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gyrB-BASED MOLECULAR IDENTIFICATION AND PHYLOGENETIC RELATEDNESS OF UROPATHOGENIC Klebsiella quasipneumoniae IN TWO TERTIARY HOSPITALS IN SOUTHWEST NIGERIA

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gyrB-BASED MOLECULAR IDENTIFICATION AND PHYLOGENETIC RELATEDNESS OF UROPATHOGENIC *Klebsiella quasipneumoniae* IN TWO TERTIARY HOSPITALS IN SOUTHWEST NIGERIA

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most frequent bacterial infections worldwide and pose a major public health problem. *Klebsiella* species are primary causes of UTIs and are often associated with drug resistance. In this study, we sequenced and compared the *gyrB* gene of uropathogenic *Klebsiella* spp. isolates from two tertiary hospitals in southwest Nigeria with reference strains of the *Klebsiella* pneumoniae complex available in GenBank to determine their phylogenetic relatedness and bridge the knowledge gap on the genetic relatedness of uropathogenic *Klebsiella* spp. in southwest Nigeria.

Method*s*: We collected 227 urine samples from University College Hospital Ibadan and Federal Medical Centre Abeokuta, Ogun State, Nigeria, and biochemically identified *Klebsiella* spp. Sequencing of the *gyrB* gene was conducted on 7 isolates based on resistance patterns. Phylogenetic analysis was done by NGPhylogeny.fr, and the phylogenetic tree was visualized by Interactive Tree of Life (iTOL).

Results: Of the cultured samples, 96 colonies appeared morphologically as *Klebsiella* spp-like on Eosin Methylene Blue agar (EMB). Biochemical identification revealed that *K*. *pneumoniae* (42) and *Klebsiella oxytoca* 22 made up the *Klebsiella-like* isolates, with the remaining (32) being *Enterobacter* spp. Seven randomly selected *Klebsiella pneumoniae* isolates were identified as *Klebsiella quasipneumoniae* using the *gyrB* gene sequence. Phylogenetic analysis revealed that the isolates from the two hospitals clustered closely with reference strains, indicating shared evolutionary lineages.

Conclusion: The uropathogenic *Klebsiella* spp. isolates from the two hospitals in southwest Nigeria are closely related to members of the K. pneumoniae complex. These findings underscore the importance of continued molecular surveillance and suggest that conserved transnational lineages are involved in proper taxonomic classification.

Keywords: *Klebsiella pneumoniae* complex; *gyrB* sequencing; *Klebsiella quasipneumoniae*; Urinary tract infection; phylogenetics; Uropathogenic; Nigeria.

INTRODUCTION

Urinary tract infections (UTIs) are significant clinical issues of the urinary system that affect the kidneys, ureters, bladder, or urethra. Bacteria are identified as a major causative agent of UTIs, followed by fungi such as *Candida* spp [31]. Nearly 35% of hospital-acquired infections (HAIs) are caused by UTIs. In Nigeria, the prevalence of UTIs varies based on demographic group, ranging from 4% to 60%, which significantly increases morbidity and is a key reason for hospital visits [16]. Individuals with neurological disorders that might impair urine flow, anatomic anomalies of the urinary system, diabetics, and hospitalized individuals are all susceptible to urinary tract infections (UTIs) [32]. Side effects from UTIs include sepsis, recurrences, and increasing kidney damage [20]. In sub-Saharan Africa, UTIs pose health challenges in vulnerable groups such as pregnant women, young adult females, and people with compromised immunity due to inaccessibility of sanitation and limited clean water, and an increase in antimicrobial resistance to first-line antimicrobial drugs.

The major bacteria causing UTIs include *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., and *Enterococcus* spp. [16]. The prevalence of antibiotic resistance has increased globally over the last few decades. Multiple drug-resistant bacteria are now known to pose a serious risk to patient safety as well as that of healthcare providers [2]. UTIs are promoted by factors such as extended hospital stays, cross-transmission, long-term antimicrobial medication, and invasive operations [15]. Multiple drug-resistant bacteria are linked to a high mortality/morbidity rate, higher medical expenses, and a variety of diseases, including wound infection, pneumonia, and urinary tract infections [50]. Antimicrobial susceptibility patterns vary based on geographical regions, individuals, and hospital types/units due to variations in environmental factors and antibiotic use [3].

