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Phylogenetic Analysis of *Klebsiella pneumoniae* Isolated from Nosocomial and Community Infection in Diyala, Iraq

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Abstract

The biological diversity of *Klebsiella pneumoniae (K. pneumoniae*) has widely been reported to be associated with pathological progress in severe nosocomial and community-acquired infections. 250 clinical specimens included sputum, urine and swabs from wound and burns samples were collected from Al-Batool Teaching Hospital (38.4%), Baqubah Teaching Hospital (61.6%) and private laboratories in Baqubah and Diyala, Iraq. Positive rates of nosocomial acquired infection were sputum 98%, urine 96%, and swabs from wound and burns 94%, while positive rates of community acquired infection were sputum 60%, urine 60%, and swabs wound and burns 30%. Positive rates of nosocomial and community acquired infections were 96% and 48%, respectively. The distribution of pathogenic bacteria of *E. coli* was 19.2% and 8.8%, and those of *K. pneumoniae* were 23.2% and 12% of nosocomial and community acquired infections respectively, which is a higher percentage than other pathogenic bacteria of nosocomial and community acquired infections. The nosocomial-acquired *K. pneumoniae* isolates were less susceptible than community-acquired *K. pneumoniae* isolates. Multi-drug resistant (MDR) and extensively drug resistant (XDR) are commonly caused by β-lactam/β-lactamase inhibitor combinations: Ticarcillin clavulanate, carbapenems and meropenem 100%; cephalosporines and ceftazidime 96%. Phylogenetic analysis of the 16S rRNA gene showed the presence of six nucleic acid variations in various samples. This variance might exhibit a clear tendency to be localized in the community acquired *K. pneumoniae* isolates. Fifteen ribosomal sequences were deposited at GenBank in accession numbers starts from OM992298 to OM992312.

Keywords: *K. pneumoniae*, antibiotic susceptible, 16S rRNA gene.

التحليل الوراثي الفيلوجيني لـ *Klebsiella pneumoniae* المعزوله من عدوى المستشفيات والعدوى المكتسبة من المجتمع في ديالى / العراق

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الخلاصة

ثبت على نطاق واسع أن التنوع البيولوجي لـ *Klebsiella pneumoniae* مرتبط بالتطور المرضي للعدوى الشديدة في المستشفيات والعدوى المكتسبة من المجتمع. . تم جمع 250 عينه من البلغم،البول ومسحات للجروح

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والحروق من مستشفى بعقوبه التعليمي ومستشفى البتول بالإضافة الى بعض المختبرات الخارجيه الخاصه في ديالى/ العراق . بلغت نسبة النمو في العينات المعزوله من المستشفيات والمجتمع 96%و 48% على التوالي.اذ بلغت نسبة انتشار بكتريا القولون 19.2% و 8.8% اما الكلبسيلا الرئوية فكانت نسبة انتشارها الاعلى و بلغت 23.2% و 12% في العينات المعزوله من المستشفيات والمجتمع على التوالي. تعد الكلبسيلا الرئويه المعزوله من المستشفيات اقل حساسيه من عزلات المجتمع .كانت الفئات الاعلى مقاومه مضادات البيتالاكتام، الكاربابينيم والسيفلوسبورينات في تكوين مقاومه متعدده للمضادات الحياتية (MDRو XDR) .

اظهر التحليل الوراثي الفيلوجيني لجين الرنا الرايبوسومي 16S rRNA عن وجود سته تباينات في الاحماض النوويه في عينات مختلفه. اظهر هذا التباين ارتباطا واضحا بعزلات الكلبسيلا الرئويه المكتسبه من المجتمع . تم تسجيل خمسه عشر تسلسلا وراثيا للحامض النووي الرايبوسومي ذات ارقام محددة تبدا من GenBank OM992298 الى GenBnak OM992312 في بنك الجينات NCBI.

1. Introduction

The exponential increase in genomes number deposited in public databases can help us to gain a more holistic understanding of the phylogeny and epidemiology of *Klebsiella pneumoniae*. The alignment of 16S rRNA prokaryotic gene sequences is used in phylogenetic tree analysis of numerous bacteria, including the fact that they are by far the most frequently used housekeeping genetic markers. The 16S rRNA gene sequence can be found in almost all bacteria, often in the form of a multigene family or operons. The 16S rRNA gene has 1550 base pairs and many polymorphisms that are utilized to differentiate it. While the variable area sequence used for taxonomy is meant to complement the target area at a specific region at the gene beginning or end. The primers used are designed to complement the target area in a specific area at the gene's beginning or gene's end [1]. Despite the fact that 500 and 1500 base pairs are commonly employed for comparing and sequencing, 16S rRNA sequence has been determined in numerous strains, including Genbank which has over 20 million sequences deposited [2].

The deposited sequences were utilized to compare the sequencing. Being a universal gene in bacteria, 16S rRNA is utilized to investigate the connections between bacteria. The use of 16S rRNA sequences to compare bacteria is not only particularly useful for detecting bacteria, but also in categorizing strains and defining the connection tree among bacteria [2]. The 16S rRNA is a structural component of the 30S ribosomal small subunit which is required for essential life. 16S rRNA has eight highly conserved nucleotide sequences, U1-U8, that are invariant across the bacterial domain. Between those conserved sections, nine variable regions, V1-V9, can also be found, which are probably less important for ribosomal function. These areas display a range of nucleotide substitution rates [3].

