SIGNALING MOLECULES IN *PSEUDOMONAS AERUGINOSA* RESPONSE TO ANTIBIOTICS AT SUB-INHIBITORY CONCENTRATIONS

ABSTRACT

Signaling molecules are N-acylhomoserine lactones (AHLs) that form the *Pseudomonas aeruginosa* cell information system, which determines gene expressions in a population-dependent manner called quorum sensing (QS). Signal molecules, which are chemically varied, control gene expressions for antibiotic resistance, pathogenicity, motility, biofilm development, bioluminescence, secondary metabolite production, and plasmid transfer. This research was aimed at identifying the signaling molecules in *the* Pseudomonas aeruginosa response to antibiotics at sub-inhibitory concentrations. One hundred and fifty (150) clinical swab specimens were collected from urinary catheter woundcatheters, wounds, and ear infections of patients, and the swabs were inoculated using the standard microbiology method. The isolates were characterized based on bacteriological methods such as morphology and biochemical tests. The isolates were further confirmed by species-specific PCR by amplification of 16S rRNA, and the amplicons were analyzed by gel electrophoresis. Further genomic sequencing was done and blast with NCBI database mining. The disc diffusion method was applied for the antibiotic susceptibility pattern. The isolated cell suspensions were analyzed by gas chromatography-mass spectrometry (GC-MS). The result of the isolation showed 22(59.46%) wound infections, 12(32.43%) ear infections, and 3(8.11%) urinary catheters. The isolates were Gram-negative, produced β-hemolysis on blood agar, and their morphology is small, pigmented, and circular in form. The isolates showed positive results in catalase, oxidase, citrate, nitrate, and negative indole tests. The amplification of the 16S rRNA gene region resulted in a band size of 1500 bp for the PCR product, and the BLAST analysis gave 99% similarity. The resistant antibiotic susceptibility showed that *Pseudomonas aeruginosa* is a multidrug-resistant bacterium. The GC-MS results obtained from urinary catheter isolates showed four (4) signaling molecules such as N-Octanoyl-L-homoserine Lactone, N-Decanoyl-DL-Homoserine Lactone, N-Dodecanoyl-DL-Homoserine Lactone, and N-Tetradecanoyl-L-Homoserine Lactone. Three (3) signaling molecules, such as N-Octanoyl-L-homoserine Lactone, N-Decanoyl-DL-Homoserine Lactone, and N-Tetradecanoyl-L-Homoserine Lactone, were obtained from wound isolates. N-tetradecanoyl-L-homoserine lactone was the only signaling molecule obtained from ear isolates. Further research should be done to develop rapid tests that could detect acyl homoserine specific to *Pseudomonas* aeruginosa present in human samples and deliver results in real time. Signaling molecule inhibitors (SMI) are potential therapeutics that could lead to the development of a new class of antibacterial drugs. The fundamental role of each signal molecule in the production of biofilms and virulent factors should also be investigated, as well as whether Pseudomonas aeruginosa needs one or more signaling molecules to form quorum sensing. Identification of the signal molecules (AHLs) of P. aeruginosaand developing signaling molecules inhibitors (SMI) can have an important prognostic, diagnostic, and therapeutic value in human medicine for the treatment of P. aeru*ginosa* infections.

Key words:*Pseudomonas aeruginosa,* Signaling Molecules, GC-MS and Antibiotics.

1. INTRODUCTION

Pseudomonas aeruginosa,a Gram-negative bacteria,produces these signaling molecules, which are diffusible chemical signals (N-acyl homoserine lactones; N-AHLs) that regulate the production of virulence determinants and secondary metabolites in a cell density-dependent manner known as quorum sensing. The complex signaling routes allow communication between cells, including within and between species (Ryan and Dow, 2008). Such signaling necessitates a transduction system that integrates this external information, resulting in the induction of antibiotic production at the right time, amount, and by the right percentage or subpopulation of cells. The nature of the molecules defines specificity for the receptor and ensures proper recognition and the expected genetic answer in the population (Horswill et al., 2007).

How low concentrations of antimicrobials affect bacterial survival and, most importantly, whether or not the molecules might have other functions at sub-inhibitory concentrations (SIC) are outstanding questions. Pioneering studies on the global transcriptome response of many important human pathogens to SICs of antibiotics have demonstrated that these molecules can affect the expression of genes related to virulence, colonization, motility, stress response, and/or biofilm formation. Further research has shown that some of these effects result from interference with bacterial cell-to-cell communication. This observation has prompted the idea that antibiotics might actually act as signal molecules in natural environments, facilitating intra- or interspecies interactions within microbial communities (Diego et al., 2011).