Klebsiella species are opportunistic, Gram-negative, rod-shaped, lactose-fermenting, and non-motile human pathogens that are commonly found in the mouth, nose, and gastrointestinal tract as normal flora. *Klebsiella* species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens [23]. The majority of human *Klebsiella* infections are caused by *K. pneumoniae* and *K. oxytoca*. Infections are more common in the elderly and those with other underlying diseases, such as cancer, and most infections occur as a result of contamination of an invasive medical device [19]. Multiple-drug-resistant *Klebsiella* spp. remain an important hospital-acquired bacterial pathogen with the prevalence of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella* spp. as high as 47.3%, especially in intensive care units and surgical wards where hospital stay is prolonged [9]. This antimicrobial resistance is thought to be associated mainly with multidrug efflux pumps, innate drug-resistant enzymes such as sulfhydryl variable-1 (SHV-1), and the acquisition of drug-resistant enzymes [50].

In Nigeria and at large, hospital-acquired infection surveillance and monitoring lag compared to developed nations, whereas in certain other nations, mandated surveillance laws are absent [4]. Control efforts have been hampered in Nigeria as well by a lack of awareness of the risks associated with hospital-acquired infections (HAI) and risk-reduction strategies. Identifying the origins and modes of illness transmission and implementing data-driven prevention guidelines and policies is essential for preventing HAIs [24]. Molecular epidemiological studies are important in elucidating transmission routes, following resistant clones, and establishing evidence-based preventive policies. Nevertheless, there is limited molecular data on Uropathogenic *Klebsiella* spp.; earlier studies have identified important clinically circulating lineages in southwestern Nigeria. Whole-genome sequencing of *Klebsiella* spp. isolates obtained from three hospitals in southwest Nigeria was conducted by [52], with their result identifying diverse *Klebsiella pneumoniae* complex members.

This study characterizes uropathogenic *Klebsiella* species by comprehensively analyzing their *gyrB* genetic relatedness comparing them to publicly available genomes. The gene *gyrB*, coding for the B subunit of DNA gyrase, possesses a higher resolution for species differentiation within the *K. pneumoniae* complex compared to the 16S rRNA gene. This research will help molecular surveillance efforts and guide targeted clinical interventions for prevention and management of UTIs in this region.

MATERIALS AND METHODS

SAMPLE COLLECTION

Study Population

The population of interest comprised patients diagnosed with Urinary Tract Infection attending University College Hospital, Ibadan, Oyo State, Nigeria, and the Federal Medical Centre, Abeokuta, Ogun State, Nigeria.

Sample type

Urine samples were collected midstream from consented patients with Urinary Tract Infection.

INCLUSION AND EXCLUSION CRITERIA

- Inclusion Criteria include: clinical urine samples collected from patients with suspected urinary tract infection attending both UCH Ibadan and FMC Abeokuta in Southwest Nigeria
- Exclusion Criteria: samples from patients who decline consent, patients outside the study area, duplicate samples, and non-urinary tract infection-related samples

Collection of samples

Consenting patients diagnosed with UTI were instructed to wash their hands before handling collection materials, and the collection container was carefully opened to avoid contamination. Three sterile towelettes were provided for cleaning the urinary opening. After urinating, the initial 20-25 mL of urine was discarded into the toilet. The midstream sample was collected in a sterile container [9], and the remainder was voided into the toilet. Samples were transported to the laboratory in an ice pack within 5 hours

Samples were collected between June and August 2025. One hundred and twenty samples were collected from UCH Ibadan and the remaining one hundred and seven samples from FMC Abeokuta, Ogun State.

Cultures and Lactose fermentation test

The samples were incubated on Eosin Methylene Blue agar (EMB) and incubated at 37 °C for 24 hours. Distinct morphological colonies were observed and recorded for distribution, and the lactose-fermenting colonies (appearing pink) were picked and stored on nutrient agar slants.

Identification of organisms

Pure cultures of bacteria were stained using Gram's method and then tested with various biochemical assays. The tests performed included: oxidase, urease, indole, methyl red, catalase, VP test, H_2S test, citrate, and motility.

Gram staining method

A small amount of sterile water was placed on a clean microscope slide, and a flamed wire loop was used to emulsify a bacterial colony from the slant to prepare a thin smear.

