Original Research A

Bioinformatics of *blaTEM*-1 and *blaSHV* genes of herbal pretreated-*Klebsiella pneum*

ABSTRACT

Background: Consumption of herbal products end up interacting with the gut microbiota which *K. pneumoniae* is a key player. **Aim**: This study investigated the effect of some herbal drugs cons the beta-lactamase genes, specifically *blaSHV* and *blaTEM*, present in a key gut patho *pneumoniae*. **Methods**: The study adopted an experimental approach. Two strains of *K. pne* were subject to treatment in four herbal medicines (Goko bitters, Goko alcoholic bitter, Ruzu bi Beta cleanser). The resistant genes, blaSHV and *blaTEM*, were amplified using polymera reaction on both plasmid and chromosomal DNA. These genes were also sequenced, and pre mutations were evaluated.**Results**: The PCR amplicon revealed the presence of the *blaSHV* ger herbal drug conditions, with bands at 477 bp, including ATCC and clinical strains treated w alcoholic bitters, Ruzu bitters, and control conditions. The *blaTEM* gene was detected only in th condition of the clinical strain, marked by a band at 867 bp. Plasmid DNA analysis further confi presence of *blaSHV* in clinical strains treated with Goko alcoholic bitters and Goko bitters, while *b* was observed in strains treated with Goko alcoholic bitters, Goko bitters, and Ruzu bitters. S alignments of the *blaSHV* and blaTEM-1 genes revealed various nucleotide substitutions, rang single nucleotide polymorphisms (SNPs) to length variations, when compared to closely relat lactamase genes. Mutation analysis indicated that herbal treatments, particularly Goko bitters a bitters, induced the highest mutation rates in both *blaSHV* (11%) and *blaTEM* (10%), with frameshift and point mutations. Conversely, non-herbal medicine-treated conditions exhibite mutations. **Conclusion**: These findings suggest that herbal drugs treated conditions exhibits a condition that promote the alteration of genes. It may also be responsible for the migration of genes from chromosome to plasmid.

Key words: Plamid, Resistant genes, mutation, herbal medicine, bioinformatics.

1. INTRODUCTION

For nearly a century, beta-lactam antibiotics have been vital in fighting bacterial int revolutionizing how we treat these diseases. However, their widespread use has led to in antibiotic resistance, a serious threat to global health (Muteebet al., 2023). This re makes these key medications less effective, endangering patients and straining he systems. To combat this menace, we need to better understand how resistance to this antibiotics develops and spreads, particularly focusing on beta-lactamases, the enzyn deactivate these antibiotics (Salam et., al 2023).

Since beta-lactams were first introduced, bacteria, especially those in the gut, have significantly, becoming incredibly numerous and diverse (Bush and Jacoby, 201 constant evolutionary struggle between antibiotics and bacteria has resulted in many re mechanisms, with beta-lactamase production being a major one.These bacterial enzyme

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down the core structure of beta-lactam antibiotics, making them have no effect pathogens (Baqueroet al.,2021). This breakdown is a key factor in beta-lactam res making it harder to treat infections caused by resistant bacteria. The fact that these e are so diverse and adaptable, with new versions constantly appearing, makes the proble worse (Uddin et al.,2021, Uddin et al., 2022).

The discovery of the first beta-lactamase genes, *blaSHV-1* and *blaTEM-1*, in *K. pneumoniae* in the 1960s was a turning point in the history of antibiotic resistance (Liak et al., 2016). The*blaSHV-1* was initially found in *K. pneumoniae*, while *blaTEM-1*, al *pneumoniae*, spread rapidly because it's located on mobile genetic elements like pl which allow bacteria to easily share genetic material (Liakopouloset al., 2016). T discovery of *blaSHV-2* in *K. pneumoniae* from an ICU patient showed how serious the was becoming (Li et al., 2022). *blaSHV-2* can break down a wide range of beta-l including penicillins, third-generation cephalosporins, and monobactams, so it was class an extended-spectrum beta-lactamase (ESBL) (Zhang et al., 2021). Another importa lactamase gene, *blaTEM-3*, also carried on plasmids, is active against many beta-lacta (Dameanti, 2023). These initial discoveries were crucial for understanding the complex behind beta-lactam resistance.

