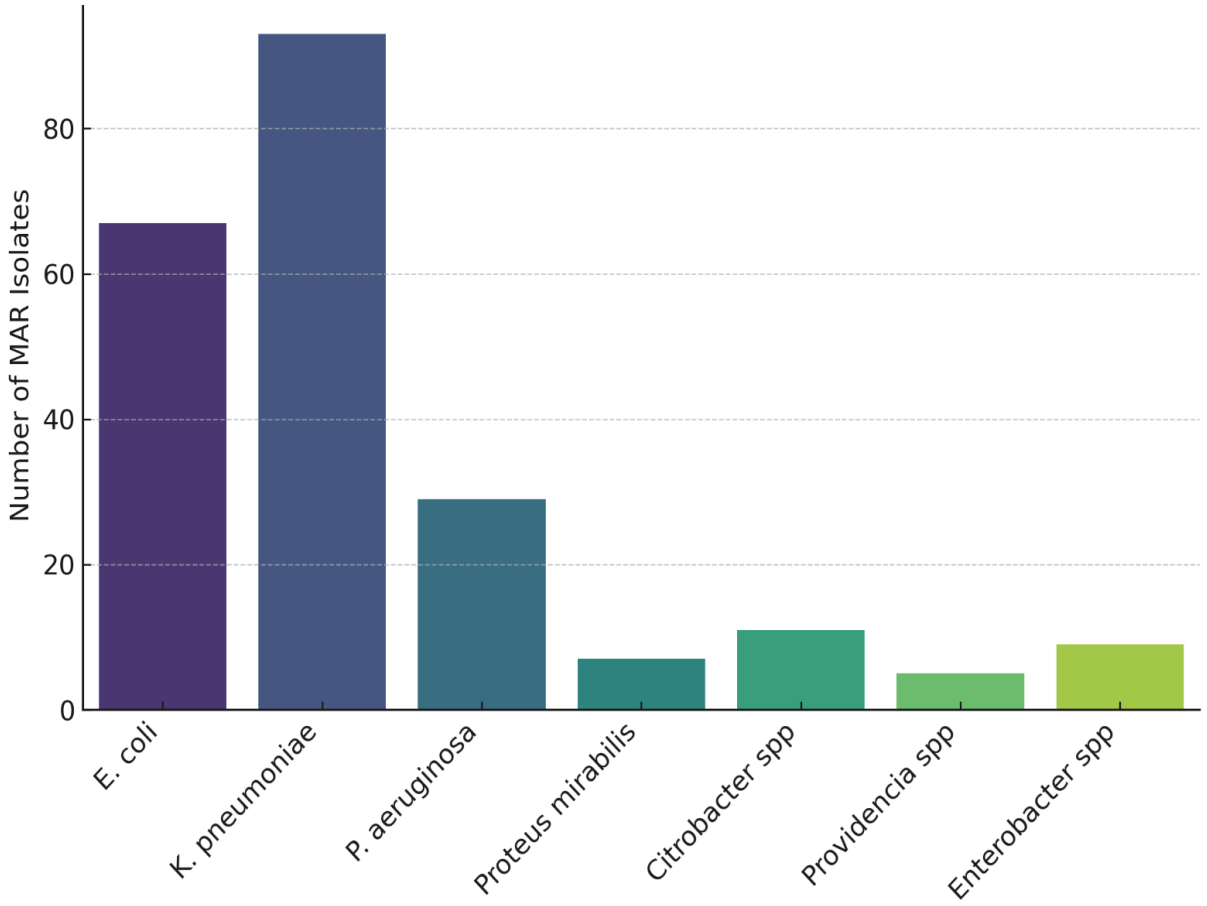

Figure 4. Stacked bars of MARI distribution per species (threshold at 0.2).

Table D. MARI distribution by species

MARI band	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*	*Citrobacter* spp.	*Providencia stuartii*	*Enterobacter* spp.	*A. baumannii*	*Serratia* spp.
0.56	13	15	5	3	5	2	2	2	1
0.67	9	13	9	2	1	2	2	2	0
0.78	21	31	2	0	2	0	1	0	0
0.89	20	16	7	1	0	0	3	0	1
1.0	**4**	**18**	**6**	**1**	**3**	**1**	**1**	**0**	**0**
Total	**67**	**93**	**29**	**7**	**11**	**5**	**9**	**4**	**2**

ESBL Prevalence and Distribution

Overall ESBL prevalence was 113/350 (32.3%). The most represented species were E. coli (46/110; 41.8%), P. aeruginosa (14/41; 36.1) and K. pneumoniae (36/122; 29.5). Mostly frequent by specimen were ESBL-producers in wound swabs (27/71; 38.0%), in urine (51/156; 32.7%); small denominators have had 100% in CSF (2/2) and pleural fluid (1/1).

