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PCR detection of quorum sensing autoinducer (AI) type one among gram negative bacteria

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Abstract: Any bacterial species is found to be regulating their activities like pathogenicity, antibiotic resistant, biofilm production, and others by phenomenon system called quorum sensing. This system is dependent on production signaling molecules called autoinducers (AI); consequently, we tested the presence of QS genes by polymerase chain reaction (PCR) among different gram negative bacterial pathogens.
Material and Methods: Fifty isolates of each of *Citrobacter spp.*, *E. coli*, *Salmonella typhimurium*, and *Pseudomonas auroginosa* were obtained from different private clinical laboratories in Baghdad and Al-Najaf cites in the period of June 2020-February 2021. Bacteria were identified using CHROM agar Orientation followed by Vitek2 system. Quorum sensing genes (*abaI* 382bp and *AHL* gene 498bp) were screened by PCR.
Results: It is showed that *E. coli* had *abaI* and *AHL* genes among 80% and 92% isolates, respectively. Also, *Citrobacter spp.* had *abaI* and *AHL* genes among 73% and 82% isolates, respectively. *Salmonella typhimurium* had *abaI* and *AHL* genes among 66% and 79% isolates, respectively. Finally, *Pseudomonas auroginosa* had *abaI* and *AHL* genes among 58% and 96% isolates, respectively.
Conclusion: It is concluded that pathogens tested had the regulatory system quorum sensing leading to the concern of causing chronic infection.
Keywords*: Quorum sensing, AHL, chronic infections, AI.*

1. Introduction

Bacterial species is found to be regulating their activities like pathogenicity, antibiotic resistant, biofilm production, and others by phenomenon system called quorum sensing or intercellular communication (1). This system is dependent on production signaling molecules called autoinducers (AI) or *N*-acyl homoserine lactone AHL (2). This regulatory system can be used to monitor the density of bacterial population in terms of harsh environment to form biofilm, pathogenesis, and other bacterial attitude (3 and 4). The activity of quorum sensing is seen by gene expression when producing AI-1 or so called type one *Lux I/Lux R* which are clustered in pair or AI-2 (type two or *lux S*) (5, 6, and 7). It is found that gram negative bacteria can communicate within each other by N-acyl derivatives of homoserine lactone (8). Gram negative *Proteobacteria phylum is known to use quorum sensing* AHL that produced by *LuxI* and sensed by *LuxR* genes (1 and 8). It is noticed that *E. coli* pathogens could sense its population density by AHL (9). *E. coli O157:H7* leads to the concern of severe gastroenteritis, septicemia, meningitis, fever, seizures, cerebral edema, coma, and Shiga toxin expression, and are studied to be related to quorum sensing system (10). *Citobacter* isolated from young pregnant female with UTI, vesico vaginal fistula, and acute pyelonephritis were found to produced different types of Acyl homoserine lactone to regulate their activities, especially those isolated from

dental plaque (11, 12, 13, 14) *Salmonella typhimurium* control virulence factor production through what so called quorum sensing (15), and also it is noticed to produce a signaling distinct from AI-2 (16). It is found that *Pseudomonas auroginosa* could lower outer membrane permeability, produce biofilm, have multidrug efflux pumps, hypermutability, and have plasmid- and transposon-encoded acquired –lactamases by having quorum sensing regulating system (17, 18,19, and 20). Having this kind of density regulation would help in increasing pathogenicity leading to chronic infections, consequently, four gram negative bacterial pathogens were screened having quorum sensing autoinducer (AI) type by PCR.

2. Material and Methods

2.1.Bacterial isolates collected: Fifty isolates of *Citrobacter spp., E. coli*, *Salmonella typhimurium* and *Pseudomonas auroginosa* were obtained from different private clinical laboratories in Baghdad and Al-Najaf cites in the period of March 2020-February 2021. Bacteria were identified using CHROM agar Orientation followed by Vitek2 system.

2.2. PCR method: Primers used in this study presented in table 1. PCR conditions were presented as follows in table 2.

Table 1: Primers used in this study (21)

Primer Name	Sequences (5'……. 3') Forward	Sequences (5'……. 3') Reverse	Predicted size (bp)
abaI	5-GTACAGTCGACGTATTTGTTGATTGGG-3	5-CGTACGTCTAGAGTAATGAGTTGTTTTGCGCC-3	382
AHL	5-TTGACCATGCAGGCGGAA-3	5-TTTGGTACCGCCGAAGCT-3	498

Table 2: PCR conditions (21)

Amplified genes	Initial denaturation	No. of cycles	Denaturation	Annealing	Elongation	Final extension
abaI	95°C/5min	40	94°C/ 30sec	66.5°C/30 sec	72 °C/ 1min	72 °C/ 10 min
AHL	95°C/5min	40	94°C/ 30sec	56 °C/ 40sec	72 °C/ 1min	72 °C/ 10 min

3. Results

3.1. Detection of quorum sensing genes among *E. coli*

It is showed that *E. coli* had *abaI* gene (382bp) (figure 1) and *AHL* gene (498bp) (figure 2) among 80% and 92% isolates, respectively.

