Study the role of N-acyl homoserine lacton formation and *luxI* gene in *K. pneumoniae* isolated from UTI

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Abstract

Background: The objective of this investigation was to find and characterize the N-acyl-homoserine lactones (AHLs) produced by different strains of *Klebsiella pneumoniae* that are resistant against antibiotic in UTIs. In this study, the *luxI gene* responsible for the formation of the homoserin lactone acyl compound was identified. **Materials and Methods:** *K. pneumoniae* identification by selective agar supplement HiCrome^{TM}agar A total of one hundred *K. pneumoniae* isolates were recovered, investigated for their antibiotic susceptibility, and screened for AHL production using the methods of Crose-Streak Assay, Soft Agar Assay, and Thin-Layer Chromatography (TLC) Assay. DNA extraction and genotyping Using a commercial purification technique (presto Minigenomic DNA extraction kit; Geneaid, Thailand), Primers for *luxI gene* (Macrogen, Korea) selection according to the supplier's recommendation. **Results:** Different short- and long-chain AHL molecules were found, according to the findings, C6-HSL, C8-HSL, and C10-HSL among 100 *K. pneumoniae* isolates recovered from UTI samples. The results of this study showed that the 59 *K. pneumoniae* isolates under study were producers of N-acyl homoserine. AHLs produce if below a threshold level (OD≥0.2). The result was positive N-acyl homoserine with a positive *luxI gene* in 33 (82.5%) of the bacterial isolates, while 7 (17.5%) were negative for both the N-acyl homoserine and *LuxI genes*. The result was highly significant differences at p-value ≤0.0001.

Keywords: *K. pneumoniae*, N-acyl-homoserine lactones molecules, *luxI* gene, UTI

INTRODUCTION

Quorum sensing (QS) was first reported in 1970 by Kenneth Nealson, Terry Platt, and Woodland Hastings [1]. The second most typical bacterial illness on the globe is urinary tract infections (UTIs). Infections in the upper urogenital tract can cause pyelonephritis and kidney infections, whereas infections in the lower tract can cause cystitis and urethritis [2]. *Klebsiella pneumoniae* is one of the bacteria related to UTIs that has been on the rise and is now a significant burden on many public health systems, particularly in hospital settings [3]. One of the virulence factors, biofilm formation is a characteristic shared by all bacteria[4]. Infections related to healthcare are caused by this bacterium's capacity to create biofilms on invasive medical devices, particularly in the lungs and urinary tracts [5]. Microorganisms that form biofilms use QS systems to control their population density, interact directly with one another, and either directly or indirectly produce chemicals that have an impact on the survival of nearby populations, also QS is essential for biofilm doublication, biofilm development, and biofilm dispersal, which regulates the number of microbes present in an area [6]. QS is used by bacteria to coordinate the expression of genes involved in group behavior. Autoinducers are extracellular signaling molecules involved in the production, release, detection, and collective response of QS [7]. N-acyl-homoserine lactones (AHLs) are a type of autoinducer QS signal molecule synthesized by gram-negative bacteria. These have been demonstrated to possess significant regulatory functions in several aspects of microbial systems. Cellular processes in microbial communities include EPS secretion, biofilm development, flocculation, and community recombination [8]. The synthesis of acyl-HSL is dependent on the genes *luxI* and *luxR*, and these two genes are essential for quorum sensing through acyl-HSL. LuxR encodes a transcriptional activator protein that is in charge of detecting the cognate acyl-HSL and causing the necessary output [9]. Typically, two regulatory tasks make up quorum sensing circuits in gram-negative bacteria. Initially, an acylated homoserine lactone (HSL) second, autoinducer reception and activation of target gene expression are mediated by a regulatory protein of the LuxR type [10]. QS enables a bacterial population to operate in concert to produce broad phenotypes, including alterations in the expression of virulence genes for competitive advantage, the acquisition of drug resistance, and production of biofilms. Multiple autoinducers associated with bacterial pathogenicity have been found [11]. A

receptor protein and an autoinducer make up the two primary parts of the quorum sensing systems in bacteria. The target gene is activated by autoinducer attaching to its receptor, and the target gene subsequently carries out the associated activity in bacteria [12]. An extensive study has been conducted on AHL-based systems. Both the luxR homolog gene, which acts as an AHL-dependent transcriptional regulator, and the luxI homolog gene, responsible for the production of AHLs, are commonly observed in these systems [13]. The *LuxR* and *LuxI genes* have homologs, wherein the R gene encodes the AHL receptor and signal transducer (R protein), whereas the *I gene* encodes the AHL signal synthase (I protein). The production of acyl-homoserine lactones (AHLs) is contingent upon the presence of the I protein [14].