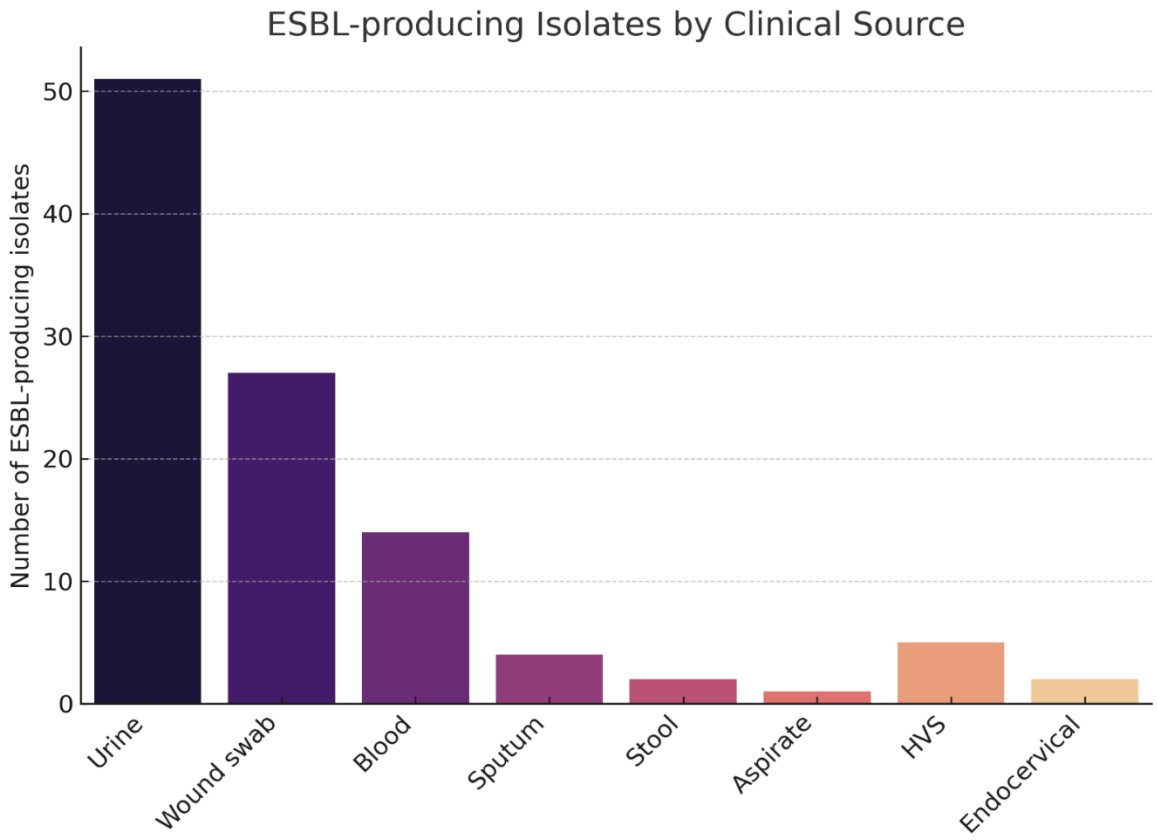

Figure 5. ESBL prevalence by specimen type (bar chart).

Table E. ESBL counts by major species

Species	ESBL+ / total	% ESBL+
E. coli	46 / 110	41.8
K. pneumoniae	36 / 122	29.5
P. aeruginosa	14 / 41	36.1
Others (combined)	17 / 77	—
Total	**113 / 350**	**32.3**

Table F. ESBL-producer distribution by specimen

Specimen	ESBL+ / total	% ESBL+
Urine	51 / 156	32.7
Wound swab	27 / 71	38.0
Blood	14 / 43	32.6
Sputum	4 / 20	20.0
CSF	2 / 2	100.0
Pleural fluid	1 / 1	100.0

Others†	14 / 57	—
Total	**113 / 350**	**32.3**
†Throat/urethral/HVS/endocervical/stool/aspirate/ascitic/ear/pus.		

comparison between ESBL and non-ESBL susceptibility: There were significant differences of ceftazidime (p = 0.004), ciproflaxacin (p = 0.012), and gentamicin (p = 0.034) but other agents indicated no significant trends.