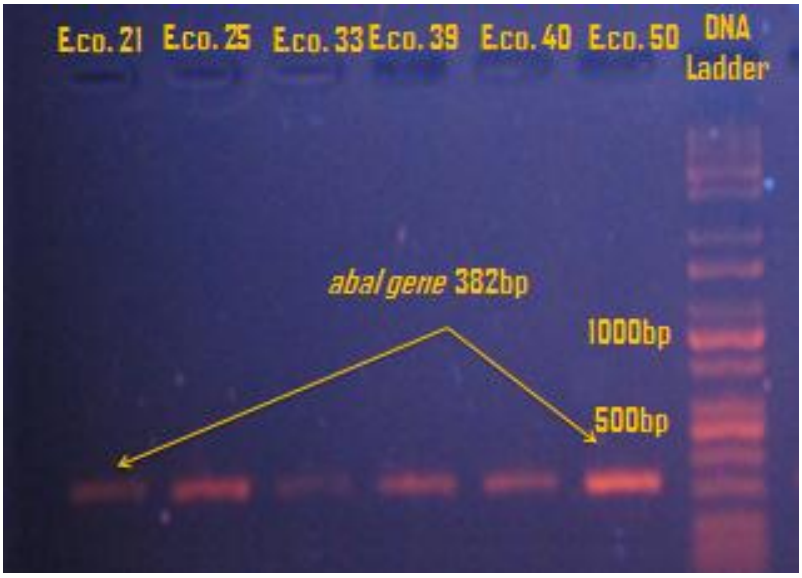

Figure 1: Gel electrophoresis (130min of 50W/cm) showed 80% positivity of *abaI* gene among *E. coli* isolates.

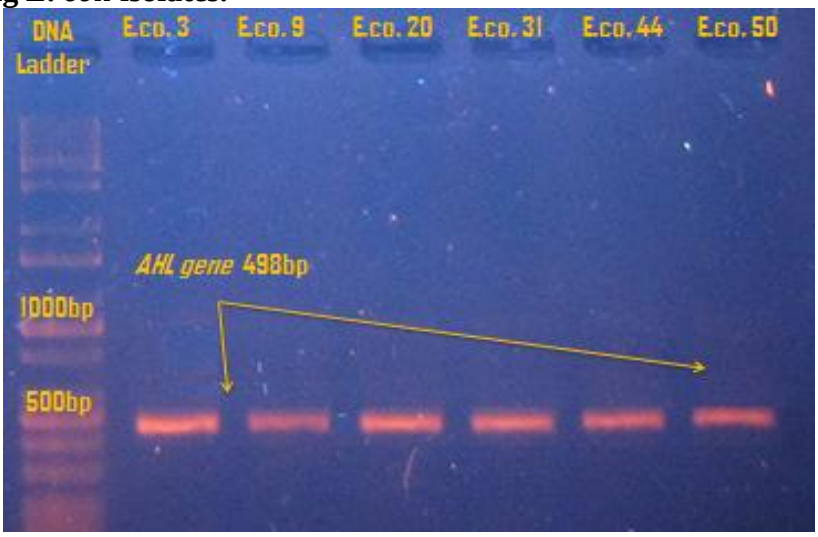

Figure 2: Gel electrophoresis (130min of 50W/cm) showed 92% positivity of *AHL* gene among *E. coli* isolates.

3.2.Detection of quorum sensing genes among *Citrobacter spp.*

It is showed that *Citrobacter spp.* had *abaI* gene (382bp) (figure 3) and *AHL* gene (498bp) (figure 4) among 73% and 82% isolates, respectively.

