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Detection Of The Presence Of Quorum Sensing Genes In Clinical Samples Of Pseudomonas Aeruginosa And Klebsiella Pneumonia

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Abstract

It has become increasingly and widely recognised that bacteria do not exist as solitary cells, but are colonial organisms that exploit elaborate systems of intercellular communication to facilitate their adaptation to changing environmental conditions Many types of signalling molecules, which regulate diverse phenotypes across distant genera.The most common signalling molecules found in Gram-negative bacteria are N-acyl derivatives of homoserine lactone (acyl HSLs), P.aeruginosa and K.pneumoniae can regulate different group actives and physiological processes through the quorum sensing mechanism. The aims of this research were to detect the presence of quorum sensing genes in ten clinical isolates of P. aeruginosa and ten clinical isolates of k.pneumoniae which represent by (pqsA,pqsR1, lasR,Luxs,LsrR and Lsrk genes)respectively by Polymerase chain reaction (PCR),Result: The DNA of 20 P. aeruginosa and k.pneumoniae isolates were successfully extracted with g-spin DNA extraction kit, the detection of pqsA,pqsR1 and lasR genes showed that all of the 10 isolates under study were positive for one or more QS genes of P.aeruginosa. By PCR technology, the results showed that all isolates were positive for all genes by 6/10 (60%) (pqsA, pqsR1 , lasR) , As for K. pneumoniae, isolates were positive for all genes with a percentage of 9/10 (90 0%) (Luxs, LsrR and LsrK), These findings suggest that PQS can function as an intercellular signal in P. aeruginosa that is not restricted only to alkyl homoserine lactones (AHL) and confirmed the existence of a relationship connection between LuxS-dependent AI-2 signals and biofilm formation in species many bacteria like K.pneumoniae.

Keywords: quorum sensing,Pseudomonas quinolone signal (PQS),LuxSgene

Introduction

Microorganisms are consistently subjected to a myriad of environmental stimuli. Such cues include changes in temperature, osmolarity, pH and nutrient availability. In response, bacteria have developed multiple systems that allow adaptation to these environmental (Stock et al., 2000).

quorum sensing is one of the mechanism bacterial populations use to communicate with their own species or across species to coordinate behaviours, for the last 20 years, synthetic biologists have recognised the remarkable properties of quorum sensing to build genetic circuits responsive to population density (Whitehead et al., 2001).

quorum sensing, use of specific small molecules as a proxy for cellular density, is commonly used by bacteria to communicate with each other and allows them to organise themselves into multi-cellular populations(Whiteley et al., 2018; .Mukherjee et al., 2019).

In nature, QS has been seen to coordinate the behaviour of groups of bacteria to produce bioluminescenc (Hammer and Bassle, 2004). To form biofilms , to regulate the production of secondary metabolites and to regulate genes involved in virulence and invasion (Boo et al., 2012).

There are three main classes of quorum sensing systems that have been identified so far in bacteria: (i) autoinducers-1 (AI-1) or acyl-homoserine lactones (AHLs) used in Gram-negative bacteria, (ii autoinducer-2 (AI-2) used in Gram-positive and Gram-negative bacteria and (iii) auto-inducing peptides (AIP) such as nisin, which is also an antimicrobial peptide(Marchand and Collins , 2013); Kong et al., 2018).

In this study, we will discuss quorum sensing in two types of Gram-negative bacteria(Pseudomonas aeruginosa, Klebsiella pneumonia) P. aeruginosa is considered one of the most gram-negative opportunistic pathogens causing a broad range of infections in healthcare settings and community(Gaynes and Edwards , 2016). P. aeruginosa it stands out as a threatening and unique microorganism, as it has the ability to causeinvasive disease, and to evade the immune defenses causing infections

hard to be treated. Infections caused byP. aeruginosa are almost impossible to treat.(Perryet al.,2016).

P. aeruginosa is responsiblefor regulating different group activities and physiologicalprocesses through the quorum sensing mechanism. In thequorum sensing mechanism, bacterial cells produce, thendetect and respond to the diffusible small signal molecule(Ali and khudhair ,2019).

Three different chemotypes of Autoinducers (AIs) have been recognized in P. aeruginosa: AHL used by las and rhl systems, alkyl quinolones (AQs) used by the PQS system, and2-(2-hydroxyphenyl) thiazole-4-carbaldehyde used by the IQS system, In the las system, N-3-oxododecanoyl-homoserine lactone, 3OC12-HSL is produced by the enzyme, which is encoded by the lasI gene(Wagner et al ., 2016).