In hospitalized patients, *K. pneumoniae* is a prevalent cause of antimicrobial-resistant opportunistic infections. The species is inherently resistant to penicillin. Individuals in the community frequently also develop resistance to a variety of antimicrobials. *K. pneumoniae* has emerged as a serious clinical and public health problem as the incidence of healthcare-associated infections caused by multidrug-resistant strains producing extended-spectrum β-lactamases and/or carbapenemases has increased. A comparable situation has evolved, including severe community-acquired infections produced by 'hypervirulent' *K. pneumoniae* strains that exhibit acquired virulence factors [4].

Klebsiella pneumoniae can behave as a "real" pathogen outside of the hospital context, causing severe community-acquired infections (CAIs) that are not considered opportunistic in otherwise healthy individuals who do not share the risk criteria for hospital-acquired infections (HAIs) [5].

The opportunistic bacterial pathogen *K. pneumoniae* is renowned for its high frequency and diversity of antimicrobial resistance genes (AMR genes). *K. pneumoniae* is the species in which numerous novel AMR genes were found before spreading to other infections, in addition to being a substantial clinical concern in its own right (e.g. carbapenem-resistance genes KPC, OXA-48, and NDM-1). While it is hard to quantify *K. pneumoniae* contribution to the overall AMR epidemic, current research showed that it has a larger ecological spread, more diverse DNA composition, more AMR gene diversity and a higher plasmid load than the other Gram-negative opportunists [6].

Among pathogens of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp* (ESKAPE) due to the advent of strains that are resistant to all or most current antibiotics. ESKAPE infections are considered the biggest concern [7]. Horizontal gene transfer (HGT), assisted by plasmids and mobile genetic elements, is the primary cause of AMR accumulation in these species [8].

Antimicrobial resistance is epidemiologically graded using conventional nomenclature in public health microbiology laboratories [9]. Multi-drug resistant (MDR) is defined as being susceptible to at least one agent from three or more antimicrobial categories. Extensive drug resistance (XDR) is defined as non-susceptibility to at least one agent in all antimicrobial categories except two or fewer. Pan drug-resistant (PDR) is defined as non-susceptibility to all agents in all categories of antimicrobials (i.e. no agents tested as susceptible to that organism) [9].

Aims of study were using 16S rRNA gene sequencing to discover mutations in local isolates which would improve our knowledge of *K. pneumoniae* taxonomy. Clinically relevant pathogenicity and antibiotic resistance characteristics demonstrated a relationship between clinical nosocomial infection and community-acquired infection samples.

2. Materials and Methods

2.1 Samples Collection and Bacterial Isolation

A total of 250 clinical specimens were collected from October 2021 to January 2022. Patients of both genders, ranging in age from 4 to 70 years, were picked from a variety of different hospitals and laboratories in Baqubah / Diyala, Iraq, including Al-Batool and Baqubah teaching hospitals and public health laboratories. Clinical specimens were obtained using cotton disposable swabs from wounds and burns, and sputum, and later transferred into the sterile medium in plastic bottles. Collection of samples was done according to ethical guidelines by the agreement of patient and by not violating their privacy and maintaining the confidentiality of their identities.

2.2 Phenotypic Identification

Phynotyping characteristics of initial identification depended on phenotypic characteristics of the colony on MacConkey agar, chocolate agar, blood agar and eosin methylene blue agar (EMB agar) incubated at 37°C for 18 or 24 hours. Pure growth colonies were produced that included the size, colour, shape and mucous after obtaining a single colony of isolated bacteria. It depended on microscopic examination after staining with Gram stain. Biochemical tests (indole, oxidase, methyl red, Voges – Proskauer, citrate utilization,urease, Kligler iron agar and catalase tests) were carried out for identifying suspected isolates according to MacFaddin [10]. Identification of *Klebsiella pneumoniae* was done by Vitek-2 Compact system which was manually loaded with VITEK 2 ID-GN cards, AST-No. 12 cards and bacterial suspension.

2.3 Antimicrobial Susceptibility of *K. pneumonia*

According to Humphries *et al.* [11] and Weinstein and Lewis [12], the susceptibility of bacterial isolates to different antibiotics was investigated using the standard disc diffusion method. The twelve different antibiotics used were: Amoxicillin clavulanate (AMG) (20/10 µg), piperacillin-tazobactam (PTZ) (100\10 µg), ticarcillin- clavulante (TIM) (75\10 µg), ceftazidime (CAZ) (30 µg), ceftriaxone (CRO) (30 µg), meropenem (MEM) (10 µg), imipenem (IMP) (10 µg), amikacin (AK) (30 µg), azithromycin (ATZ) (15 µg), levofloxacin (LEV) (5 µg), ciprofloxacin (CIP) (5 µg), trimethoprim-sulfamethoxazole (TS) (1.25 µg).

2.4 Genomic DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of GeneAid extraction kit from USA /Promega. The concentration and purity of the DNA extract were measured by using the quantitation fluorometer from USA /Promega.

2.5 Standard Sequencing

The resolved PCR amplicons were commercially sequenced from the forward direction, following the instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, SouthKorea).