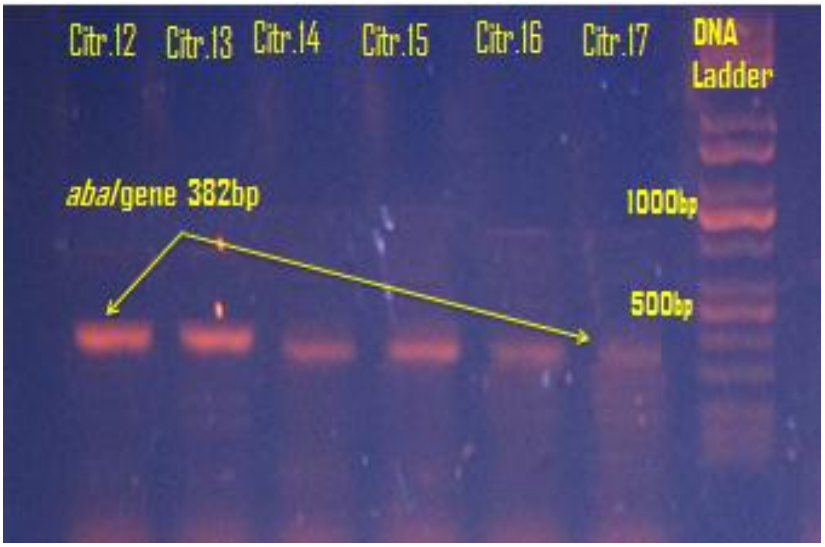

Figure 3: Gel electrophoresis (130min of 50W/cm) showed 73% positivity of *abaI* gene among *Citrobacter spp.* isolates.

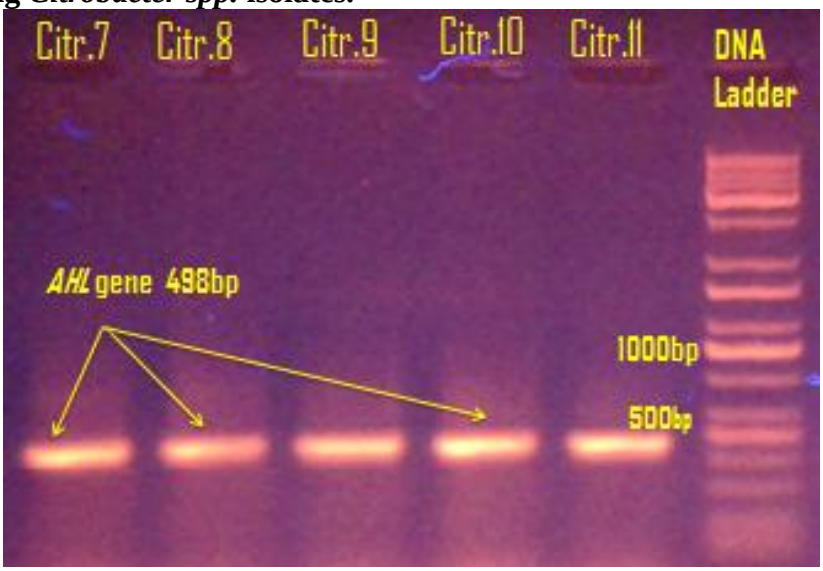

Figure 4: Gel electrophoresis (130min of 50W/cm) showed 82% positivity of *AHL* gene among *Citrobacter spp.* isolates.

3.3.Detection of quorum sensing genes among *Salmonella typhimurium.*

It is showed that *Salmonella typhimurium* had *abaI* gene (382bp) (figure 3) and *AHL* gene (498bp) (figure 4) among 66% and 79% isolates, respectively.

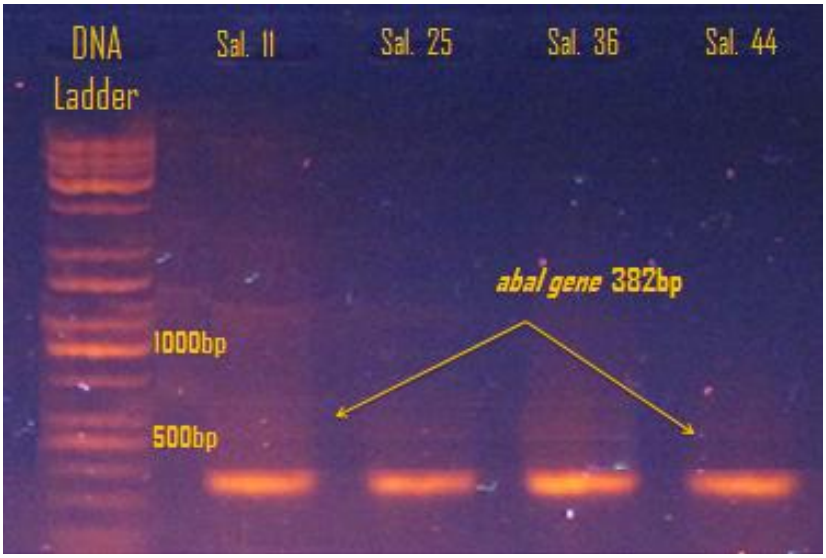

Figure 5: Gel electrophoresis (130min of 50W/cm) showed 66% positivity of *abaI* gene among *Salmonella typhimurium* isolates.