In the 1990s, ESBL-producing *K. pneumoniae* became a major threat in hospitals, significant outbreaks and making treatment very difficult. ESBL prevalence rose to a levels, with some regions reporting that up to 40% of *K. pneumoniae* isolates from h produced these enzymes (Navon-Venezia et al., 2017). During this time, *K. pneumoniae* commonly carried *blaTEM* and *blaSHV* β-lactamases (Chong et al., 2011), with many versions spreading worldwide (Liakopoulos, 2016). The rapid spread of these ESBLs limited treatment options, often leaving doctors with few effective antibiotics. This highlig urgent need for new strategies to fight the growing problem of antibiotic resistance.

There is a rise in antibiotic resistance is becoming more prominent in developing natio as Nigeria which has been reported by many studies (Mbada et al., 2015, Aagaard et a Monsi et al., 2018, Orokor et al., 2025). However, due to the financial implication conventional antibiotics, most low-and-middle income earners in Nigeria adopt locall antimicrobial agents. Besides, recent studies are demonstrating the use of traditional r to treat resistant pathogens (Xue et al., 2023). The effect of these locally produced bacterial resistance development is yet to be investigated. These drugs are only tested ability to kill the agent of interest without understanding the untoward effect on other flora within the human system. The observation from previous studies that exposures of previously sensitized to antibiotic results in the acquisition of resistance to that class serves as the basis for the choice of exposure factors in this research (Monsi et al., Monsi et al., 2019, Monsi et al., 2021).

Bioinformatics is now essential for studying how resistance genes like *blaTEM-1* and evolve and spread in *K. pneumonia* (Rozwandowicz et al., 2018).This provides information about how resistance spreads and how new variants emerge. Research interaction between herbal drug pretreatments and *K. pneumoniae* could reveal how compounds affect gene expression, mutation rates, and resistance, potentially leading ways to combat beta-lactamase-mediated resistance (Sun et al., 2021). Investigating h inhibitory antibiotic concentrations contribute to resistance evolution is also important, a help us understand how low-level exposure contributes to the selection and spread of r

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strains (Martinez, 2008). These diverse research approaches are crucial for developing
strategies to combat the increasing threat of antibiotic resistance.

This study aims to investigate the bioinformatics characteristics of *blaTEM-1* and *blaSH*
in *K. pneumoniae* pretreated with herbal drugs. Through sequence analysis this researc
to provide insights into the genetic adaptations and potential therapeutic implications o
based interventions against resistant *K. pneumoniae* strains.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Herbal medicines

Locally made drugs used in this study (Beta cleanser [Bet], Goko alcoholic bitters [Gab
bitters [Gob], Evacuation solution [Eva], Danko solution [Dan], Ruzu bitters [Ruz], and n
herbal mixture [Ruz]) were purchased from Mile 3 market, Port Harcourt, Rivers State,
while antibiotics (ceftriaxone, gentamycin,piperacillin/tazobactamand ciprofloxacin
purchased.

2.1.2 Bacteria strains

The study used a combination of laboratory and clinical strains of *K. pneumonia*
laboratory strains used was *K. pneumoniae* WCDM 0097 purchased from Sigma
Kingdom). A clinical strain was obtained from the River State University Clinic and confi
performing a polymerase chain reaction amplification of 16S rRNA.

2.2 Exposure Studies

An overnight bacterial culture at an optical density (OD) of 0.5 was diluted by a ten-fo
dilution from the overnight culture concentration to 10^9 in TSB medium. This final di
equivalent to 10^2 CFU/ml when enumerated on TSA. Five different concentrations o
made drugs were obtained by serial dilution of locally made drugs in TSB medium co
the overnight bacteria broth as diluents. The final concentrations of the antibacterial ag
the first to fifth Bijou bottle was 100%, 50%, 25%, 12.5% and 6.25%, respectively
different concentrations were then plated in a 24-well plate (**Figure 1**).