The prevalence of Pseudomonas *aeruginosaaeruginsa i*nfections has been on the rise globally as a result of risk factors such as aging groups, severe chronic infections, overuse of medical devices, and immune compromised patients (Qin *et al*., 2023). Therefore, the determination of N-AHLs is essential and of great interest to combat the recalcitrant nature of Pseudomonas aeruginosa. The identification of AHLs in a clinical setting can have important prognostic, diagnostic, and therapeutic value in human medicine.

Quorum sensing is a system employed by Pseudomonas aeruginosa that depends on cell concentrations to regulate gene expressions (de Kievit and Iglewski, 2000). Christiaenet al. (2014) reported that *Pseudomonaaeruginosa**Pseudomonasaeruginu*ses quorum sensing to control virulence-determining factors and biofilm creation. P. aeruginosa employed Las and Rhl, the main quorum-sensing systems that are in charge of secreting signaling molecules such as N-3-oxododecanoy-L-homoserine lactone and N-butanoyl-L-homoserine lactone, respectively. According to Rutherford and Bassler (2012), these signaling molecules initiate associated transcription regulators LasR and RhlR, thereby enhancing biofilm production and the expression of virulence-determining factors. The virulence-determining factors are elastases, proteases, pyocyanins, lectins, rhamnolipids, and toxins. Kang et al. (2017) reported that besides LasI-LasR and RhlI-RhlR, P. aeruginosa consists of another quorum-sensing system, such as PQSMvfR, which enhances the production of biofilm. Activation of transcriptional factors (MvfR) such as PqsR by the control of the pqsABCDE operon leads to the formation of the pseudomonas quinolone signal 2 heptyl-3-hydroxyl-4 quinolone (Lee and Zhang, 2015). Kang et al. (2017) also reported that the following proteins,PqsA and PqsD, are involved in biofilm production.

A potentially effective approach for the management of P*seudomonas* aeruginosa ailments is the impeding of quorum sensing. According to Reuter et al. (2016), this strategy has a minimal chance of leading to the development of bacterial resistance, can avoid or reduce the production of biofilms, and can also diminish bacterial pathogenicity. Rasmussen and Givskov (2006) also reported that this method has a slim range and is not likely to have unpremeditated impeding effects on favorable bacteria. According to Kalia (2013), Las and Rhl systems can be either ordinary or artificial inhibitors, and they have the ability to decrease AHL synthase activity, limit AHL formation, degrade AHLs, or compete with AHL receptors for binding. Quasi-sensing signaling inhibitors have received rigorous research in the past few years. Gokalsinet al. (2017) reported that carotenoid zeaxanthin, which was

gotten from plants, lichens, and algae, is one of the inhibitors that has the capacity to decrease biofilm production in P*seudomonas* aeruginosa by locking to the signal receptor sites and preventing the expression of virulence-determining factors such as lasB and rhlA. Paczkowskiet al. (2017) also affirmed that plant primary metabolites such as flavonoids serve as transcriptional regulator contenders and, importantly,decreased their binding capacity to control regulated genes responsible for quorum sensing in P. aeruginosa. Las and Rhl systems are inhibited by N-decanoylcyclopentylamide, an artificial quorum sensing impeder, by impeding the locking of 3OC12-HSL and C4-HSL to their associated receptor sites, thereby preventing the creation of biofilm and the production of virulence-determining factors (Ishida et al., 2007). An artificial substance that is a constituent of halogenated furanone was reported by Hentzeret al. (2002), and it was observed to inhibit quorum sensing-controlled virulence gene expression in P*seudomonas* aeruginosa. It was also observed that this artificial substance was capable of infiltratingP*seudomonas* aeruginosamicroniches, dislocating the biofilm organization, and enhancing bacterial cell dispersion from the solid layer. Kitao et al. (2018) reported that MvfR is an essential target to inhibit the pseudomonas quinolone system. Maura and Rahme (2017) reported that a benzamide-benzimidazole substance was applied chemically to impede P*seudomonas* aeruginosaMvfR, thereby decreasing biofilm formation and enhancing the sensitivity of P*seudomonas* aeruginosato antimicrobials such as meropenem and tobramycin. Thus, benzamide-benzimidazole also served as a competitive rival of MvfR by locking to its ligand-binding area, thereby decreasing the DNA binding capacity of MvfR (Kitao et al., 2018). The first quorum sensing inhibitor that has undergone clinical testing is the macrolide antibiotic azithromycin. Patients with cystic fibrosis who have chronic lung ailments brought on by P. aeruginosa have proven that azithromycin treatment is effective (Imperiet al., 2014). According to a number of studies, azithromycin suppressed P. aeruginosa's pathogenicity and greatly decreased the generation of quorum sensing signal molecules (Balaet al., 2011). It is interesting to note, nevertheless, that P. aeruginosa'sMexCD-OprJ efflux pump confers azithromycin resistance when a biofilm is forming. Azithromycin treatment efficacy may therefore be decreased by up-regulated MexCD-OprJ expression brought on by mutation (Gillis et al., 2005).