MATERIALS AND METHODS

K. pneumoniae Identification by selective agar supplement HiCrome^{TM}agar. CHROM^{TM} agar was prepared according to the manufacturer's instructions.

Three methods that use for Detection of N-Acyl homoserine Lactone .

Crose-Streak Assy

Klebsiella pneumoniae biosensor strain was inculated into 2 ml of LB containing 30 ug/ml of gentamicin and then grown motionless at 28 °C over night. Create 80 ul of 20 ug/mL (X-gal) per 20 mL LB agar plate. Dry the plate for at least an hour at 37 °C without the lid. 30 ul of the *K. pneumoniae* biosensor strain to the plate while holding it at an angle (OD600 = 0.2), and then wait for a line of cells to appear as the culture runs down on the plate's surface. After that, put *K. pneumoniae* in a loop alongside the biosensor strain, and another strain will be examined for AHL synthesis. Alternately, spot 5ul of the culture will be evaluated for its ability to produce signals that the spot absorbs into the agar as it is placed close to the biosensor strain. The plates were incubated at 28 °C for 12 hours, and the biosensor strain started to turn blue. The presence of the AHL signal is shown by the *K. pneumoniae* biosensor's blue coloration close to the strain(s) of interest.

Soft Agar Assay method

Inject 2 ml of LB containing the biosensor strain of *K. pneumoniae*, which contains 30 ug/ml of gentamicin. grow motionless at 28 °C over night. 250 ul of the *K. pneumoniae* culture (OD A600 = 0.2) should be combined with 20 ml of melting agar, along with 80 ul of 20 ug/ml (X-gal) for every 20 ml of cooled agar. Mix and pour the dish right away. The plate should be allowed to set before drying inverted at 37 °C for 30–45 minutes. Bacterial cultures, colonies, or supernatants can be plated or patched as required. Plates are incubated at 28 °C. Interest-generating strains that secrete AHL will have a blue halo.

Thin-Layer Chromatography (TLC).

On a plate of reversed-phase C18 TLC, arrange the bacterial cultures to be analyzed. Ideally, spots should be 2–3 cm from the TLC plate's bottom. Permit stains to completely dry. Fifty mL of 60% methanol and a TLC plate should be placed in a glass chamber for incubation. The solvent should be allowed to rise all the way to the plate's top. Place the plate in a fume hood and let it stand at room temperature for 60 minutes to let the methanol evaporate. Tape should be used to completely enclose the TLC plate on all four sides, leaving at least a 1/2-inch lip on each side. This is a good use for laboratory tape. LB soft agar in a volume of 50 mL was prepared. 400 ul of the *K. pneumoniae* culture and 160 ul of 20 ug/mL X-gal are added after cooling to 45 °C. The agar solution should be blended immediately, followed by flowing over the TLC plate to cover the whole thing. 30 minutes are needed for the agar to solidify. Place the TLC plate on top of an 8 x 8 glass pyrex dish with one hundred mL of water in the bottom after removing the tape border. The sealed pyrex plates should be incubated at 28 °C for 24-48 hours, or until blue spots show up to show that the biosensor is engaged.