The air-dried smear was placed on the staining rack and flooded with crystal violet stain for 1 minute. The crystal violet was then gently washed off with water, drained, and the excess water was dried with a paper towel by gently tapping the slide on the towel. The smears were flooded with iodine and retained on the slide for 1 minute, followed by washing the iodine off with slow-running tap water. Excess water was then drained, and the slide was decolorized with acetone-alcohol (1:1). Immediately afterward, it was washed with tap water, drained, and counterstained with safranin for 2 minutes, then rinsed with tap water. The slide was observed under bright field microscopy with oil immersion at 1000x magnification [26].

Indole Test

Bacteria were inoculated into 4 ml of peptone broth and then incubated at 37°C for 24-48 hours. 0.5 mL of Kovac's reagent was added to the broth culture; production of indole ring within 30 seconds to 1 minute indicates a positive result [46]

Methyl-red Test

The medium was allowed to reach room temperature before being inoculated with a pure loopful culture, then incubated for 24 to 48 hours at 37°C. After 24 hours of incubation, a 1 mL aliquot of the broth was transferred to a clean test tube, and 2-3 drops of methyl red indicator were added to the aliquot. Development of a distinct red color indicates a positive result [5]. The remaining broth containing the organism was re-incubated for an additional 24 hours. Immediate observation of the red color was recorded.

Voges-Proskauer Test

Five milliliters of MR-VP broth from the preincubated culture were transferred into a clean test tube, 0.6 mL of Barritt's reagent A was added, followed by 0.2 mL of Barritt's reagent B. The tube was gently shaken for approximately 1 min to allow adequate aeration and then allowed to stand at room temperature. Pink to red coloration was observed indicating positive result [5].

Citrate Test

Simmons citrate medium agar slant was aseptically inoculated with the isolate's colony. The cultured slant was then incubated at 35°C for 24-48 hours [26].

Urease Test

Christensen's urea agar slant was adequately inoculated over the entire surface by streaking in a zigzag pattern, followed by incubation at 35-37°C for 24-48 hours of the inoculated Agar slant with its cap loosened [8].

Oxidase Test

A sterile swab stick was used to obtain a growth from the slant; a dropper pipette was then used to drop 1% tetramethyl-*p*-phenylenediamine dihydrochloride onto the culture on the swab [51].

Motility and sulfur utilization Test

A sterile inoculating needle was used to pick and stab deeply into the sulfur, indole, and motility (SIM) agar; the agar containing inoculum was then incubated at 37 °C for 24 hours. Production of black color was observed, indicating H_2S production, and motility was observed under the microscope to complement the migration pattern observed in the agar [45].

Molecular Characterization:

Genomic DNA Extraction

Genomic DNA was extracted from bacterial isolates using the ZR Fungal/Bacterial DNA MiniPrep™ Kit according to the manufacturer's instructions. Briefly, 100 mg of bacterial cells suspended in 200 µL of sterile water were transferred into a ZR BashingBead™ Lysis Tube containing 750 µL of Lysis Solution. The samples were homogenized using a bead beater at 4,000

rmp for 5 min and centrifuged at 10,000 × g^m for 1 min. Approximately 400 µL of the clarified supernatant was transferred to a Zymo-Spin™ IV Spin Filter and centrifuged at 7,000 × g^m for 1 min. The filtrate was mixed with 1,200 µL of Fungal/Bacterial DNA Binding Buffer supplemented with 0.5% (v/v) β-mercaptoethanol. The mixture was loaded onto a Zymo-Spin™ IIC Column in two successive aliquots of 800 µL, with centrifugation at 10,000 × g^m for 1 min after each loading. The column was washed with 200 µL of DNA Pre-Wash Buffer followed by 500 µL of Fungal/Bacterial DNA Wash Buffer, centrifuging at 10,000 rpm for 1 min after each wash. Finally, the column was transferred to a sterile 1.5 mL microcentrifuge tube, and genomic DNA was eluted with 100 µL of DNA Elution Buffer by centrifugation at 10,000 × g for 30 s. The purified DNA was stored at −20°C until further molecular analyses [6].