P. aeruginosa bacteriawhen reachesto threshold density, 3OC12-HSL will bind to LasR, whichacts as a transcriptional activator; LasR, in turn, dimerizesand binds to target promoters to control gene expression ofmany virulence genes, The rhl system is responsiblefor controlling the las system at the transcriptional and post transcriptional levels(Latifiet al ,2010).

In klebsiella pneumoniae bacteria, the autoinducers ofsignal system I (AI-1) have been identified as derivates of anacyl homoserine lactone (acyl-HSL) backbone with species specific substitutions. These molecules diffuse freely in and outof cells. Two genes are crucially involved in quorum sensingthrough acyl-HSL: the synthesis of acyl-HSL is dependent onluxI, while luxR encodes a transcriptional activator protein thatis responsible for the detection of the cognate acyl-HSL andinduction of the appropriate output (Bassler, 1999).

Many gram-positive and gram-negative bacteria contain highly conserved luxS homologues and produce autoinducer molecules that are functionally similar to V. harveyi AI-2(Wen and Burne, 2002).

The LuxS-dependent quorum-sensing system has been referred to as an interspeciescommunication system and may operate as a universal quorumsystem for many bacteria possessing the characteristic luxSgene. Several reports have shown the involvement of this typeII QS in the regulation of expression of virulence-related factors, motility, secretion systems, regulatory proteins, andpolypeptides involved in the acquisition of hemin ,It was shown that bacterial communicationmediated by LuxS is also involved in biofilm formation byStreptococcus gordonii (Blehertet al., 2003), Streptococcus mutans, and Salmonella entericaserovar Typhimuriumin their natural environment(Proutyet al., 2002).

The objective of this research was to detect the presencequorum sensing genes in P. aeruginosa, which is representedby (pqsA,pqsR1 and lasR) and K.pneumoniae by(Luxs,LsrR and LsrR)genes, by using PCR technique andinteraction between them.

Material and Methods

Isolation and Identification

20 clinical isolates were used, 10 isolates of which were P. aeruginosa and 10 isolates of K. pneumoniaen obtained from Al-Hussein Teaching Hospital and Bint Al-Huda Teaching Hospital in Dhi Qar Governorate.VITEK 2 compact system GN cards were carriedout to confirm the diagnosis ofP.aerougenosa and K.pneumoniae to species level, according to manufacturer's instructions(Biomerieux, France).

DNA Extraction and Detection of Quorum Sensing Genesby a Conventional Polymerase Chain Reaction.

The nucleic acid extraction of the P. aeruginosa and K.pneumoniae isolates was performed using a commercial DNA extraction kit (Quick DNA extraction kit), according to the manufacturer'sinstruction (Intron Biotechnology). Determination of DNAquality and quantity is done by using nanodrop at 260/280 nmand gel electrophoresis(Proutyetal., 2002). PCR protocol was used to detect thepresence of quorum sensing genes (pqsA,pqsR1, lasR,Luxs,LsrR and Lsrk)genes.Tables (1) show Sequence primers and molecular size ofPCRproduct andTable (2) showannealing temperature, sequences, and the expected size of amplicon for PCR assay.

Tables (1) Sequence primers and molecular size of PCR product

Bacterial spices	Primer target	Primers sequence '5→'3	Product bp	References
P. aeruginosa	pqsA	CTGGACGACAACCAGATCCT -' 3 '5-F- -ATGTGCGAGGGAATCTGTTC- '3'5R-	bp71	)Sejeong et al,2015(
	LasR	ATGGCCTTGGTTGACGGTT -' 3'5'F- '3R-5-GCAAGATCAGAGAGTAATAAGACCCA-	bp 725	(Mowafy et al,2021)
	pqsR1	-GTCACCCGCTGTTCGACG-'3'5F- ACGATCAAGCAGGACAACGC-'3'-5R-	806	)Pachecoet al,2019(
K. pneumonia	Luxs	F-5'- GGAACGCGGTATCCACAC -' 3' 5'- TGAGCTCCGGGATCTGGT-'3'	226bp	)Balestrino et al ,2005(
	LsrR	-CTTCCGTTACCAGGCCATTA - '35' 5'- CTATATCAGCGAGGGCGAAC-'3	248	)Araujo et al ,2010(
	LsrK	CAGGGTACCGGTCTCTTTGA - '35' TACGAGGTCTCGGGACAAAC -' 35'	267 base pair	)Araujo et al ,2010(

Table 2: Thermal cycling conditions of (pqsA,pqsR1 , lasR,Luxs,LsrR and LsrR) genes used for PCR .