ESBL and Other Resistance Genes

There were 106 ESBL gene targets found in at least one target ESBL gene in populations containing at least one target ESBL gene (n=113), ESBL-phenotypic isolates. General prevalence: blaTEM 35.4% blaCTX-M 31.0% blaSHV 13.3%. Co-carriage blaCTX-M/blaTEM, blaTEM/blaSHV, and all three (blaSHV/blaCTX) (n=1, 3, 9, respectively). Other resistance genes: sul1(n=45; 40.0) and tet (n=13; 11.5).

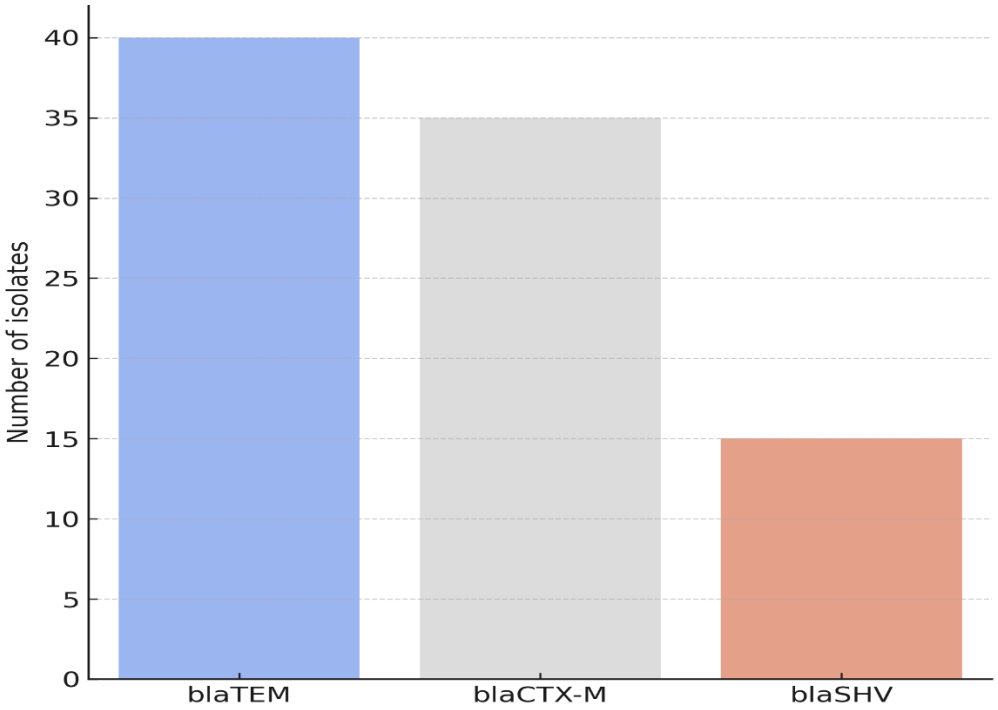

Figure 6. ESBL gene carriage (single and combined) with adjunct bars for sul1 and tet.

Table G. ESBL gene patterns among ESBL-phenotypic isolates (n=113)

Gene pattern	n	% of ESBL+
blaTEM	40	35.4
blaCTX-M	35	31.0
blaSHV	15	13.3
blaCTX-M + blaTEM	9	8.0
blaTEM + blaSHV	3	2.7
blaCTX-M + blaSHV	1	0.9
blaCTX-M + blaTEM + blaSHV	3	2.7

Demographic relationships: No significant ESBL status to sex/age overall, blaTEM ($p = 0.038$) and blaCTX-M ($p = 0.040$) difference by age present greater frequency with increasing age, where there was higher frequency in 185 y and 3660 y.

Virulence Genes in Selected E. coli

Virulence genes were identified in 30 ESBL-positive E. coli in the following manner: ST1/sta (16.7%), aggR (13.3%), stx1 (10.0%), bfpA (6.7%).