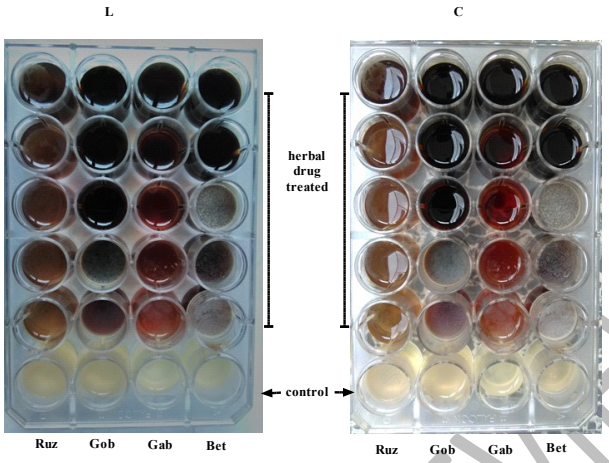

Figure 1. Pattern of the plating of bacteria cultures

2.3 Genomic DNA extraction

Comment [a13]: beta-mercaptoethanol was added

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Comment [a15]: A fungal/bacterial DNA binding buffer (1,200 µl) was added to the filtrate in the collection tube from above step.

For optimal performance, add beta-mercaptoethanol (user supplied) to the Fungal/E DNA Binding Buffer to a final dilution of 0.5% (v/v) i.e., 500 µl per 100 ml. A wet weight 50-100 mg of *K. pneumoniae* colonies that have been resuspended in up to 200 µl (phosphate buffered saline) was added into a ZR Bashing Bead Lysis Tube. Lysis So 750 µl was added to the tube. This was secure in a bead beater fitted with a 2 ml tub assembly (Disruptor Genie) and process at 12,000 rpm for 5 minutes. The ZR Bashir Lysis Tube spun at 10,000 rpm for 1 minute. Supernatant (400 µl) was transferred to SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 rp minute. The base of the Zymo-Spin IVTM Spin Filter was snapped off prior to use. Add of Fungal/Bacterial DNA Binding Buffer to the filtrate in the Collection Tube from abo Eight hundred microlitre of the mixture from the above step was added to a Zymo-Spi Column in a Collection Tube and Centrifuged at 10,000 rpm for 1 minute. The flow throu discarded from the Collection Tube and the above step was repeated. Two hundred m of DNA Pre-Wash Buffer were added to the Zymo-SpinTM IIC Column in a new Collecti and centrifuge at 10,000 rpm for 1 minute. Five hundred microlitres of Fungal/Bacter Wash Buffer were added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 r minute. The Zymo-SpinTM IIC Column was transferred to a clean 1.5 ml microcentrifu and 100 µl (25 µl minimum) DNA Elution Buffer was added directly to the column ma centrifuged at 10,000 rpm for 30 seconds to elute the DNA.

2.4 Plasmid DNA extraction

Comment [a16]: Fifty microlitres

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Overnight bacterial culture of 0.5-5 ml in a clear 1.5 ml tube was spun at 12,000 rpm fo seconds and the supernatant discarded. Two hundred and fifty microlitres of ZymoPUR (Red) was added to the bacterial cell pellet and resuspended completely by vortexi hundred and fiftymicrolitres of ZymoPURE™ P2 (Green) was added and immediately n gently inverting the tube 6-8 times; no vortexing was involved. The reaction was all temperature for 2-3 minutes. Cells are completely lysed when the solution appears clear