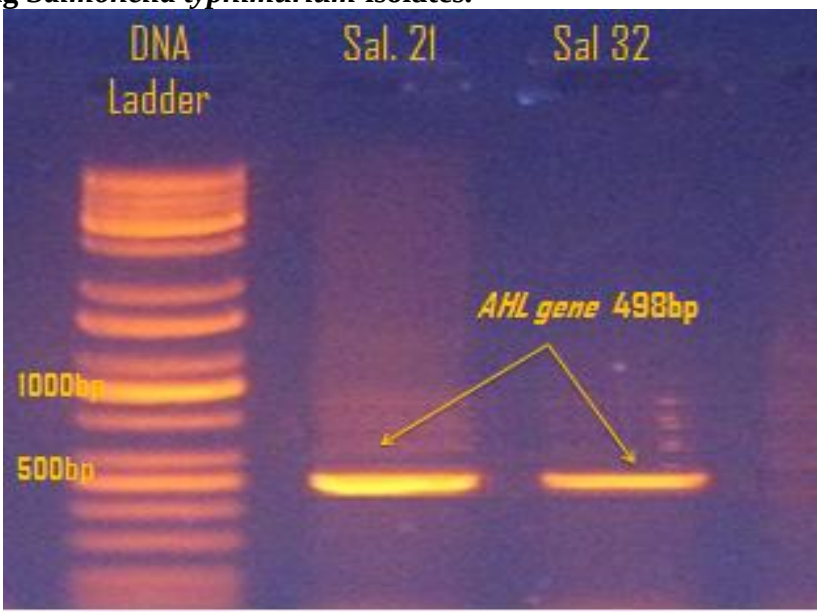

Figure 6: Gel electrophoresis (130min of 50W/cm) showed 79% positivity of *AHL* gene among *Salmonella typhimurium* isolates.

3.4.Detection of quorum sensing genes among *Pseudomonas auroginosa*

It is showed that *Pseudomonas auroginosa* had *abaI* gene (382bp) (figure 7) and *AHL* gene (498bp) (figure 8) among 58% and 96% isolates, respectively.

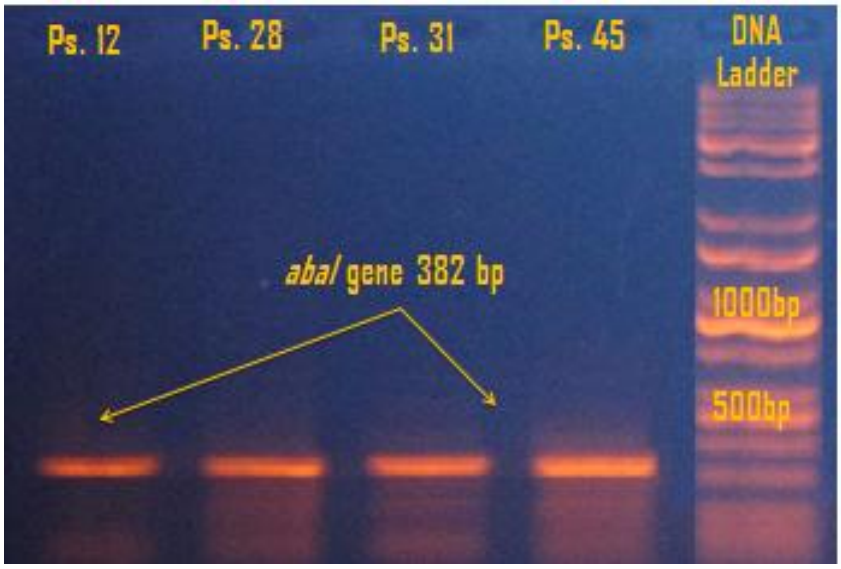

Figure 7: Gel electrophoresis (130min of 50W/cm) showed 58% positivity of *abaI* gene among *Pseudomonas auroginosa* isolates.