DNA extraction and genotyping

Using a commercial purification technique (presto Minigenomic DNA extraction kit; Geneaid, Thailand), Primarily, selecting and preparing According to the supplier's recommendation, these primers (Macrogen, Korea), which were supplied in lyophilized form, were dissolved in nuclease-free water to give a final concentration of 100 picomole/µl. To prepare a 10 pmol concentration, work primers were resuspended in 90 µl of nuclease-free water to reach a final concentration of 10 pmol/µl. The primer stock

solutions were kept in the tubes and frozen at -20 degrees Celsius until needed. A specific pair of primers was used in conventional PCR to detect *LuxI genes*. The forward and reverse primers were :F:5´TTCATATGAATATTATTGCTGGATTTC3´.And,R:5´TCGAGCCTATCTAAATACACATCAATCA - 3´. As previously described, the initial denaturation stage took place at 95 ℃ for 2 min, and then the amplification process began with 30 cycles of denaturation at 95 ℃, annealing at 58 ℃ for 45 s, and extension at 72 ℃ for 1 min. Primer 3 software was used to compute annealing temperatures [15].

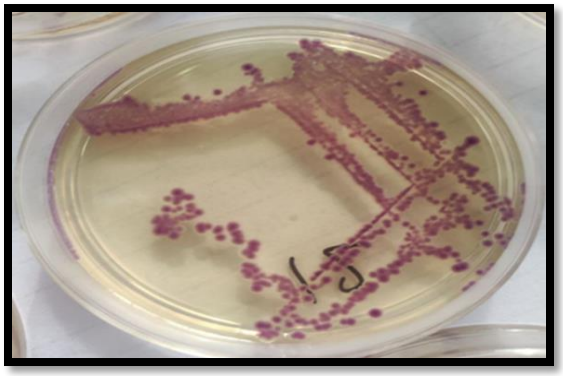
Statistical analysis

The data analysis utilized the IBM SPSS-29 statistical tool (IBM Statistical Packages for Social Sciences, version 29, Chicago, IL, USA). The data were presented using basic metrics of frequency and percentage and a significant correlation of greater than 0.7. The outcome yielded statistically significant differences with a p-value of ≤0.0001.

RESULT

K. pneumoniae Identification

The utilization of selective agar supplement HiCrome^{TM} agar was employed in this study. The sample was collected and afterward cultivated on HiCrome *Klebsiella* selective agar supplement, which contained Carbenicillin at a concentration of 25 mg per 500 mL. The culture was incubated at a temperature of 37°C for 24 to 48 hours to isolate *Klebsiella spp*. Colonies. These colonies exhibited a purple-magenta coloration, as depicted in Figure 1.



Figure(1): *K. pneumoniae* grown on HiCrome^{TM}agar for 24 hrs at 37˚c.

Detect N-acyl homoserine lactone

The results of this study showed 59 *K. pneumoniae* isolates by the methods of Crose-Streak Assay, Soft Agar Assay, and Thin-Layer Chromatography (TLC) Assay. These methods can be seen in figure 2,3.

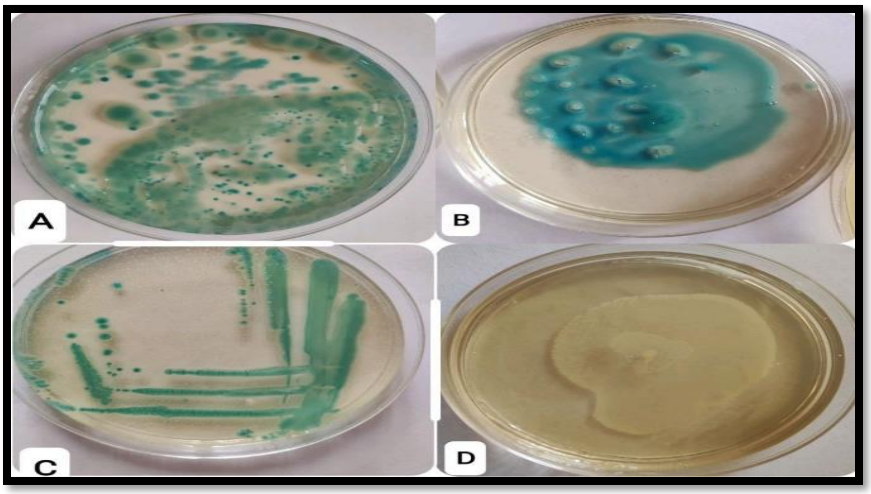


Figure 2: A,B. Soft Agar Assay blue color and positive AHLs. C: Crose-Streak Assay blue color and positive AHLs.D: Soft Agar Assay not be color and negative AHLs.