Gene	Initial denaturation	Denaturation	Primer annealing	Primer extension	Final extension
pqsA	°C94	°C94	°C64	°C72	°C72

LasR	94°C	°C94	°C62	°C72	°C72
pqsR1	°C94	°C94	°C62	°C72	°C72
Luxs	°C94	°C94	°C64	°C72	°C72
LsrR	°C94	°C94	°C62	°C72	°C72
LsrK	°C94	°C94	°C64	°C72	°C72

The primers used in the interaction

The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).

Primers Selection

The primers used in PCR amplification included, P. aeruginosa, which is represented by (pqsA,pqsR1 and lasR) and K.pneumoniae by(Luxs,LsrR and Lsrk)genesas illustrated in Table (1).

Results

Isolation and Identification of P. aeruginosa and K.pneumoniae, 20 clinical isolates were used, 10 isolates of which were P. aeruginosa and 10 isolates of K. pneumoniaen were identified biochemically byusing Vitek 2 compact system GN cards (BioMerieuxFrance). According to the 46 biochemical reactions includedin cards, all the isolates were identified successfully with anidentification probability of 93–99%.

Molecular Analysis

The DNA of 20 P. aeruginosa and k.pneumoniae isolates were successfullyextracted with g-spin DNA extraction kit. Purity andconcentration were confirmed with nanodrop, and the intactDNA bands were confirmed through gel electrophoresisFigure (1)Show Gel electrophoresis of genomic DNA extraction from Bacteria.

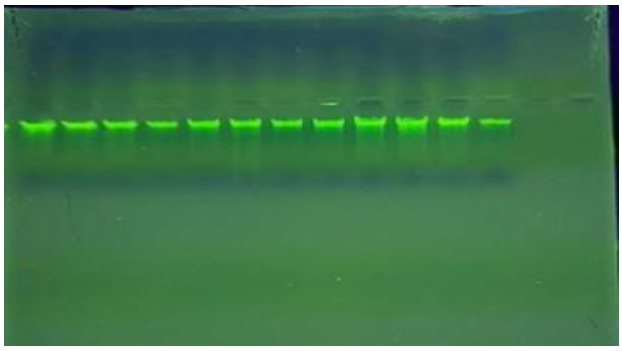


Figure 1: Gel electrophoresis of genomic DNA extraction from Bacteria, 1% agarose gel at 5 vol /cm for 1 hour.

Detection of QS Genes by Conventional PCR Techniques

The detection of pqsA,pqsR1 and lasR genes showed thatall of the 10 isolates under study were positive for one ormore QS genes of P.aeruginosa. By PCR technology, the results showed that all isolates were positive for all genes by 6/10 (60%) (pqsA, pqsR1 , lasR)Table(3),Figure(2).

As for Klebsiella pneumoniae, the results showed that all isolates were positive for all genes with a percentage of 9/10 (90 0%) (Luxs, LsrR and LsrK) genesTable (3)Figure(3).

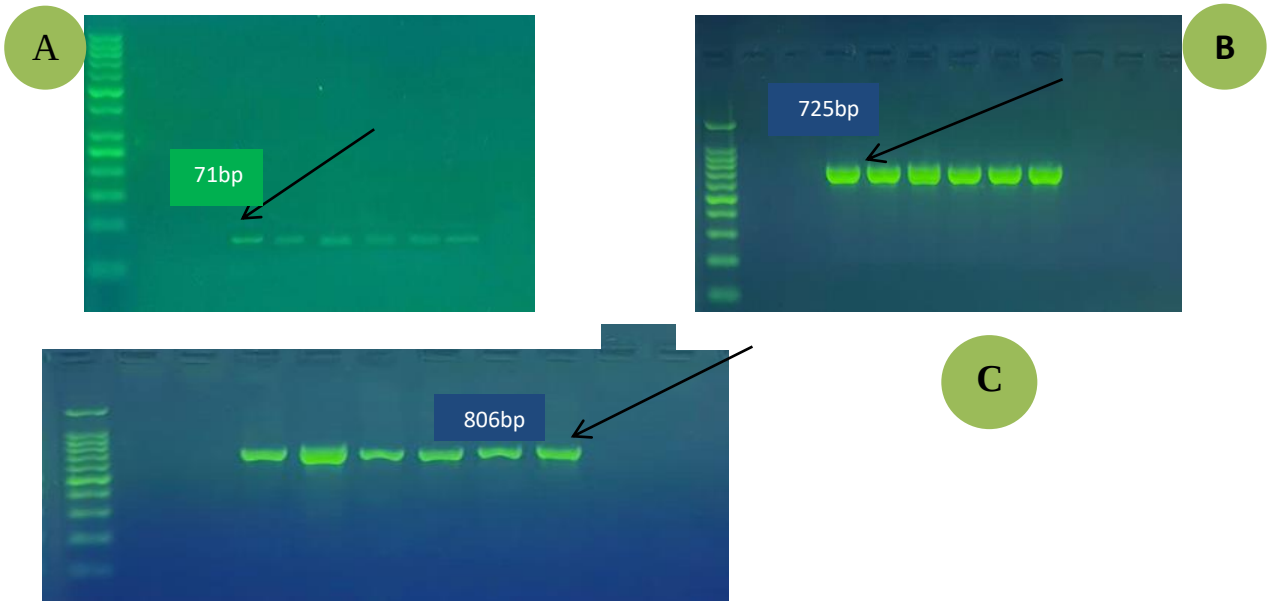
Table(3) Distribution of quorum sensing genes among A.P. aeruginosa and B.K .pneumonia isolates