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted at the University of Abuja Teaching Hospital in the Gwagwalada Area Council of the Federal Capital Territory of Abuja (N 8^{o} 57’ 1.4976”, E 7^{o} 3’ 45.4212), Nigeria.

2.2 Sample Collection

The casualty and surgical wards of the Teaching Hospital were the source of 150 clinical samples from various sexes. The samples came from the urinary catheter, burns on the skin, and ear infections. According to Perry, (2001) adopted method for the recovery live bacterial pathogen at 4^{o}C and nutrient agar inoculation and incubations at 37^{o}C for 48hours; all swab samples were kept at 4^{o}C, which is better for recovering live bacterial pathogens.The clinical specimens were swiftly inoculated on nutrient agar and incubated overnight at 37°C for 48 hours aerobically.

2.3Isolation, Identification and Biochemical Characterization of *Pseudomonas aeruginosa*

The experiment method described by was used to evaluate and classify the growth mediums, three isolates, one from each clinical sample source, were randomly chosen for characterization based on

morphological and biochemical tests like Gram stain characteristics, methyl and nitrate test, oxidative-fermentation test, catalase, citrate and oxidase tests, and indole production (table 3).

2.4 **Extraction of Genomic DNA**

The phenol-chloroform kit was used to perform the DNA extraction technique according to the experiment of Barker, K. in 1998. The steps were completed in accordance with the kit's instructions. 200µl of the sample was added into a 1.5ml tube.400 µl of lysis buffer was added to the sample contained in the 1.5ml tube and 10µl of proteinase K was added to the tube, and incubate at 55°C for 10-60 minutes. 400µl 0f equilibrated phenol (pH 7.8) was added, vortexed, and centrifuged at 12000rpm for 5minute. Supernatant was taken and 700µl of Chloroform was added: Isoamyl alcohol (24:1) (672µl Chloroform: 28µl Isoamyl alcohol) mixed well and centrifuged at 12000rpm for 5 minute and the layer was transferred to a fresh tube.40µl of 3M sodium acetate (PH 5.2) was added. 400µl of 100% ethanol was added and incubate for a minimum of 1hour. It was centrifuged for 15 minutes at 4°C at 14000rpm to pellet the DNA. Supernatant was carefully removed without disturbing the DNA pellet.150ul of 70% cold ethanol was added and sample was centrifuged at 4°C for 2 minutes at 14000rpm and the supernatant was discarded. The DNA pellet was dried at room temperature for 5-10 minutes. The DNA pellet was re-suspended in 100 µl of TE buffer or molecular grade water by pipetting up and down. It was centrifuged briefly to collect the sample and the tube was placed on ice (Plate 1).

2.5 PCR Assay

For molecular confirmation of the isolates validated by conventional testing, a PCR assay was carried out. All of the isolates that were presumed to be *P. aeruginosa* by morphological and chemical testing had their species identity further confirmed by PCR. The 16S rRNA gene was amplified using the universal primers 27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-GGTTACCTTGTTACGACTT-3′. According to the kit instructions, in a final volume of 20µl, the PCR was conducted using the following master mix parameters: Master Mix (10µl), MGW (3µl), Primers (F/R) (2µl), and DNA Template (5µl). The denaturation of the template occurred over 40 cycles of 30 seconds each at 94°C during the PCR process, 40 seconds at 50°C for annealing, and 1 minute at 72°C extension prior to 10 minutes of final extension at 72°C for 40 cycles (Plate 1) (Barker, K. 1998).

2.6 Analysis of PCR Products (amplicons)

The results of the PCR reaction were analyzed using trisborate-EDTA buffer (pH = 8.0), and the amplicons were then subjected to 1.5% agarose gel electrophoresis. The agarose gel was next stained with ethidium bromide and examined using a UV transilluminator device running at 120 volts for 30 minutes. The estimated size of the amplicons was established by comparing the size of the amplifications (PCR products) to a pertinent DNA ladder of 25/100 bp (Lilit and Nidhi, 2013).

2.7 Genomic Sequencing

The genomic sequencing of *Pseudomonas aeruginosa* was carried out and blast with NCBI database mining to identify the strain (Tamura, 2021) (figure 1).