and viscous and 250 µl of ice cold ZymoPURE P3 (Yellow) was added and mixed thoro inversion but was not vortex. The tube was inverted an additional 3-4 times after the turned completely yellow. Complete neutralization is indicated by the sample turn yellowish precipitate. The lysate was incubated on ice for 5 minutes and centrifuge minutes at 16,000 rpm. Six hundred microliters of the supernatant of the centrifuged lysa aliquoted into a clean 1.5 ml Eppendorf tube. Care was taken in order not to disturb th pellet so that cellular debris was not transferred into the new tube. To the clear lysate, 2 ZymoPURE Binding Buffer was added and mixed by inverting the capped tube 8 tim Zymo-Spin II-P Column was placed in a collection tube and the clear lysate was transfe the Zymo-Spin II-P Column. The Zymo-Spin II-P/Collection tube assembly was incub room temperature for 2 minutes and centrifuged at 5,000 rpm for 1 minute. The flow was discarded and 800 µl of Zymo-PURE Wash 1 was added to the Zymo-Spin II-P colu spun at 5,000 rpm for 1 minute. The flow through was discarded and 800 µl Zymo-PUR 2 was added to the Zymo-Spin II-P column and spun at 5,000 rpm for 1 minute. T through was discarded and 200 µl of Zymo-PURE Wash 2 was added to the Zymo-S column and centrifuged at 5,000 rpm for 1 minute. The flow through was discarded an Spin II-P column was spun at 12,000 rpm for 1 minute to remove any residual wash buf Zymo-Spin™ II-P column was transferred into a clean 1.5 ml eppendorf tube and 25 µl o PURE™ Elution buffer was added to the column matrix. The sample was incubated temperature for 2 minutes and then centrifuged at 12,000 rpm for 1 minute. The eluted DNA was stored at ≤ -20ºC. The entire procedure of plasmid extraction was performed temperature (25ºC).

Comment [a18]: Genes *blaSHV*

2.5 Amplification of Resistance Genes*blaSHV* and *blaTEM*-1

Comment [a19]: µM eoxynucleoside

Primers (**Table 1**) were designed based on the most conserved sequences at the en *blaSHV* and *blaTEM*-1 genes in *Klebsiella*. These were used to amplify resistant gen the *Klebsiella* strains. The PCR mixture used contained 1× PCR buffer (50 mM KCl, 10 r HCl [pH 8.3]), 2.5 mM $MgCl_2$, 200 µMdeoxynucleoside triphosphate, 1.0 U T polymerase (TaKaRa Biotechnology [Dalian] Co. Ltd., China), 10 nM each primer, and 1 the DNA template in a final volume of 50 µl. PCR conditions were as follows: initial dena at 95°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72 minute; and a final extension at 72°C for 5 minutes. An aliquot (2 µl) of PCR products wa an agarose gel to check for amplified fragments.

Table 1. Primers Used for *K. pneumoniae* 16S rRNA Gene, TEM-1 and SH Amplification

Gene	Forward Primer	Reverse Primer
blaTEM-1	ATGAGTATTCAACATTTCCG	CTGACAGTTACCAATGCT
blaSHV	CCGCAGCCGCTTGAGCAAA	GCTGGCCGGGGTAGTGGTGTC
16S rRNA	AGAGTTTGATCCTGGCTCAG	GGTTACCTTGTTACGACTT

2.5.1 Preparation Of 1.0% Agarose Gel

Comment [a20]: Please mention the concentration of ethidium bromide

It is normally 1µg/ml

One gram of agarose powder was added to 100 ml of TBE (Tris Base, Boric Acid, running buffer and heated in a microwave for 3 minutes for complete dissolution of the a The solution was allowed to cool to 56ºC and 2 µl of ethidium bromide was added.

solution was cast in a mould with the gel comb properly inserted. The agarose gel was to solidify for 45 minutes at room temperature.

2.5.2 Agarose Gel Electrophoresis

Ten microlitres of DNA ladder (molecular marker) mixed with loading dye were loaded in well of the solidified gel immersed in TBE buffer in Gel electrophoresis chamber. Whe load PCR master mix was used and 10 µl of PCR products (amplicon) were loaded in e of agarose gel. The reaction was performed at 90 volts for 60 minutes. Gel was viewe documentation system with UV trans-illuminator.