PCR Amplification

Amplification of the target genes (*leuS, rpoB, gyrB, pyrG, and rplB*) was performed using primer sets listed in Table 1. Each amplification was conducted in a 24.5 µL reaction mixture containing 2 µL of extracted DNA template, 12 µL of 2× PCR Master Mix, 8 µL of primer mix, and 2.5 µL of nuclease-free water. Polymerase chain reaction was carried out in a thermocycler under the following conditions: an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 50°C for 10 s, and initial extension at 72°C for 60 s. A final extension was performed at 72°C for 7 min, after which the reaction was held at 4°C until the samples were retrieved [34].

DNA sequencing and analysis

Purified *gyrB* gene amplicons from selected *K. pneumoniae* isolates were bidirectionally sequenced using the same primers used for amplification; the analysis was conducted using an ABI 3500XL genetic analyzer at Inqaba Biotec, Pretoria, South Africa.

Raw chromatograms were observed for sequence quality, and the bidirectional sequences were assembled into a consensus sequence using BioEdit version 7.2.5. Bases with low quality were trimmed at both ends. Consensus sequences were BLASTed against the National Center for Biotechnology Information (NCBI) database for determination of species identity based on percentage similarity.

The edited Nucleotide sequences were uploaded to the NCBI GenBank database using the Bankit online portal, where all metadata were included before the allocation of an accession number.

Phylogenetic analysis

MAFFT alignment algorithm was used to perform multiple sequence alignment using the NGPhylogeny.ft web server. Reconstruction of the phylogenetic tree was done using FastTree, employing the general time reversible (GTR) mode, branch support were estimated using Shimodaira-Hasegawa-like local support value. The resultant tree was visualized using iTOL version 6.

Table1: List of House-keeping gene Primers used in Amplification [21].

Genes	**Primers**	**Expected band size (bp)**
gyrB	'F- 5'-TCGACGAAGCGCTCGCGGGTCACTGTAA -3'	400-1000
	R- 5'- GCAGAACCGCCCGCGGAGTCCCCTTCCA 3	
leuS	F- 5'-GATCARCTSCCGGTKATCCTGCCGGAAG-3'	500- 800
	R- ATAGCCGCAATTGCGGTATTGAAGGTCT-3'	
pyrG	F- 5'-AYCCBGAYGTBATTGCRCAYMAGGCGAT-3'	400-900

	R-5’-GCRCGRATYTCVCCCTSHTCGTCCCAGC-3’	
rplB	F-5’-GTAAACCGACATCTCCGGGTCGTCGCCA-3’	500-700
	R-5’-ACCTTTGGTCTGAACGCCCCACGGAGTT-3’	
rpoB	F-5’-CCGAACCGTTCCGCGAACATCGCGCTGG-3’	300 -700
	R-5’-CCAGCAGATCCAGGCTCAGCTCCATGTT-3’	

Results

Bacterial isolation and distribution.

Of the 227 urine samples collected and cultured on Eosin Methylene Blue agar, 214 urine samples were recovered. Eighty-one of the isolates were morphologically identified as *Escherichia coli*, followed by *Klebsiella*-like organisms with 96 (i.e *Klebsiella* spp and *Enterobacter* spp). While the non-lactose fermenters are 37 and Thirteen plates showed no growth as shown below.

Table 3:

	Morphological appearance on EMB agar	**Number of Sample**	**Lactose Reaction**	**Probable Organism**
1	Dark purple colonies with metallic sheen	81	Lactose Fermenter	*Escherichia coli*
2	Pink mucoid colonies	96	Lactose Fermenter	*Klebsiella* spp *and Enterobacter* spp
4	Colourless to pale colonies	37	Non-lactose Fermenter	*Proteus spp, Pseudomonas* spp., *Providencia* spp, *Acinetobacter* spp etc
5	No growth observed	13	No growth	No growth
	Total = 227			

Appearance and distribution of Bacterial isolates from urine samples.

Biochemical characteristics of *Klebsiella* isolates.

Of the 96 *Klebsiella*-like isolates, 42 of them were identified as *Klebsiella pneumoniae*. 22 as *Klebsiella oxytoca* and 32 were identified as *Enterobacter* spp. The indole test was used to distinguish *K. pneumoniae* (-) from *K. oxytoca* (+). A motility test was also employed to distinguish *Klebsiella* spp. from *Enterobacte*r spp. as shown in the Table 4 below.