Used universal primer 27F (5`-AGAGTTTGATCC TGGCTCAG-3`), 1540R (5`-GGAGGTGATCCAACCGCA-3`). PCR Steps included initial denaturation at 95°C for 05.00 of one cycle, the thirty cycles for three steps: Denaturation 95°C for 00.30, annealing 60°C for 00.30 and extension72°C for 01.00, one cycle for final extension 72°C for 07.00, and hold 10°C for 10.00, product size 1500bp [13].

2.6 Statistical Analysis

The statistical analysis was performed using version 23 of the Statistical Package for Social Sciences (SPSS) was used for. Chi-square test was used for comparing the antibiotic resistance between different isolates.

3. Results and Discussion

3.1 Distribution of Growth Pattern of Bacterial Isolates

The distribution of investigated clinical specimens consisted of 250 samples was divided into two identical parts: nosocomial acquired infections and community acquired infections. Nosocomial samples were isolated from Baquba and Al-Batool hospitals at a percentage of 61.6% and 38.4%, respectively. Community samples were isolated from private laboratories. Positive bacterial growth of nosocomial infection was approximately 120/125 (96%) of total positive bacterial growth, while community infection was 60/125 (48%) of positive bacterial growth. There were significant differences between community-acquired and nosocomial *K. pneumoniae* bacteremia, with nosocomial infections having a non-growth rate that was more than twice that of community-acquired infections [14]. A total of 250 samples were positively grown from clinical specimens from three distinct infectious sources (wounds and burns, urine and respiratory sputum) as shown in Table 1. All samples were exanimated by a variety of microbiological detection methods, including enrichment, differential, and selective media, colony morphology, cell microscopy using Gram stain, and several biochemical assays.

Table 1: Distribution of growth pattern of bacterial isolates (n = 250).

		Growth	*E. coli*	*K. pneumoniae*	*Pseudomonas aeruginosa*	*Proteus* spp.	*Acinetobacter* spp.	Total	*Staphylococcus spp.*	*Streptococcus spp.*	Total
Nosocomial acquired (n=120)	Urine n=50	98%	28 %	26 %	12 %	12 %	12 %	90 %	4 %	4%	8 %
	Sputum n= 25	96%	0%	36 %	20 %	20 %	20 %	96 %	0 %	0 %	0 %
	Wounds and burns n=50	94%	20 %	14 %	24 %	12 %	14 %	84 %	10%	0 %	10%
	Total	96%	19.2 %	23.2%	18.4%	13.6%	14.4 %		5.6%	1.6%	
Community acquired (n=60)	Urine n=50	60%	22 %	14 %	2 %	0 %	16 %	54 %	6 %	0 %	6 %
	Sputum n= 25	60%	0 %	16 %	16 %	12 %	8 %	52 %	8%	0%	8 %
	Wound Wounds and burns n=50	30%	0 %	8 %	6 %	8 %	8 %	30 %	0 %	0%	0 %
	Total	48%	8.8%	12%	6.4%	5.6%	11.2 %		8.3%	0	

3.2 Isolation and Identification of Bacteria

All usual bacteriology tests revealed that 180 clinical specimens yielded positive growth when cultured and developed on blood, MacConky and chocolate agar. It is noteworthy that the 120 nosocomial growth samples were classified as 111/125 (88.8%) gram-negative bacteria and 9/125 (7.5%) gram-positive bacteria.

On the other hand, the 60 growth community samples were classified as 55/125 (44%) gram-negative bacteria and 5/125 (4%) gram-positive bacteria when compared, which appeared statistically significant (p.value < 0.03).

The distribution of pathogenic bacteria of *E. coli* and *Klebsiella spp.* with a higher percentage of other pathogenic bacteria in both nosocomial acquired and community acquired infections was shown in this study. *K. pneumoniae* belongs to the group of "ESKAPE," which is an important causative agent of both hospital and community-acquired infections [15]. This result is similar to those reported by Al-aajem [16] concerning that the most causative agents of UTI are *E. coli*, *K. pneumoniae* and *Enterococcus* faecalis, and this result is also similar to those reported by Koksal *et al*. [17]. The rise of infections caused by ESBL-positive *E. coli* and *Klebsiella* spp. is bringing together a number of issues, including antibiotic resistance and a reduction in outpatient treatment choices. Different environmental circumstances, host characteristics, healthcare practices and hygiene habits in each nation may cause variances and similarities in the kind and distribution of uropathogens.

3.3 Identification of *K. pneumoniae* by VITEK 2 Compact System GN

The GN ID Card of the VITEK 2 was used to confirm the diagnosis of the 44 isolates of *K. pneumoniae* screened in the current investigation.

3.4 Distribution of *K. pneumoniae* According to the Sources of Infection

Of all 250 clinical samples of wounds and burns, urine, and sputum, 44 isolates were positive for *K. pneumoniae*. 23.2% (29/125) of clinical samples tested for *K. pneumonia*e from nosocomial acquired infection. These positive isolates were obtained in a high percentage; urine samples constituted 26% (13/50), 36% (9/25) from sputum samples, and a low percentage was obtained from wound and burn samples which achieved 14% (7/50), as previously shown in Table 1.
Community-acquired infection accounted for 12% (15/125) of *K. pneumoniae* clinical samples. These positive isolates were obtained in high percentages; sputum samples constituted 16% (4/25), 14% (7/50) from urine samples, and the wound and burn samples 8% (4/50). The percentage of *K. pneumoniae* isolates according to sample source is shown in Table 1.