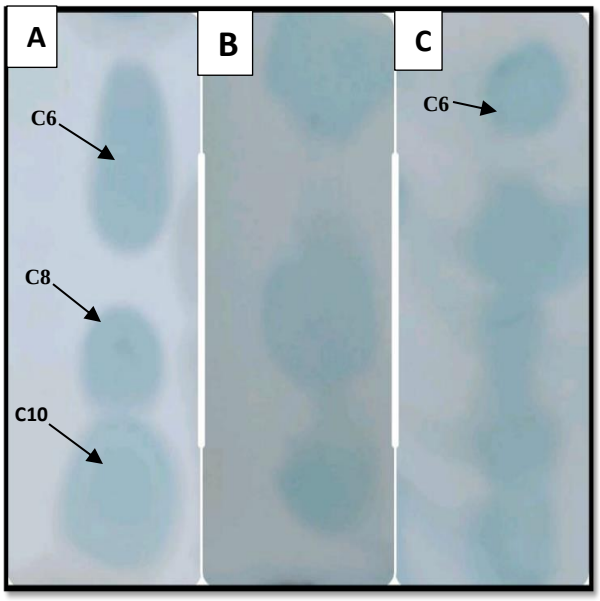


Figure (3): Thin-Layer Chromatography (TLC) Assay, blue color and positive AHL

detection of genes that involved in biofilm formation

The *LuxI gene* presented in the DNA of *K. pneumoniae* isolates with a PCR product size of single amplicon 370 bp was shown in figures 4,5 and 6. It was found that the positive N-acyl homoserine with the positive *LuxI gene* was found in 33 (82.5%) of bacterial isolates, while 7 (17.5%) were negative for both the N-acyl homoserine and *LuxI gene*. The result was highly significant differences at p-value ≤0.0001.

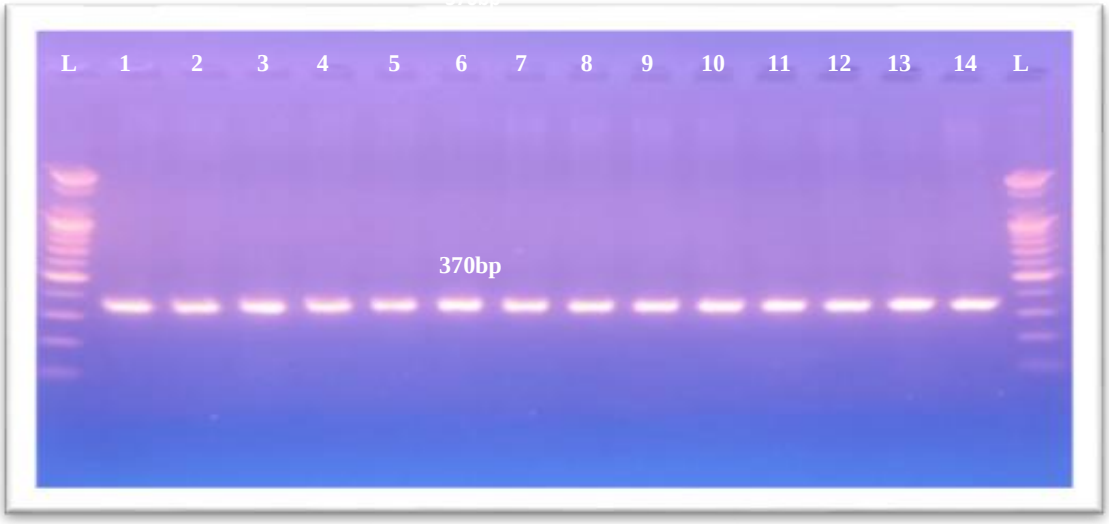


Figure 4: The amplification of the *LuxI gene* in *K. pneumoniae* samples was fractionated on a 2% agarose gel (75 min at 45 volts) by electrophoresis stained with ethidium bromide. M: 100-bp ladder marker. Lanes 1–14 represent positive bands for 370 bp PCR products.