Table 4: **Biochemical characteristics of *Klebsiella* spp *and Enterobacter* spp. isolated from urine samples.**

S/N	Biochemical Tests	*K. pneumoniae*	*K. oxytoca*	*Enterobacter spp*
1	Lactose	+	+	+
2	Catalase	+	+	+
3	Urease	+	+	+
4	Citrate	+	+	+
5	Methyl red	-	-	-
6	Voges-Proskauer	+	+	+
7	H_2S	-	-	-
8	Indole	-	+	-
9	Oxidase	-	-	-
10	Motility	-	-	+

Detection of housekeeping and ESBL genes

Amplification of the five housekeeping (*leuS, gyrB, rpoB, rplB, and pyrG*) genes from the seven selected isolates were successful, with the amplicon band size of each gene falling into expected band size (*leuS*= 862-890bp, *gyrB*= 856-880bp, *rpoB* = 100-108bp, *pyrG*= 890-899bp and *rplB*= 905-953bp) .

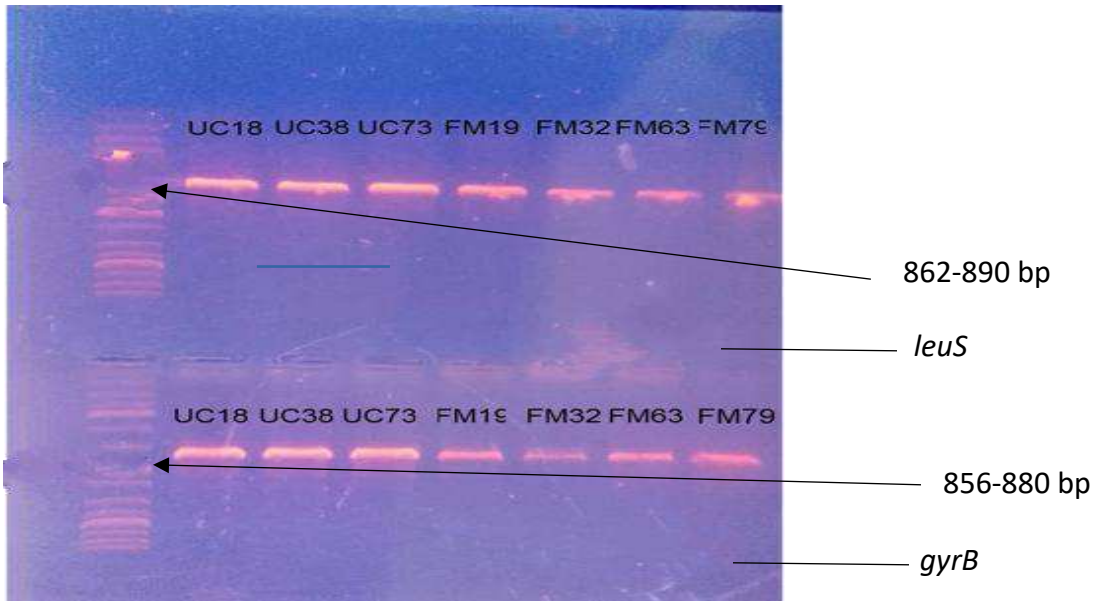