2.6 Sequencing of the PCR amplified products

PCR products were purified using EXOSAP-IT (Ambion, CA) prior to bi-directional seque Purified products were injected on the ABI3500XL analysers (Applied Biosystems) with a array, using POP7.

2.7 Ethical Consideration

Collection of samples of *Enterobacteriaceae* microorganisms were performed i accordance with the ethical recommendations of the Ethical Committee under the jurisd the Rivers State Ministry of Health in Port Harcourt of Nigeria.

2.8 Data Analysis

All experiments were performed at least in duplicate and on at least two independent oc Results were presented as mean ± SD where necessary. Where appropriate, s analyses were performed using an unpaired t test in which a two-tailed *P*-value was ca (GraphPad Prism Software Version 5.03, San Diego, CA). Statistical significance was as a *P*-value of less than 0.05 at 95% confidence interval. Initially nucleotide sequenc observed and corrected manually in Chromas-Lite, Version 2.1.1. Geneious version 9. used to analyze the sequence data generated.

3. RESULTS

3.1 Amplification of *blaTEM* -1 and *blaSHV*in *K. pneumoniae* isolates exposed to h drugs

The amplicons of the *blaSHV* and *blaTEM* genes of the beta–lactamase family are s **Figures 2-4**. The electrophoretic patterns of the PCR products revealed that the presenc *blaSHV* gene was found in five conditions of herbal drugs (**Figure 2**). For Genom amplification templates, *K. pneumoniae* strain ATCC in beta cleanser and control cc demonstrated the presence of the *blaSHV* gene on the chromosome with bands at 477 other hand, the clinical strain demonstrated the presence of the *blaSHV* on Goko a bitters, Ruzu bitters and control conditions.For genomic DNA amplification only the condition of the clinical strain demonstrated visible a band at 867 bp which signi detection of the *blaTEM* gene (**Figure 3**). However, conditions: clinical Beta cleanser Goko alcoholic bitters, control ATCC, and ATCC beta cleanser showed faint bands that as smears across the agar. For the plasmid detection of beta-lactamase (**Figure 4**), the gene was present in the clinical strain of *K. pneumoniae* treated in Goko alcoholic bitter bitters conditions with a band size of 477 bp which are shown on the left side of the g *blaSHV*while the *blaTEM*-1 gene was present in the clinical strains of *K. pneumoniae* tr Goko alcoholic bitters, Goko bitters, and Ruzu bitters which are shown on the right sid band size at 867 bp.

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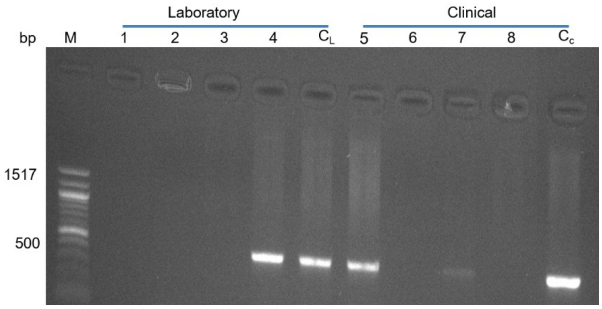

Figure 2. PCR amplification products of *blaSHV*gene in *K. pneumoniae* exposed to herbal d
Lane M: DNA ladder (molecular marker). Keys: 1 - ATCC Gab, 2 - ATCC Gob, 3 - ATCC Ruz, 4- .
Bet, C_L- ATCC control, 5- clinical Gab, 6clinical Gob, 7- clinical Ruz, 8- clinical Bet and C_c -clinica