Table H. Virulence gene occurrence in *E. coli* (n = 30)

Gene (pathotype)	n	%
ST1/sta (ETEC)	5	16.7
aggR (EAEC)	4	13.3
stx1 (EHEC)	3	10.0
bfpA (EPEC)	2	6.7

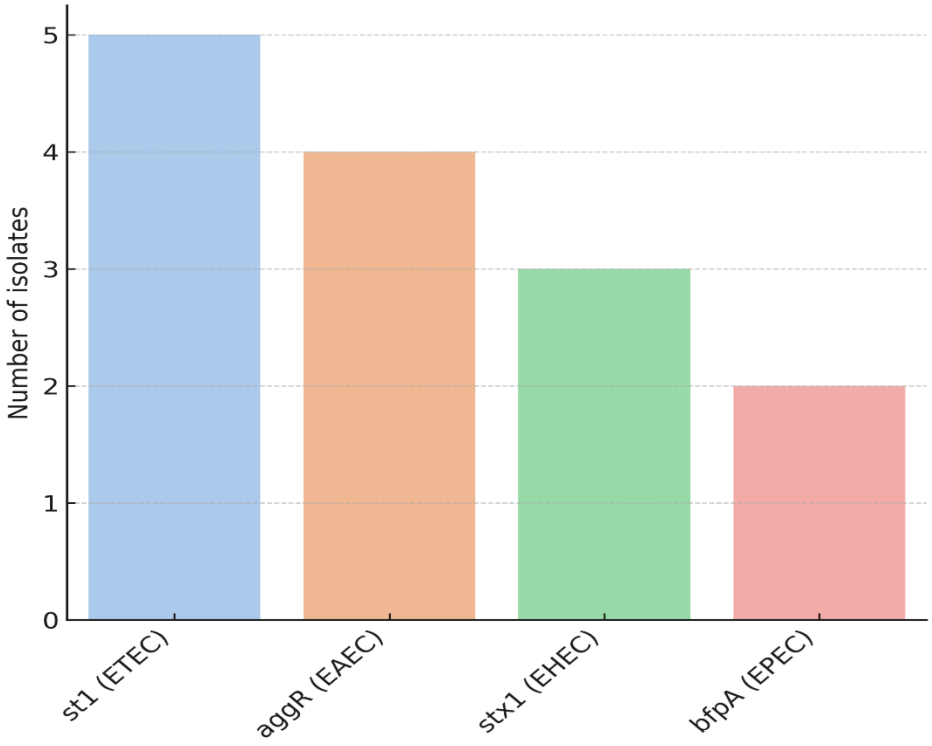

Figure 7. Virulence gene profile of selected *E. coli* isolates (stacked bar).

Sequencing and Phylogeny (Selected Isolates)

Sixteen-S rRNA sequencing confirmed species-level identities with **>99.5–100%** similarity for representative *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates with deposited accessions.