Fig 1: Gel electrophoresis image of amplified *leuS* and *gyrB*

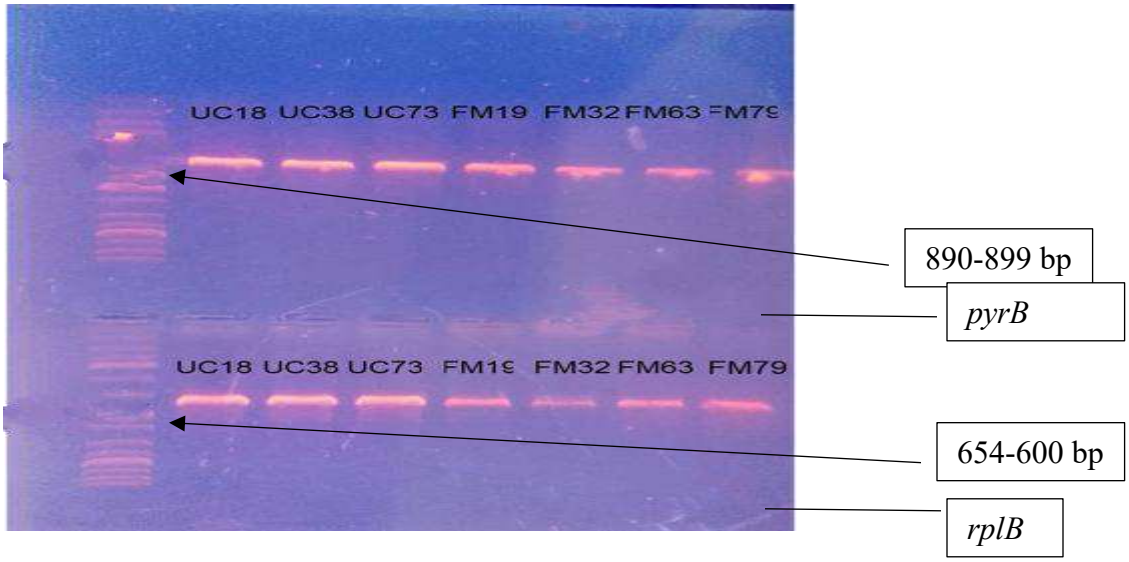
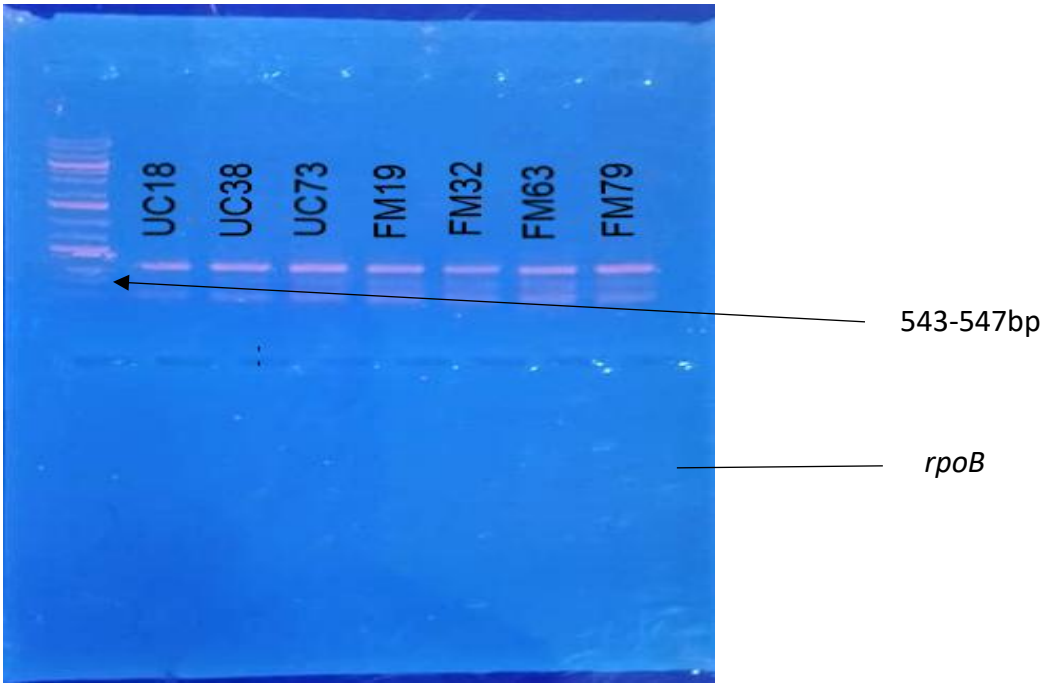


Fig 2: Gel electrophoresis image of amplified *pyrB* and *rplB*



gyrB gene sequence, similarity, and phylogenetic analysis

The BLAST results confirmed a close match between our isolates and *Klebsiella quasipneumoniae* reference strains, with a high similarity of 99.69% (Table 5). The phylogenetic tree shows short branch lengths and tight clades, indicating that these locally isolated *Klebsiella quasipneumoniae* are highly conserved and closely related to one another and to other international members of the *Klebsiella pneumoniae* complex as shown below.

Table 5: gyrB BLAST analysis result of the seven sequenced isolates from urine samples.