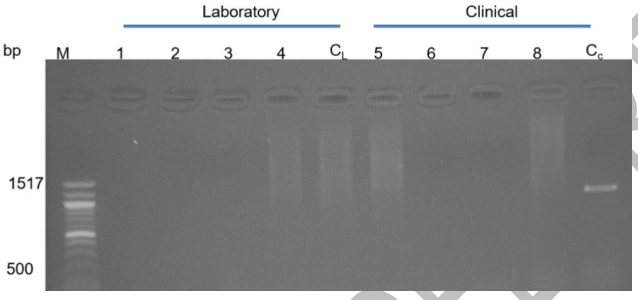

Figure3. Amplification products of *blaTEM*-1 gene of *K. pneumoniae*. M is a 100-1517
ladder (molecular marker). Keys: 1 - ATCC Gab, 2 - ATCC Gob, 3 - ATCC Ruz, 4 - ATCC Bet, C
control, 5 - clinical Gab, 6 clinical Gob, 7 - clinical Ruz, 8 - clinical Bet and C_c - clinical control.

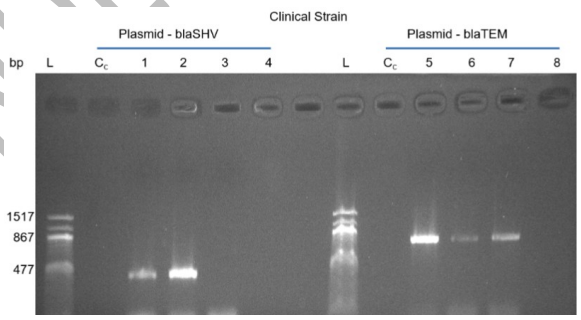

Figure 4. Amplification of plasmid *blaTEM*-1 and *blaSHV* genes in clinical *K. pneumoniae*.
1517 bp DNA ladder (molecular marker). Keys: 1 - clinical Gab, 2 clinical Gob, 3 - clinical Ruz, 4
Bet and C_c - clinical control, 5 - clinical Gab, 6 clinical Gob, 7 - clinical Ruz, 7 - clinical Bet.

3.2 Analyses of nucleotide sequence alignment *blaSHV* and *blaTEM*-1 Genes

Appendices 1 to 8 show the alignment of nucleotide sequences of *blaSHV* and *bl* genes in *K. pneumoniae* with genes closely related to the beta-lactamase genes. Ge most sequenced genes had nucleotide substitutions in the beta-lactamase genes. Thes from single nucleotide substitution (also known as single nucleotide polymorphism [S several length nucleotide substitutions. The resistance genes were compared to sever related genes. Dots represent nucleotides conserved in all gene sequences while dash gaps that have been inserted to generate optimal sequence alignment. Summarized in are the number and types of mutations observed in the beta-lactamase gene in *K. pneu* clinical and laboratory strains exposed to different herbal medicines. The highest nu mutations were noted in the GOB condition (11%) in the *blaSHV* gene of the clinical iso laboratory *K. pneumoniae* treated in Ruzu bitters *blaTEM* (10%). Both conditions frameshift and point mutations. The lowest mutations observed were noted in no medicine-treated conditions.

Table 2. Number and types of mutations beta-lactamase in genes

Isolate	Resistant genes	Mutations n (%)	Nucleotides range
Clinical control	*blaSHV*	1 (1.3%) (Point)	80 (1-80)
Laboratory control	*blaSHV*	-	- (60-110)
Clinical GAB	*blaSHV*	-	- (160-310)
Clinical GOB	*blaSHV*	11 (11%) (Frameshift/Point)	100 (1-100)
Clinical Ruz	*blaSHV*	5 (7.1%) (Frameshift/Point)	70 (70-140)
Laboratory GAB	*blaTEM*	11 (7.9%) (Point)	140 (10-150)
Laboratory GOB	*blaTEM*	9 (10.6) (Point)	85 (1-85)
Laboratory Ruz	*blaTEM*	10 (10%) (Frameshift/Point)	100 (1-100)