A

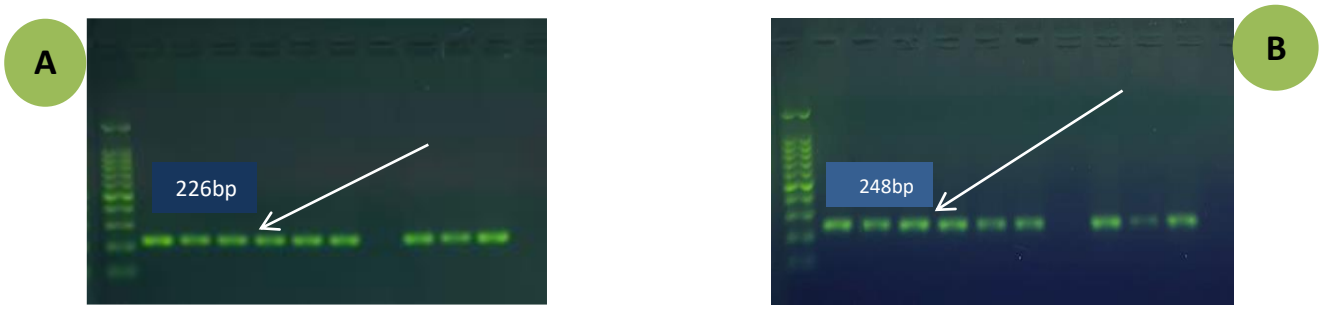
ple	DNA Result	PCR PqsA	PCR LasR	PCR PqsR1
	+	-	-	-
	+	-	-	-
	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	-	-	-
)	+	-	-	-

B

ple	DNA Result	PCR Luxs	PCR LasR	PCR LsrK
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	-	-	-
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+



Figure(2): Gel electrophoresis for quorum sensing genes of P. aeruginosa isolates; A: for pqsA gene amplicons(71 bp);B for LasR (725 bp); C: forpqsR1(806bp);The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder 100).



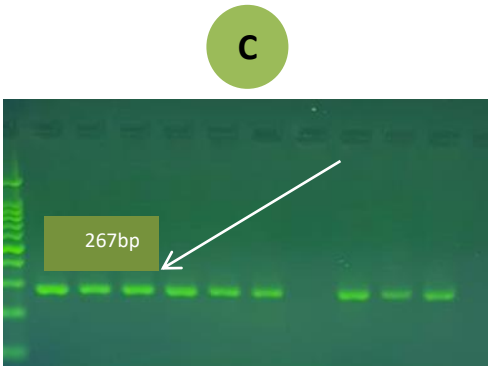


Figure 2: Gel electrophoresis for quorum sensing genes of K. pneumonia isolates; A: forLuxs gene amplicons (226 bp); BforLsrR (248 bp); C: for LsrK (267);The product was electrophoresis on 1.5% agarose at 70 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder 100).