The results showed that *K. pneumoniae* is the most common hospital pathogen causing UTIs. Many studies have shown that *K. pneumoniae* is one of the most important of clinically isolated nosocomial pathogens. *Klebsiella pneumoniae* is a pathogen and a common cause of HA-UTI and CA-UTI. A total of 81 isolates (31 HA-UTI and 50 CA-UTI) were found in Portugal [18]. It is noticeable that the results are closer to Paterson's *et al* [19] in term of the percentage of *K. pneumoniae* (18.7%) due to ESBL-producing organisms and Al-aajem [16] regarding the percentage of *K. pneumoniae* isolates from UTI which was 22.73%. The differences in these results may be attributed to sample types, isolating source, sampling time, method, number of collected samples, season of sampling, geographical area from which the samples are collected and other factors that differ among studies of this type, the presence of *K. pneumoniae* varies among infected isolates.

3.5 Antibiotics Susceptibility of *K. pneumoniae*

To determine possible resistance of *K. pneumoniae* isolates to 12 antibiotics from various classes, all 44 isolates were tested using the Clinical and Laboratory Standards Institute's antibiogram method (CLSI) [11] and [12], using the disc diffusion method (Kirby-Bauer method) against twelve classes of antibiotics as indicated in Table 2.

Table 2: Distribution of antibiotic resistance according to the nosocomial acquire infection and community acquire infection

Class		Nosocomial acquired (n=29)	Community acquired (n=15)	*P. value*
1	**AMG**	89.65	86.6	0.06
	PTZ	72.42	66.6	0.02*
	TIM	100	100	0.08
2	**CAZ**	96.66	100	0.08
	CRO	79.32	86.66	0.05*
3	**MEM**	100	80	0.03*
	IMP	58.62	66.66	0.05*
4	**AK**	41.37	53.32	0.05*
	ATZ	41.37	60	0.02*
5	**LEV**	55.17	66.6	0.05*
	CIP	58.62	66.66	0.05*
6	**TS**	58.62	73.33	0.05*

P-value was calculated using the Chi-square test in terms of the R, I & S group.

Class (1) B-Lactam-B-lactamase inhibitor combinations, (2) [Cephalosporines GIII, GIV], Sub-class (3) [Carbapenems], Class (4) [Aminoglycosides] Class (5) [Fluoroquinolones], subclass (6) florate pathway antagonists [12], Classes=class and sub-class.

According to the study, nosocomial-acquired *K. pneumoniae* infections are less susceptible than community-acquired infections, and there is a higher rate of resistance for different types of β-lactam/ β -lactamase (AMG, TIM, and PTZ) in nosocomial infections than in community infections. Carbapenems MEM resistance is highest in nosocomial infections while IMI resistance is highest in community infections. The rates of resistance for community infection to third-generation cephalosporin (CRO, and CAZ), aminoglycosides (ATH and AK), fluoroquinolones (CIP and LEV), and florate pathway antagonists (TS) has shown higher susceptibility for nosocomial *k. pneumoniae*.
Al–Zubaidi and Al–Taai [20] showed that *K. pneumoniae* had high resistance to various antibiotics - Lactam groups. AMC 73.36%, AMP 100%, PIP 81.16%, CAZ 62.32%, CRO56.5%, ATM 72.46%, IPM27.53%, MEM26.19%, aminoglycoside groups (AK 47.82%), quinolones groups LEV 31.82%, CIP 24.63. *K. pneumoniae* is a relevant pathogen that can present acquired resistance to almost all available antibiotics, thus representing a serious threat to public health.

Liu *et al.* [21] reported that gram-negative significant bacterial resistance to numerous medically essential antibiotics is low in both community-acquired and nosocomial isolates. Fluoroquinolone-resistant community-acquired carbapenemase-producing *K. pneumoniae* infection exceeded hospital-acquired *K. pneumoniae* infection [22].

It might be due to increasing antibiotic exposure in hospital environments, which would enhance the selection pressure for antimicrobial resistance. Juan *et al.* [23] reported that in nosocomial infections (41.8%) and CA infections, the proportion of multidrug-resistant (MDR) isolates was greatest (5.7%).

The treatment of *K. pneumoniae* infection varies depending on the kind of infection (e.g. surveillance for occult infection, source control, site-specific antimicrobial therapy) [21].

Antibiotic resistant genes are developed in these bacteria during their transmission across bacteria. The molecular mechanisms of resistance have been increasingly revealed as technology progressed. Recent advances in the mechanisms include the ability to hydrolyze antibiotics (e.g., extended spectrum β-lactamases), AmpC β -lactamases, carbapenemases), (iii) avoid antibiotic targeting (e.g., mutated *van*A and *mec*A genes), (iv) prevent antibiotic permeation (e.g., porin deficiency), and (v) excrete intracellular antibiotics (active efflux pump) ability to acquire and transfer genetic determinants, including virulence genes, pathogenicity islands, and antimicrobial-resistance genes [24].