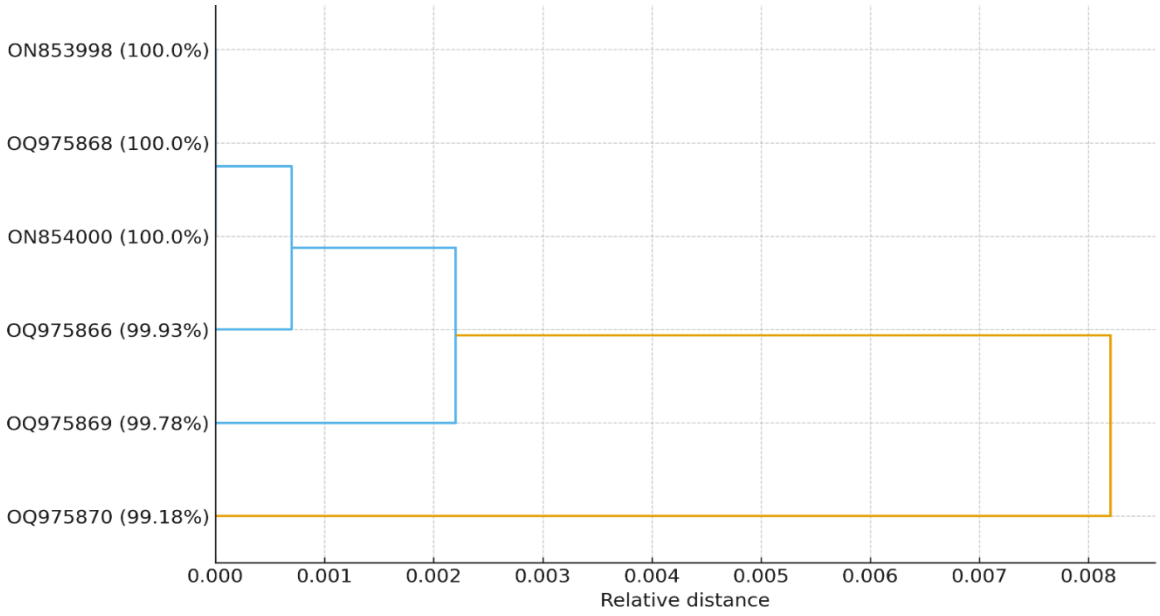

Figure 8. Phylogenetic tree of *K. pneumoniae* isolates (a *P. aeruginosa* tree was not constructed due to limited numbers).

Brief summary

These included K. pneumoniae, and E. coli; resistance levels with older cephalosporins were high in the oral area but the most active was imipenem (7090 percent susceptible). The prevalence of the blaTEM, esBLCTX-M, and common sul1 co-carriage were 32 percent with urine and wound isolates predominant. The presence of MARI > 0.2 in 3/4 isolates supported the fact that significant pressure is present as of multidrug resistance.

4. DISCUSSION

The research results indicate that Gram-negative bacterial isolates obtained at different types of clinical samples have a high level of antimicrobial resistance in one of the tertiary hospitals, South-West Nigeria. The prevalence of Klebsiella pneumoniae and Escherichia coli is in line with the global incidence of Enterobacterals which assert control in healthcare associated and community-based infections. Detailed descriptions of the high count of urinary and blood isolates highlights clinical importance of same among at-risk patients including those harboring invasive devices or those admitted to hospitals at a longer time period.

The domain of our findings on the high prevalence of resistance to popular antibiotics, such as amoxicillin, cefuroxime, cefixime, and ceftazidime, underlines the loss of 20-year-old Third, the entire domain of 2 -lactam efficiency. The indexing of the multiple antibiotic resistance (MAR) indicated that a majority of the samples harbored, had, MAR indices above 0.2, which shows that they have been exposed to high levels of antibiotic selection pressure in the hospital. The trend is similar to the one found in other low- and middle-income settings with the spread of resistance being engineered by empirical prescribing ease of availability to the antibiotics bought over the counter, as well as lack of proper infection control.

This was evident in the production of extended-spectrum 2 -lactamase (ESBL) that was widespread and evident in K. pneumoniae and E. coli, with the TEM, SHV, and CTX-M family of 2 -lactamase uses confirmed via the practice of molecular analysis. Even more complicated, two or more ESBL determinants are present in individual isolates, which further complicates treatment to the point that carbapenems are yet other effective interventions. Alarmingly, a few of the isolates were too carbapenin-resistant that indicates the emergence of potential appears of carbapenemase producers, which advises the concern of diminishing therapeutic choices.

Phylogenetic comparison between representative isolates did not only confirm genetic diversity of circulating isolates, but also demonstrated clustering of closely related lineages within the hospital, indicating indications of local transmission of resistant lineages, or protean resistance to extinction of resistant lineages. The articles reveal how important the genomic surveillance results are to track the trends in the resistant strains and guide infection prevention measures.

Our results allude to the significance of the solid antibiotic stewardship measures, including the guided empiric therapy based on the revised local susceptibility information, the restrictive intake of high-end antibiotics, and also strict subsequent

infection control actions. An earlier prediction of therapeutic failure could be conducted than that provided by phenotypic mechanisms, though the incorporation of genuine surveillance systems through the use of molecular identification of resistance determinants.

5. CONCLUSION

This paper is convincing evidence that Gram-negatives in a primary healthcare facility in Nigeria express worrying patterns of multidrug resistance and ESBL production, which occurs due to a combination of heterogeneous genetic determinants with healthcare-associated selective pressure. K. pneumoniae and E. coli are the most common culpable with high levels of resistance to first line antibiotics and apprehensive indications of carbapenam resistance. Global and local monitoring of resistance, assimilation of molecular diagnostic, and antimicrobial stewardship of infection is urgent in order to reduce the rate of resistance transmission and require left over therapeutic choices.

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