Isolate	Accession Number	Gene	Close match	Accession Number
UC18	PZ111488	gyrB	*Klebsiella quasipneumoniae* strain 68-17 chromosome complete genome	CP042319
UC32	PZ111489	gyrB	*K. quasipneumoniae subsp Similipneumoniae* strain ACESH00366hy	CP138833.1
UC73	PZ111490	gyrB	*Klebsiella quasipneumoniae* S1174-1	CP063874
FM19	PZ111491	gyrB	*Klebsiella quasipneumoniae* Strain 174-1	CP063874
FM32	PZ111492	gyrB	*Klebsiella quasipneumoniae subsp Similipneumoniae* strain ACESH00366hy	CP138833.1
FM63	PZ111493	gyrB	*Klebsiella quasipneumoniae subsp Similipneumoniae* strain ACESH00366hy	CP138833.1
FM79	PZ111494	gyrB	*Klebsiella quasipneumoniae subsp Similipneumoniae* strain ACESH00366hy	CP138833.1

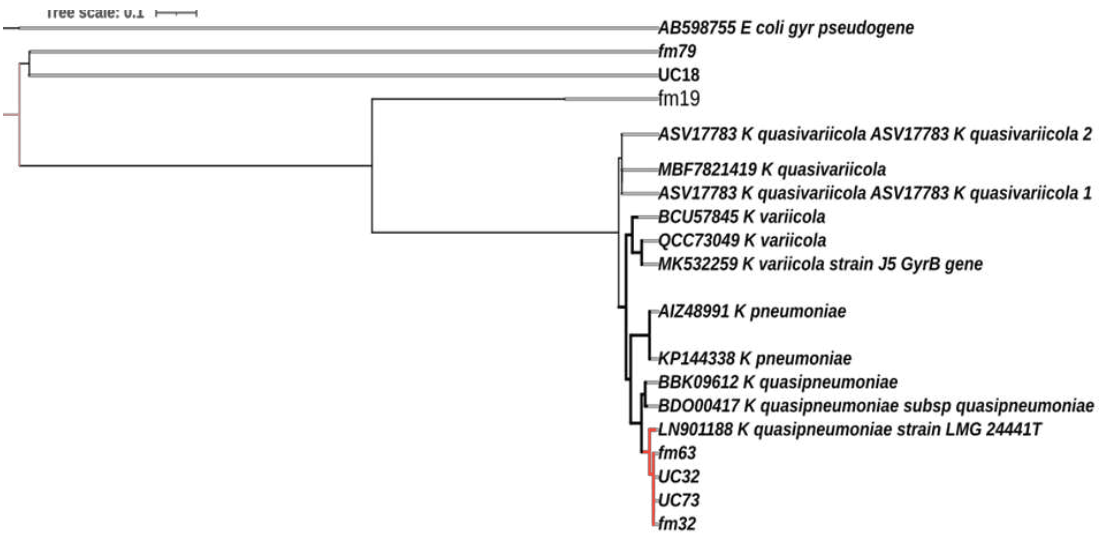


Fig 3: Dendrogram showing *gyrB* genetic relatedness of *Klebsiella quasipneumoniae* isolated from urine samples with international clones.

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DISCUSSION

The distribution of organisms recovered from 227 urine samples cultured on EMB agar showed that *Escherichia coli* accounted for 35.7% of all isolates, making it the most prevalent, followed by *Klebsiella* spp. (28.2%), *Enterobacter* spp*., Proteus* spp., and *Pseudomonas* spp. (7.5%, 14.1%, and 8.81%, respectively). The dominance of *E. coli* and *Klebsiella* spp. could be a result of their possession of multiple virulence determinants that facilitate colonization of the urinary tract. *Klebsiella pneumoniae* produces a capsule, uses a siderophore-mediated iron acquisition system, and can form biofilms, which enhance persistence in the urinary tract. The high prevalence may also be due to pressure from the indiscriminate use of antibiotics. This pattern is consistent with earlier research, despite *E. coli's* lower prevalence compared with some reports. [4] reported that *Klebsiella* spp. accounted for 15% of isolates, whereas *E. coli* was the most common uropathogen, comprising 50%. [28] similarly reported that *E. coli* is the most prevalent cause of UTI, followed by *K. pneumoniae*. Therefore, this result aligns with the existing epidemiological trends, with *E. coli* and *Klebsiella species* as the leading causes of urinary tract infections.