3.6 Multi-drug Resistance of *K. pneumonia* Isolates

MDR, PDR, and XDR were studied in 44 *K. pneumoniae* isolates. Multi-drug resistance is now defined as follows: in nosocomial infection, 11 (37.93%), 7 (24.31%), and 11 (37.93%) isolates were confirmed as MDR, XDR, and PDR, respectively. The most commonly used agents in MDR and XDR were β-lactam/ β-lactamase inhibitor combinations (TIM), carbapenem (MEM), and cephalosporines (GIII, GIV) (CAZ) and moderate (CRO).
Community acquired infections were 4(26.67%), 4 (26.67%) and 7 (46.66%) of isolates were confirmed as MDR, XDR, and PDR, respectively. MDR and XDR are most commonly caused

by β-lactam/ β-lactamase inhibitor combinations (TIM) and moderate (AMG), as well as cephalosporines (GIII, GIV) (CAZ) and moderate (CRO).

K. pneumoniae isolates significantly (P. value 0.05) of MDR isolates from nosocomial infection was the highest at 41.8%, followed by isolates from community infection at 23.5%, with a similar tendency among three categories that could be recognized in isolates resistant to ciprofloxacin, ceftriaxone, and levofloxacin. Wang *et al.* [25] reported that MDR isolates from nosocomial were the highest 53.1%, followed by isolates community infection 23.3%. Practices in infection control and empirical treatment of MDR, XDR and PDR notified in *K. pneumoniae* are one of the most common risks to public health. Typically, PDR *K. pneumoniae* is associated with nosocomial infections (the high prevalence of the usage of antibiotics in Diyala hospitals is well regulated). However, some PDR strains of *K. pneumoniae* are common causes of community-acquired infections.

3.7 Molecular Study of *Klebsiella pneumoniae* DNA.

3.7.1 Total DNA Extraction of *Klebsiella pneumoniae* Isolates

Using a genomic DNA purification kit (Promega, USA), total genomic DNA was extracted from *K. pneumoniae* isolates. DNA extracted from 18 clinical isolates XDR was transferred by gel electrophoresis based on band size. The Quantus™ Fluoromete was used to determine the concentration and purity of DNA. The DNA content in the extracts ranged from 13 ng/μl to 25 ng/μl, according to the findings. The output is dependent on culturing methods, bacterial category, amount of pellet, and type of extraction kit. All these have an effect on the quality and properties of nucleic acid. The genotypic identification findings of 18 isolates revealed that 100% of them were *K. pneumoniae* with 1500 bp 16S rRNA gene amplicons. The results Figure 1 show a bright band of positive isolates when compared to a 1500 bp DNA ladder; the genotypic research utilizing a PCR reaction with a specific 16S rRNA gene cleared up any uncertainties regarding the diagnosis.

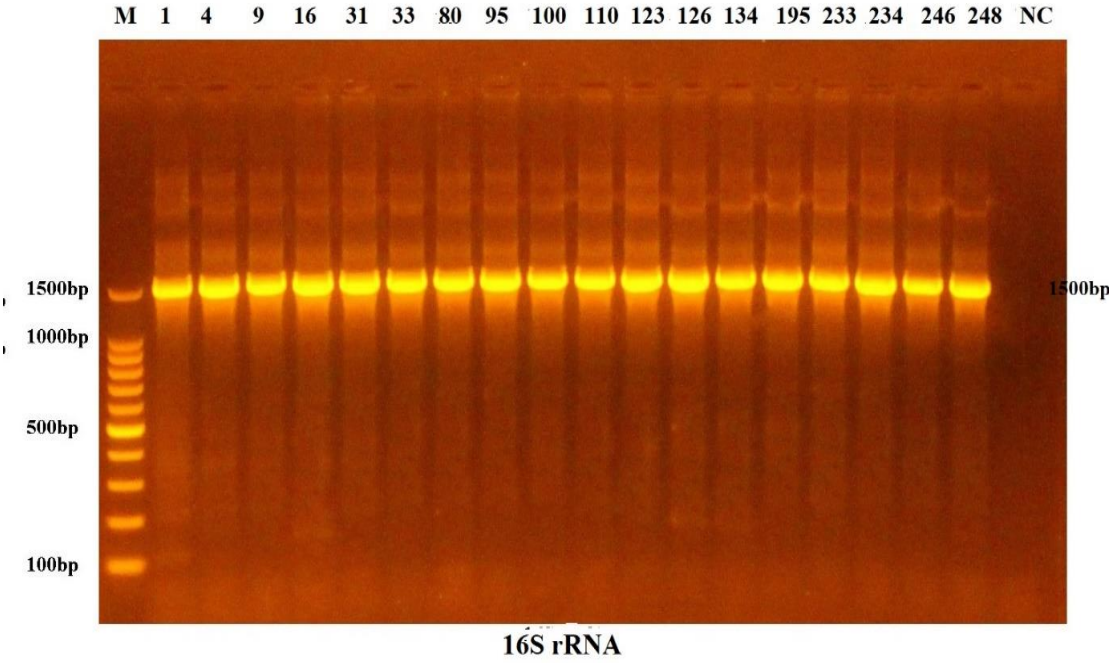

Figure 1: 16S rRNA primer amplification in *K. pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker, NC: negative control 60 minutes of electric current at 100 volts.