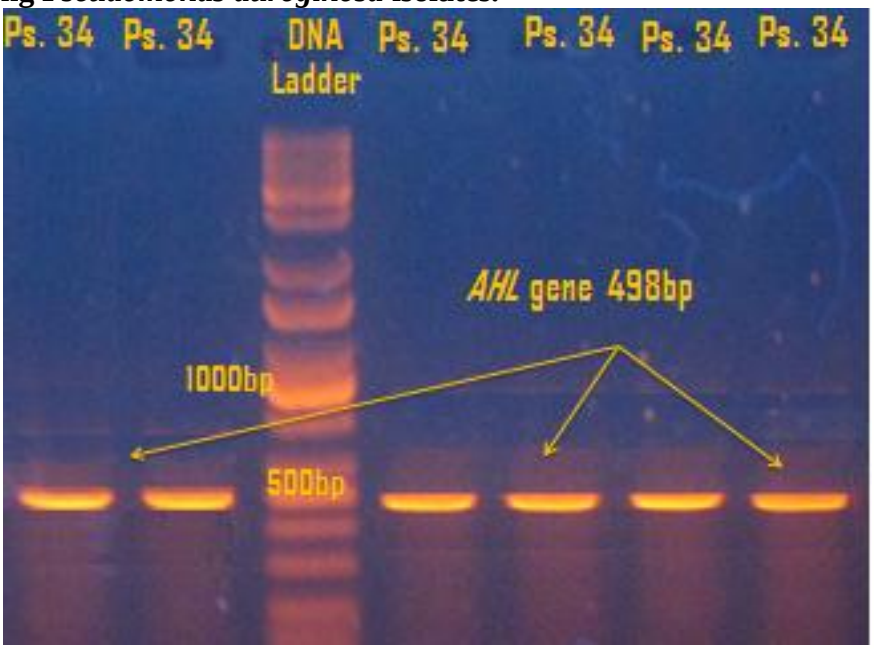

Figure 8: Gel electrophoresis (130min of 50W/cm) showed 96% positivity of *AHL* gene among *Pseudomonas auroginosa* isolates.

4. Discussion

It is studied in literature that bacteria can communicate with each other depending on density of their population and this process or system is called quorum sensing, for example when environment become hard in lung during cystic fibrosis, *P. auroginosa* produces biofilm to survive. This biofilm production as matter of fact is controlled by AHL signaling molecule (22). In our study, PCR assay showed that different gram negative bacterial isolates could harbor QS genes (*abaI*) and (*AHL*) in wide range. For example, *E. coli* had *abaI* and *AHL* gene among 80% and 92% of isolates, respectively. These findings resemble what has been revealed in literature in detecting quorum sensing systems among such pathogens. It was found that *E. coli* depends on QS to regulate virulent factors and colonize in a host and have *SdiA* system that is a

type of *Lux R* to sense AHL (N-acyl homoserine Lactone molecule) (23). Also, it was reported that high concentration of AHL molecules produced by E. coli in un tolerated conditions were found and strongly activate 10 different *Lux R* type receptors (24). Our PCR detection further revealed that 73% and 82% of *Citrobacter spp* isolates had *abaI* and *AHL* gene, this would be similar to Goh *et al.* (11). They demonstrated that *Citrobacter amalonaticus* isolated from dental plaque had various quorum sensing types of AHL signaling molecule such as N-hexadecanoyl-L-homoserine lactone, N-butyryl-L-homoserine lactone N-octanoyl-L-homoserine lactone N-hexanoyl-L-homoserine lactone (11). It is also found that even mice pathogen like *Citrobacter rodentium* used QS system to regulate virulence and deep lesions (25). Our results further showed *Salmonella typhimurium* had *abaI AHL* genes among 66% and 79% isolates, respectively. Findings resemble Gnanendra *et al.* (26). He and his colleagues reported that *Lux R* family which sense AHL molecule was found in wide range among *Salmonella typhimurium* isolated from chronic infections (26). It is demonstrated that this bacterium could respond to AHL signaling molecules in vitro (37°C) (27 and 28). Final results obtained in this study showed 58% and 96% of *P. auroginosa* isolates had *abaI* and *AHL* genes, respectively. It is well studied that *P. auroginosa* can regulate biofilm production and pathogenesis through different QS systems for example LasI/LasR (29 and 30).

5. **Conclusion:** Gram negative bacterial species is found to be regulating their activities like pathogenicity, antibiotic resistant, biofilm production, and others by quorum sensing (AI-1). Our findings revealed that *E.coli*, *Citrobacter spp.*, *S. typhumrium*, and *P. auroginosa* pathogens had the ability to monitor their density and regulate their activities through having quorum sensing genes, leading to the concern of becoming the chronic infections hard to be treated.

6. Reference

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