Discussion

QS mechanisms are used by numerous species ofbacteria to regulate virulence factor expression or phenotypicchanges,bacteria control gene expression on a community-widescale by producing, secreting, detecting, and responding to extra cellular signaling molecules called autoinducers,they accumulatein the environment in proportion to cell density, and two majorclasses of quorum-sensing molecules have been described forgram-negative bacteria(Michaelet al., 2001; Stroeheret al., 2003).

of QS genes, indicates that they can have different patternsPCR analysis of P. aeruginosa under study for the presenceAccording to quorum sensing genes production, gene pattern includes the P. aeruginosa isolates 3, 4, 5, 6, 7, and 8were all positive for pqsA, pqsR1 , lasR systems, as shown in Table (3)Previous study showed that lasR and rhl system is the firstQS system discovered in P. aeruginosa, which is controlledvia the AHL(Woese , 1987).

Another study showed that clinical P. aeruginosa isolateswere deficient in QS genes(Karatuna et al,2010).While there was a study confirming of PQS biosynthetic genes pqsABCDE are all present, speciallyMvfR gene, which is considered a master regulator of PQS biosynthesis genes(Dezielet al, 2004).

Our result also showed isolates number 1, 2, 9and 10, Table (3) hasdefect for the production of pqsA, pqsR1, lasR genes, which areconsidered as transcription regulators of quorum system thatwhen companied with their specific autoinducers, activatetranscription of different virulence factors(Medinaet al,2003). This defect canbe caused by mutation or sother reason that cause the geneto be silent.

That the regulation of PQS biosynthesis by LasR (3-oxo-C12AHL) occurs through transcriptional regulation of MvFR that is responsible for converting HHQ to PQS,32 but under certain growth situation, there can be a considerable LasR independent biosynthesis of PQS genes(Diggle et al,2002).

As for K. pneumonia bacteria, the results of the PCR analysis a isolates 1, 2, 3, 4, 5, 6, , 8 ,9 and 10 were all positive for (Luxs, LsrR ,LsrK) systems, as shown in Table (3) .Showed a Functional type II QS system was identifiedin K. pneumoniae, where luxS was shown to be critical forAI-2 synthesis(Balestrino et al,2005) ; Herring et al,2003),and mutations in quorum sensingrelated genes induced changes in biofilm formation and LPS synthesis.

Signaling system 2requires the luxS gene for the synthesis of V. harveyi AI-2anon-HSL AI, the structure of which is unknown (Surette, and Bassler, 1999;Suretteet at,1999). Theprimary sensor for V. harveyi AI-2 is thought to be LuxP, andthe LuxP–AI-2 complex interacts with LuxQ to initiate signaltransduction(Lilley and Bassler, 2004).

Thebacterium K. pneumoniae has emerged as a multidrug-resistant pathogen that has spread globally and is acknowledged as a cause of invasive blood-borne infections, as well as pneumonia and urinarytract infections, particularly in healthcare settingsCalfee, 2017;Zaidiet al,2005).(

Biofilmsprovide conditions that physically protect cells from hostileinfections(Stickler and Bacterial, 2008). These sessile communities, where environmental factors, antimicrobials and are the cause of many chronic bacteria can reside in close proximity with each other, also creates increased opportunities for chemical signalling and gene transfer to occur between bacterial cells of the same or different species(Cook and Dunny, 2014). This is an especially important genetic mechanism that is largely responsible for the phenotypes and related infections ,Recent efforts to develop alternative strategies to combat bacterial infections have led to the identification of novel compounds that target bacterial processes, including quorum sensing and biofilm formation(Brackman and Coenye, 2014). For instance, a ‘quorum quenching enzyme that inactivates AI-2 molecules has been discovered that inhibits K. pneumoniae biofilm formation(Weilandet al,2016).

Conclusion

QS is one of the mechanism bacterial populations use to communicate with their own species or across species to coordinate behaviours. For the last 20 years, synthetic biologists have recognised the remarkable properties of quorum sensing to build genetic circuits responsive to population density. This has led to progress in designing dynamic, coordinated and sometimes multi cellular systems for bio-production in metabolic engineering and for increased spatial and temporal complexity in synthetic biology, qS systems in P. aeruginosa play a main role in the pathogenesis of infections, Our study revealed that the regulation PQS system was mediated by the las and rhl system.

These findings suggest PQS can act as an intercellular signal in P. aeruginosa that is not restricted only to AHL. This can be an important tool for further future studying of quinolone signaling in more specific in P. aeruginosa bacteria, in the K.pneumonia the luxS gene of quorum-sensing ,Vibrio harveyi is required for type 2 autoinducer production,harveyi AI-1 quorum-sensing circuit is species specific, the AI-2system can be used for interspecies cell-cell signaling and mayconfer upon bacterial cells the ability to monitor the totalbacterial density of mixed populations.

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