3.7.2 Genetic Identification *Klebsiella pneumoniae* on 16S rRNA Sequences

15 clinical isolates of *Klebsiella pneumoniae* (assigned S1 to S15) were isolated from various communities (S1, S2, S4, S6, S8, and S12) and nosocomial (S3, S5, S7, S9, S10, S11, S13, S14and S15) sources. Isolates showed a similar single band of 1500 bp size using agarose gel electrophoresis according to amplification of the 16S rRNA gene. Results of Sanger sequencing were checked and aligned using nucleotide BLAST to ascertain the sequence similarity in the NCBI database. For a variety of reasons, 16S rRNA gene sequences have been the most frequently utilized housekeeping genetic marker in the study of bacterial phylogeny and taxonomy. 16S rRNA gene is present in almost all bacteria, often as a multigene family or operon. The function of the 16S rRNA gene has not changed over time, implying that random sequence changes are a more accurate measure of time (evolution), and that the 16S rRNA gene (1,500 bp) is large enough for informatics purposes [26].

The NCBI BLAST engine found up to 99.5% sequence similarity between the sequenced samples and *K. pneumoniae* reference target sequences for the 1531bp amplicons. By comparing the observed nucleic acid sequences of the examined samples to the nucleic acid sequences of the retrieved samples (GenBankacc.CP052181.1), the accurate positions and other details of their retrieved PCR fragments were found. The overall length of the targeted loci was determined using the NCBI server, as well as the start and end positions of the targeted locus inside the most similar bacterial target, as shown in Figure 2.

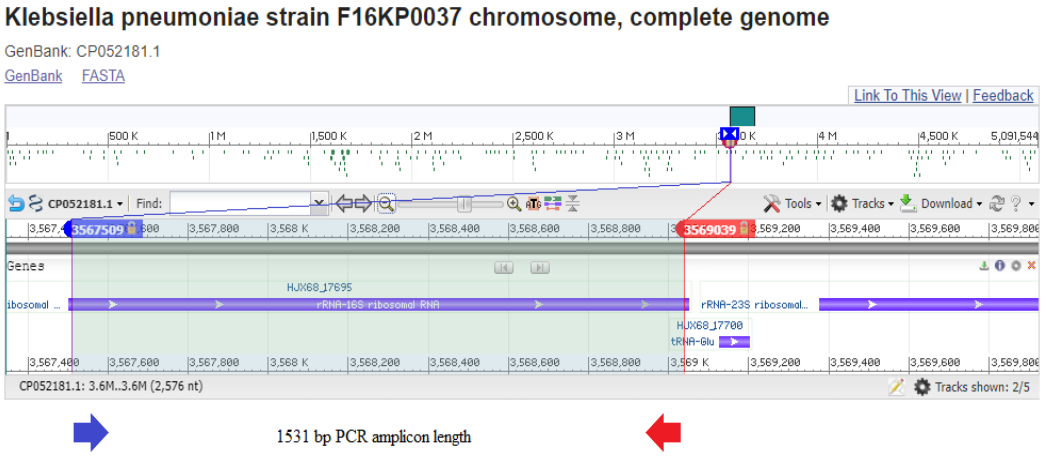

Figure 2: 16SrRNA sequences inside *K. pneumoniae* genomic sequences were partially covered by the exact site of the recovered 1531bp amplicon (GenBankacc.no.CP052181.1). This amplicon beginning point is shown by the blue arrow, while its terminus is indicated by the red arrow.

Interestingly, the results of the 1531 bp samples discovered the presence of six nucleic acid variations in the various samples when compared with the most similar referring reference nucleic acid sequences (GenBankacc.no.CP052181.1).

Therefore, 16S rRNA has been comprehensively used to reconstruct the phylogenetic evolution of microorganisms. The genetic proximity between *K. pneumoniae* strains has also been observed by various other researchers [27].

Results indicated the presence of six nucleic acid variations (174T > C, 448A > G, 448A > T, 450A > G, 466G > T, and 806A > G) in some of the investigated samples. The observed 174T > C variance was detected in the investigated S2, S8 and S12 samples. The observed 448A > G variance was detected in S8, while the neighbouring 450A > G variance was identified in S4, S5, S8, and S14 samples. The detected 448A > T variance was identified in

S2–S7, S10, S11, S13–S15 samples, less frequently, the 466G > T variance was detected in both S8 and S12 samples. Meanwhile, S2-S4 and S12-S14 samples showed 806A > G variance. To confirm these variations, the studied samples sequencing chromatograms, as well as their extensive annotations, were validated and documented, and the quinces' chromatograms were displayed according to their locations in the PCR amplicons, as shown in Figure 3.