Biochemical identification showed that *Klebsiella* species make up 51.85%, with *Klebsiella oxytoca* accounting for 27.16%. The distinction between these two species was mainly based on their indole reaction, as *K. pneumoniae* is typically indole-negative, while *K. oxytoca* is indole-positive. Classical biochemical methods remain useful despite increased availability of advanced diagnostic tools; reactions such as indole production and motility are still helpful for the identification of closely related species, particularly in low-income communities. This difference

aligns with the findings of [40], who demonstrated that traditional phenotypic tests—especially indole—remain reliable compared with PCR and MALDI-TOF for differentiating *K. pneumoniae* from *K. oxytoca*. Additionally, motility testing was used to differentiate *Klebsiella* species from *Enterobacter* species, which account for 20.98%, since *Klebsiella* spp. are non-motile, whereas *Enterobacter* spp. show active motility. This approach aligns with the classic differentiation method described by [42], who used motility along with other biochemical markers to differentiate *Klebsiella* spp, *Enterobacter* spp, and *Serratia* species apart. The findings in this study therefore confirm that classical biochemical tests are still reliable for accurately distinguishing closely related Enterobacteriaceae, even with the availability of newer molecular tools.

Amplification of housekeeping genes (*gyrB, leuS, pyrG, rplB, and rpoB*) signifies the presence of conserved loci appropriate for phylogenetic analysis due to slow evolution of housekeeping genes under purifying selection; detection of sequence polymorphisms after sequencing provides higher discriminatory power than phenotypic method

This result revealed high similarities (over 90%) among all the isolates with exception in FM 19 with 84.06 % similarity with *Klebsiella quasipneumoniae* member revealing it divergence, signifies superior discriminatory capacity in comparison with 16SrRNAfor *Klebsiella pneumoniae* complex members, because of rapid evolution of *gyrB* in comparison to ribosomal genes while high conservation within species.

The phylogenetic tree displays lineage-based clustering within the *Klebsiella pneumoniae* complex, with tight clades and short branch lengths indicating strong genetic relatedness among most isolates. This suggests that a conserved lineage may be circulating within the two tertiary hospitals, pointing to a recent common ancestor or shared sequence type. [44] reached a similar conclusion, using phylogenomics to track the spread of dominant sequence types within the

Klebsiella pneumoniae species complex (KpSC) and identify specific lineages capable of extensive dissemination. Although phylogenetic closeness does not necessarily indicate a transmission pathway, finding the same isolate species in different hospitals does not imply hospital-to-hospital transmission. This aligns with [22], who noted that relying solely on genetic similarity can lead to false-positive inferences.

To emphasize divergence within the larger Enterobacteriaceae family, the *E. coli* that appeared as an outlier was intentionally included to represent a separate lineage from the sampled Klebsiella pneumoniae complex isolates. This serves as a reference point for clear ancestral separation within the same taxonomic group rather than indicating misclassification. Similar observations have shown that lengthy branch distances can result from high SNP accumulation, even among individuals of the same species, due to long-term adaptation or antimicrobial selective pressure [44]. Intra-species phylogenetic variation can result from the slow accumulation of single-nucleotide polymorphisms, which remains a key factor in evolution.

Conclusion

This finding reveals high prevalence rate of uropathogenic species of *Klebsiella pneumoniae* in the two tertiary hospitals in southwest Nigeria and display a relatively close *gyrB*- based genetic relatedness between isolated organisms and members of *K. pneumoniae* complex globally reported. High resistance to the available antibiotics pinpoints the limitation in therapeutic options available for UTI treatment in the study area. These studies underscore the importance of routine molecular surveillance.

Declarations

Ethical approval/ participant informed consent

This research was approved by the research and ethical committee of both University College Hospital, Ibadan, Oyo State, and Federal Medical Centre, Abeokuta, Ogun State, Nigeria, with reference numbers UI/EC/24/0844 and NHREC/08/10-2015, respectively. All participants were provided with verbal and written informed consent before urine samples were collected.

Consent for Publication

Not Applicable

Materials and Data Availability

Generated/ analyzed data during research are available in the NCBI database with the temporary accession numbers PZ111488-PZ111494. Dendrogram data and supporting files are available from the corresponding author upon request at sadiq.so@fmcabeokuta.org

Competing Interest

All authors have no competing interests

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Authors Contributions

S.O: Conceptualization, Methodology, Visualization, Curation of data and writing; W.E: Conceptualization, Supervision, and Data Curation; A.R: Supervision and Administration; O.E: Supervision, Administration, and data curation; and I.O Molecular design and anlysis .

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