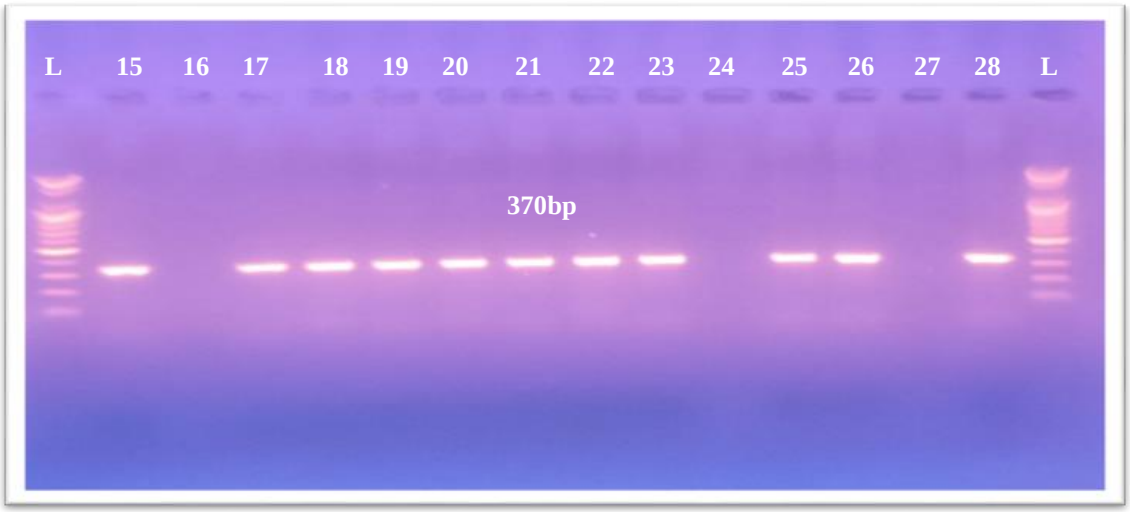


Figure 5: The amplification of the *LuxI gene* in *K. pneumoniae* samples was fractionated on a 2% agarose gel (75 min at 45 volts) by electrophoresis stained with ethidium bromide. M: 100-bp ladder marker. Lanes 15, 17, 18, 19, 20, 21, 22, 23, 26, and 28 represent positive bands for 370 bp PCR products.

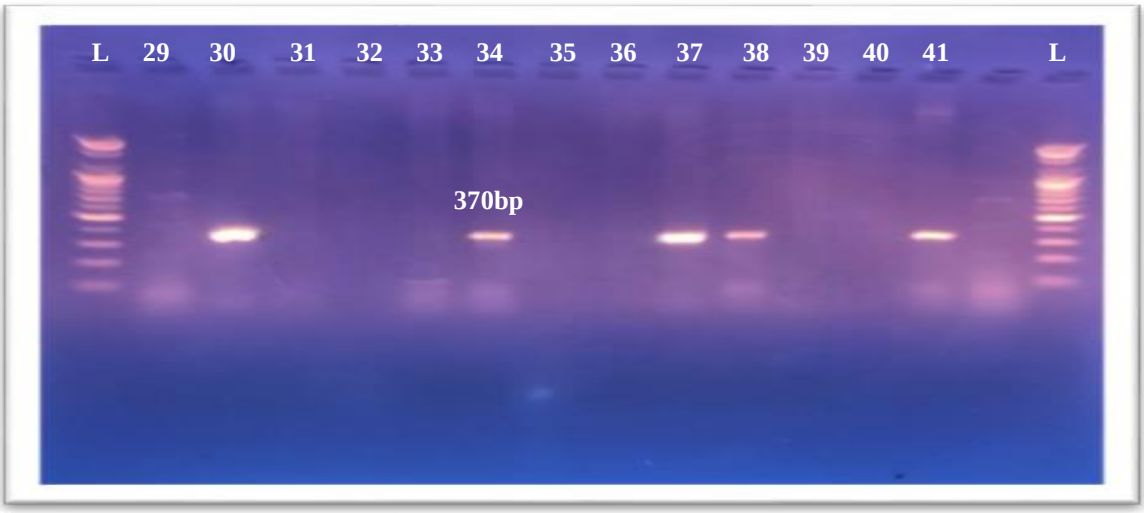


Figure 6: The amplification of the *LuxI gene* in *K. pneumoniae* samples was fractionated on a 2% agarose gel (75 min at 45 volts) by electrophoresis stained with ethidium bromide. M: 100bp ladder marker. Lanes 30, 34, 37, 38 and 41 resemble positive bands for 370bp PCR products.