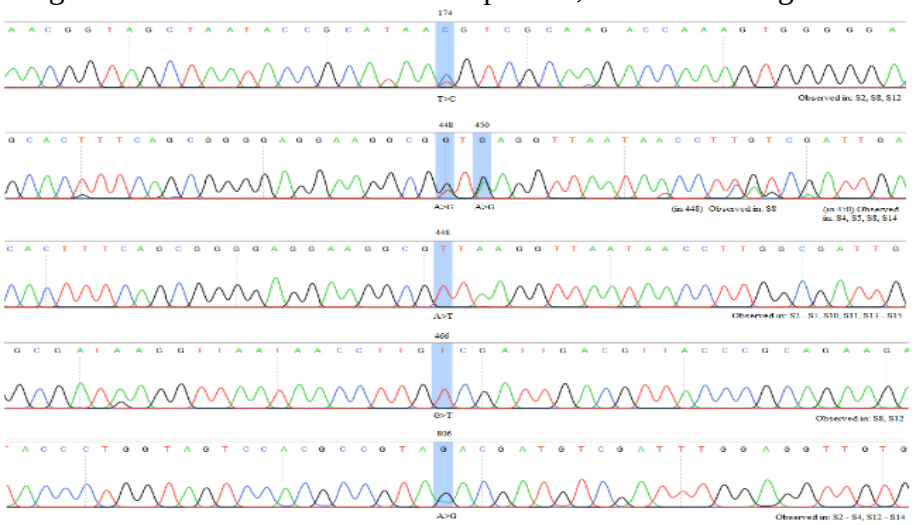

Figure 3: Chromatogram of the investigated human-infecting bacterial samples of *K. pneumoniae*. Letter "S" denotes to the code of the investigated samples. The presence of a double- peak indicates possible double infection issue in the investigated sample.

In 2020, Mohammed *et al.* [28] showed the appearance of two isolates (MH119051.1) and (MH119052.1) which gave closer matches with some global stains such as KM203760.1, an Indian isolate.

To study the phylogenetic of the investigated S1–S15 samples, a comprehensive phylogenetic tree was generated according to nucleic acid sequences observed. In the amplified 1531bp of the Ribosomal amplicons, the phylogenetic tree was generated to incorporate these fifteen investigated samples alongside other relative nucleic acid sequences of human infecting *Klebsiella pneumoniae* sequences.

All the investigated samples were aligned alongside other related sequences inside this tree to form the cladogram's currently integrated sequences. In this comprehensive tree, there were a total of 60 aligned nucleic acid sequences. Within the *K. pneumoniae* sequences, the included samples were grouped into seven closely related phylogenetic clades in the produced cladogram.

As shown in Figure 4, the biggest clade consisted of the majority of incorporated samples of *K. pneumoniae* sequences, as 14 sequences of variable strains of *K. pneumoniae* were incorporated within extremely close phylogenetic distances within this clade. Variable strains were found to be deposited from variable Asian and American sources, such as China (GenBankCP028583.1, CP028797.1, CP027068.1, CP028783.1, and CP029384.1), Japan (AP024795.1, AP024788.1), and the USA (GenBankCP043047.1).

In the vicinity of this major clade, a small clade was also incorporated. Within this clade, six sequences were incorporated, with close homology among them. As in the case of the major clade, the investigated samples were not incorporated into this clade.

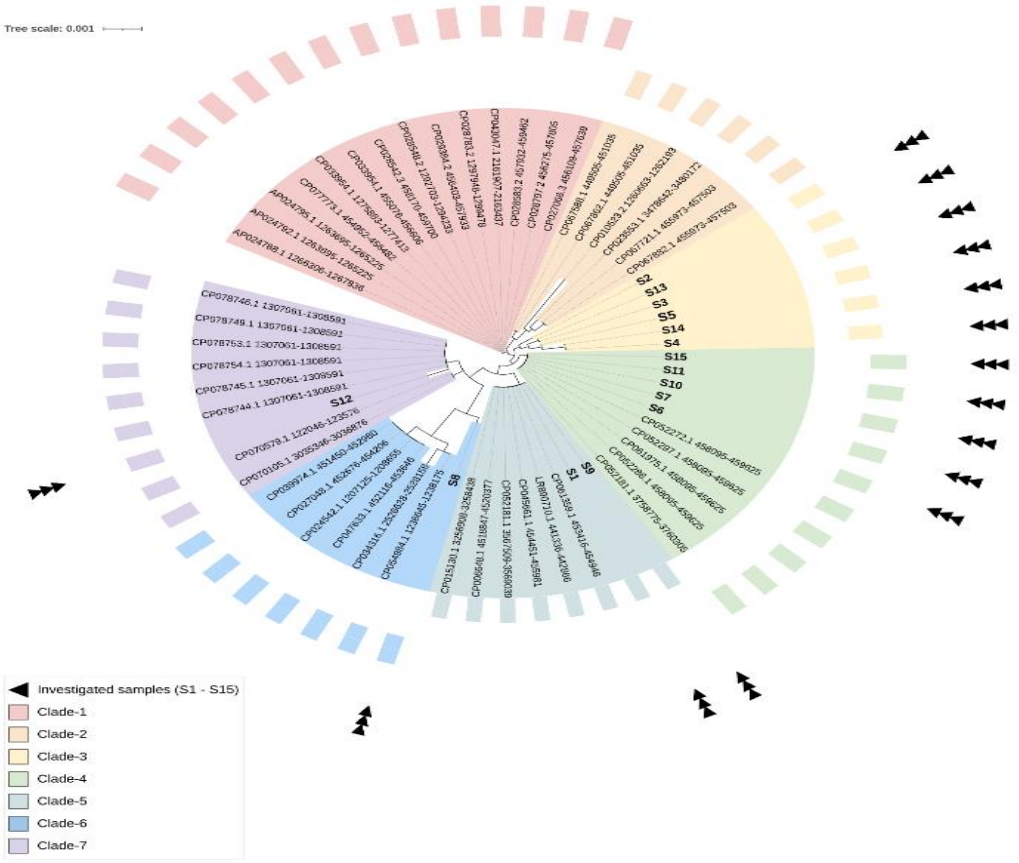

Figure 4: A cladogram phylogenetic tree comprising genetic variations of the 16S ribosomal segment from fifteen *Klebsiella pneumoniae* samples was constructed. The triangle with the black colour represents the sequences of microorganisms that were examined. All of the numbers related to each referencing species' GenBank entry number The number "0.001" at the top of the tree denotes the degree of scale range among the creatures classified by the comprehensive tree. The letter "S #" stands for the code of the sample under investigation.