3. DISCUSSION

Gram-negative pathogens possess complex resistance gene regulatory systems that rapid adaptation to several environmental signals that have not yet been studied (Bhag al., 2019, Manisha et al., 2024). The *blaSHV* and *blaTEM* genes encode 204 and 75 acid long polypeptides respectively. The alignment of nucleotide sequences of the *blaS* *blaTEM* genes in *K pneumoniae* with closely related beta-lactamase genes revealed sig genetic variability, primarily in the form of single nucleotide polymorphisms (SNP nucleotide substitutions in all *K. pneumoniae* isolates exposed to the herbal drugs. The lactamase genes are common genes widely known to be associated with resistance t lactamase class of antibiotics especially in gram-negative bacteria (Pishtiwan and 2019). These SNPs were mostly found in herbal medicine-treated conditions except 1.3% rate notedfor one of the controls. This finding is consistent with previous studies th reported mutations in beta-lactamase genes as a mechanism for antibiotic resista Nascimento et al., 2020, Rocha et al., 2022). The observed nucleotide substitutions included both point mutations and frameshift mutations, suggest that these genetic alt may contribute to the evolution of resistance against beta-lactam antibiotics in *K. pneum*

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The highest mutation frequency was recorded under the GOB condition (11%) in the gene of the clinical isolate, while the *blaTEM* gene exhibited a 10% mutation rat *pneumoniae* exposed to Ruzu bitters. The occurrence of both frameshift and point muta these conditions suggests that exposure to certain herbal medicines may induce alterations, potentially impacting beta-lactamase expression and function (Kumarasam 2010). This aligns with previous research highlighting the role of environmental and s pressures in shaping resistance determinants in bacterial populations (Monsi et al., Monsi et al., 2018, Anga et al., 2020).Their studies demonstrated that when there is a ch the environmental condition of a pathogen, such as exposure to herbal drugs, *S. aureu* *coli*showed alteration in some virulent genes. This implies that pathogenic bacteria ca environmental changes and alter the gene expression that will promote their pathogenici

Since *K. pneumoniae* commonly reside in the gut which makes it to be exposed to thes drugs become sensitized. This indicates that herbal drugs act as a stressor that alters t sequence of the beta–lactamase drug. These polymorphisms seen in some of the her treatment conditions could be responsible for these mutational changes for the bac survive through natural selection. This is because according to the principle of natural s when an organism is in an environment or condition that is harsh, they survive exhibiting adaptive changes such as production of new virulence factors, alteration c sites and migration from the region of high exposure, all of which could be detrimenta host. In addition, our study agrees with an *in vitro* study by Webber *et al.,* (2015) on the biocide on *Salmonella*. Their study demonstrated that mutations were detected in thre (*fabI*, *ramR*, and *gyrA*) after exposure to biocide.

Conversely, the lowest mutation rates were observed in non-herbal medicine-treated co indicating a reduced selective pressure in the absence of external compounds. These suggest that exposure to herbal medicines may contribute to genetic modifications in re genes, warranting further investigation into the molecular mechanisms driving these c Future studies should explore the functional consequences of these mutations c lactamase activity and their potential clinical implications.

A noteworthy observation in our study is that beta–lactamase was present or chromosome or plasmid DNA or both. A proposed phenomenon to this observation is th of the *blaTEM*-1 and *blaSHV* to act as transposable genetic elements. This study sho resistance inducement due to herbal drugs exposure could either be chromosomal or mediated. The chromosomal-mediated resistance has previously been repo fluoroquinolones due to amino acid substitution in DNA gyrase gene (Jacoby, 2009 Domokos et al., 2018 identified several genes (*qnrB, qnrS, qnrC*and*qnrD*) to be respon fluoroquinolone resistance. Similar substitutions were noted in this current study.

However, resistance to beta-lactam antibiotics is not limited to mutations in betala enzymes. A report has revealed that efflux pumps located on the membrane of mos negative bacteria cause resistance. Their main function is to remove antibiotics substances from within the bacteria cell. Mutations of these proteins have been identifi responsible for the resistance of *Enterobacteriaceae* to beta-lactam drugs (Ogbolu*et al.,*

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5. CONCLUSION

Herbal medication has the potential of inducing bacterial resistance through migration lactamase gene from chromosome to plasmid and vice versa. The observation of muta exposed isolates implies this could alter the enzymatic activity based on the exposed co

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REFERENCES