DISCUSSION

A total of 100 urine samples were diagnosed with *K. pneumoniae*. *K. pneumoniae* grown on HiCrome^{TM}agar. purple-magenta-colored mucoid colonies [16]. The results of this study was showed that the 59 *K. pneumoniae* isolates under study were producers of N-acyl homoserine. AHLs produce if below a threshold level (OD≥0.2) is positive. According to the resercher's Tierney and Rather 2019 method, Crose-Streak Assay, Soft Agar Assay, and Thin-Layer Chromatography (TLC) Assay [17]. Among the several AHL biosensors developed thus far, the *K. pneumoniae* biosensor has the most extensive sensitivity to AHLs, even at exceedingly low concentrations. The biosensor, which utilizes galactosidase, is very suitable for the analysis of thin-layer chromatography (TLC). Due to its remarkable sensitivity to various AHLs, only a small quantity of AHL extracts from discarded supernatants is necessary [18]. During the investigation of the extracts obtained from the culture supernatants of T2-1-1 and T2-1-2, three distinct spots were observed on the TLC plate. These spots exhibited relative migration factor (Rf) values that were consistent with those of 3-oxo-C6-HSL, C6-HSL, and C8-HSL. It was observed that strain T2-1-2 exhibited a higher production of 3-oxo-C6-HSL compared to strain T2-1-1 [19]. Only 12 (23%) of the 52 different isolates did not produce any AHLs or AHL-like activity that could be detected in the bioassays utilized. 28 (70%) of the 40 isolates that exhibited AHL-like activity tested positive in multiple assays [20]. Proteobacteria and Vibrio spp.

were the most common strains that were shared with other investigations. AHLs were generated by 38% of α-Proteobacteria in this investigation. The present study reported Ruegeria AHL-producing members for the first time, but of the Proteobacteria, 40% of Vibrio and 35% of Pseudoalteromonas produced AHLs [21]. study characterised 11 AHLs generated by 47 V. alginolyticus strains and examined their effects on biofilm formation. We found AHLs and the major AHL signals produced by the 47 V. alginolyticus strains, and we suggested that 3-oxo-C10-HSL affects biofilm development [23]. In the soft agar bioassay, it was shown that 18 out of the total 68 strains exhibited the ability to produce AHL. In the extract bioassay conducted, it was shown that 21 out of 68 strains exhibited AHL production. Moreover, several extracts displayed faint coloring or inhibitory effects on the reporter strains [21]. Among the 43 species belonging to the Halomonadaceae family, the production of AHLs is observed. The distribution of *LuxI*-type synthase was examined in 29 species through the amplification of a 300-400 bp segment using the polymerase chain reaction (PCR) technique [22]. QS of Type 1 is facilitated through the involvement of autoinducers, also known as AI-1. These AI-1 molecules are species-specific AHLs that are synthesized and controlled by AHL synthases encoded by the *luxI* gene [24]. The results of this study highlight the significant relationship between the *LuxI* gene and biofilm formation in *Klebsiella*. The strong correlation between *LuxI* gene presence and varying degrees of biofilm formation reinforces the role of quorum sensing mechanisms in coordinating biofilm-related behaviors [25].

Conclusion

The study concluded that Acyl Homoserine Lactone (AHL) was not produced by all isolates of *K. pneumoniae*, but it has a role in bacterial pathogencity by increasing the production of virulence factor. In conclusion, the presence of Acyl Homoserine Lactone (AHL) and the *LuxI gene* in *K. pneumoniae* from urinary tract infections (UTIs) suggests the involvement of quorum sensing mechanisms in the pathogenesis of UTIs caused by this bacterium. The production of AHLs is regulated by the *LuxI gene*, which encodes for an enzyme responsible for AHL synthesis.

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