All the investigated fifteen ribosomal sequences were deposited in the NCBI web server and unique accession numbers were obtained for all analyzed sequences, starting from GenBank OM992298 that deposited to represent the S1 sample, to the GenBank OM992312 that deposited to represent the S15 sample.

A smaller clade is located in the vicinity of this six-sequence clade. Within this clade, six *K. pneumoniae* strains were incorporated, which were deposited from variable regions in America (GenBank CP067588.1), South America (GenBank CP067862.1, CP067721.1 and CP067882.1), and South Africa (GenBank CP023553.1). The most interesting fact observed in the investigated bacterial isolates is correlated with the positioning of six of the investigated samples in a separate *K. pneumoniae* clade located beside this small clade.

Bacteria are vulnerable to mutations brought on by their environment. This, however, is a natural part of their development, allowing them to change their genetic code on a regular basis [29].

As in the case of these six (S2, S3, S4, S5, S13, and S14) samples, another five investigated community acquired and nosocomial acquired samples (S6, S7, S10, S11, and S15) were found

not to be affected by the currently observed variations. These samples reside in a clade made of 10 sequences of *K. pneumoniae*. This clade consists of various strains of *K. pneumoniae* isolated from various Asian sources (such as GenBank CP052272.1, CP052297.1, CP052286.1, CP052181.1) from South Korea, and GenBank CP061975.1 from China). Besides this clade, both S1 and S9 samples are suited to another clade. Within this clade, 10 different sequences of *K. pneumoniae* are incorporated. However, no obvious effect of the origin of isolation was reported. Alsanie [30] reported that a total of 23 isolates of *K. pneumoniae* were identified using a microbiological approach and validated using 1365 bp of 16S rRNA gene sequences from a total of 134 clinical samples. The BLAST results revealed that partial 16S rRNA sequences are similar to *K. pneumoniae* strains, with MN-314311 and MK713647 having a matrix of 98% and 97% respectively.

Furthermore, a small clade made of eight sequences is observed. The most important notion in this clade is represented by the determined positioning of all investigated S1 and S9 samples in *K. pneumoniae*. This observation indicated that both investigated samples shared the same biological distributions in the cladogram. Both (S8 and S12) samples are found to share the same mutation, 466G > T. Since the observed 466G > T is not found in any sample isolated from a nosocomial acquired source, this variance may exhibit a clear tendency to be localized in the community acquired *K. pneumoniae* isolates. As in the case of the other five incorporated clades of *K. pneumoniae*, these two clades have exhibited another multinational distribution of these bacterial sequences. This is due to the presence of several Asian Americans, such as GenBank CP054984.1 (deposited from Hong Kong), GenBank CP047633.1 (deposited from China), GenBank CP034316.1 (deposited from Tanzania) and GenBank CP070105.1 (deposited from the USA). *K. pneumoniae* into the nosocomial is a vital spread that leads to increased morbidity, mortality, health care costs, and antibiotic use. The factors related to the community spread of MDR and PDR *K. pneumoniae* overlap but differ from those related to nosocomial. Preventing the further community spread of PDR *K. pneumoniae* is of the utmost importance and requires a multidisciplinary approach involving all investors. These results highlight that the genomic surveillance focus should remain a priority in the hospital environment [18].

The presence of this tree has confirmed sequencing reactions because it explains the actual neighbour-joining-based positioning in such investigated sequences. In total, in addition to the Asian origins of these samples, several American and African origins were also observed. Thus, the multiple origins of the investigated samples could not be excluded from the explanation.

4. Conclusion

Phylogenetic analysis the 16S rRNA gene is important for identifying mutations in local isolates. In this study, the positive rates of nosocomial acquired infection and community acquired infection were 96% and 48% respectively. The distribution of the pathogenic bacteria *E. coli* was 19.2% and 8.8%, and of *K. pneumoniae* was 23.2% and 12%, which is a higher percentage than other pathogenic bacteria of nosocomial and community-acquired infections. Nosocomial *K. pneumoniae* isolates were less susceptible than community-acquired *K. pneumoniae* isolates, and have higher rates of resistance to different types of -lactam/-lactamase (AMG, TIM, and PTZ). Phylogenetic analysis of the 16S rRNA gene showed the presence of six nucleic acid variations in various samples (174T > C, 448A > G, 448A > T, 450A > G, 466G > T, and 806A > G). Since the observed 466G > T was not found in any sample isolated from a nosocomial acquired source, implying that this variance might exhibit a clear tendency to be localized in the community acquired *K. pneumoniae* isolates. The fifteen

ribosomal sequences from OM992298 to OM992312 are the unique accession numbers that were deposted in NCBI-GenBank.

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