2.8 **Antimicrobial Susceptibility**

The disk diffusion technique was applied to determine antibiotic susceptibility, and it was carried out in accordance with Clinical Laboratory Standard Institute (CLSI) standards. A commercially available antimicrobial disc is used to test against *Pseudomonas aeruginosa* isolates. This disc contained known antibiotics such as Piperacillin/tazobactam (10 ug), Cefoperazone (10 ug), Ofloxacin (5 ug), Tetracycline (10 ug), Amikacin (5 ug), Gentamycin (10 ug), Bacitracin (10 ug), Clarithromycin (5 ug), Levofloxacin (10 ug), and Cefpirome (15 ug). They were kept in an environment (a candle jar) for 48 hours at 37 °C, and the percentage of resistance was determined (Gencer*et al*., 2002).

2.9 Signaling Molecules Detection through GC-MS

Pseudomonas aeruginosa cell suspensions that were recovered from antibiotic susceptibility by disk diffusion technique with commercially prepared antimicrobial discs with known antibiotics at sub-inhibitory concentrations were sterilized using sterile water for 20 minutes at 130 °C and then centrifuged for 15 minutes at 4000 rpm. Three times, an equal volume of chloroform—five mL each time—was used to extract the cell-free supernatant solution from the sample. After being washed with an equal amount of water, the mixed organic phases were dried at room temperature under a stream of nitrogen.The leftover material was re-dissolved in 1 mL of chloroform before being gradually dried off in smaller vials. Computer software was used to control both the single-quadrupole mass spectrometer and the gas chromatography system that was used for the analysis. The injection of the sample was carried out in split mode using a capillary column covered with a 0.25 lm film of 5% Phme Siloxane. A flow rate of 0.8 mL/min was used using helium as the carrier gas. The GC injector had a fixed 200 °C temperature. The recommended oven temperature program instructs retaining the temperature at 150 °C for three minutes before raising it by 15 minutes to 275 °C. 280 ^{o}C was chosen as the new temperature for the transfer line. For mass spectrometry, the following parameters were used: a quadruple MS at 150 oC, an MS source at 230 oC, an electron ionization source set to 70 eV, and an emission current of 500 lA. The mass spectrometer was used in full-scan mode, which covered the range of m/z 15 to 800, and in SIM mode, which covered m/z 143 (Cataldi*et al.,* 2007).

3. RESULTS

3.1 The clinical specimens:

The clinical specimens were received from casualty and the surgical wards from different age groups and urinary catheter from University of Abuja Teaching Hospital between June, 2021 and August, 2022 and a total of 37 isolates of *Pseudomonas aeruginosa* were identified from the specimens (Table1).

The three sources of the clinical specimens such as urinary catheter tip swabs wound swabs and ear infection swabs showed 3(8.11%), 22(59.46%) and 12(32.43%) respectively and the percentage gender distribution of the isolates showed 22(64.71%) from male patients and 12(35.29%) from the female patients (Table 2)**.**

3.2 Morphological and Biochemical Identification of the Isolate

Table 3 shows the morphological and biochemical characters which identify the isolates. The isolates are area small pigmented circular, which shows positive, results to catalase, oxidase, citrate and nitrate testes; negative test to Indole and methyl red test.

Table 1: Demography of the clinical specimens and the number of *P. aeruginosa* obtained from the different age group and catheter from University of Abuja Teaching Hospital, Nigeria

Age	No of clinical samples (%) N= 150		No of *Pseudomonas spp*. N=37	
	Sample No	(%)	Sample No	(%)
<10	6	4.00	6	16.22
11-20	20	13.33	4	10.81
21-30	50	33.33	2	5.41
31-40	32	21.33	3	8.11
>40	27	18.00	19	51.35
Catheter	15	10.00	3	8.11

Table 2: Distribution of clinical isolates from the sample's sources and the percentage gender distribution in UATH, Nigeria

Sources	No of the isolates (%) N=37N=34	Gender	No of the isolate by gender (%)
Wound swabs	22 (59.46)	Male	22(64.71)
Ear swabs	12(32.43)	Female	12(35.29)
Urinary Catheter	3(8.11)		

Table 3: Morphological and Biochemical Identification of the Isolates

S/N	Source of Isolate	Colony Morphology	Gram Reaction	Oxidase	Catalase	Indole	Citrate Test	Methyl Red	Nitrate
1.	Wounds	Small Pigmented circular	Gram –ve Rods	+	+	-	+	-	+
2.	Ear	Small Pigmented circular	Gram –ve Rods	+	+	-	+	-	+
3.	Catheter	Small Pigmented Circular	Gram –ve Rods	+	+	-	+	-	+

Key: + (Positive) – (Negative)

3.3 The Result of PCR Amplification of 16s rRNA Gene (Plate 1)

At the molecular level, *Pseudomonas aerugi*nosa was recognized and validated. The band size of the amplification of the 16S rRNA gene region is 1500bp (Plate 1). According to the BLAST analysis, 98 sequences from the NCBI data exhibited 99% similarity. The phylogenetic tree produced l other species genetically.

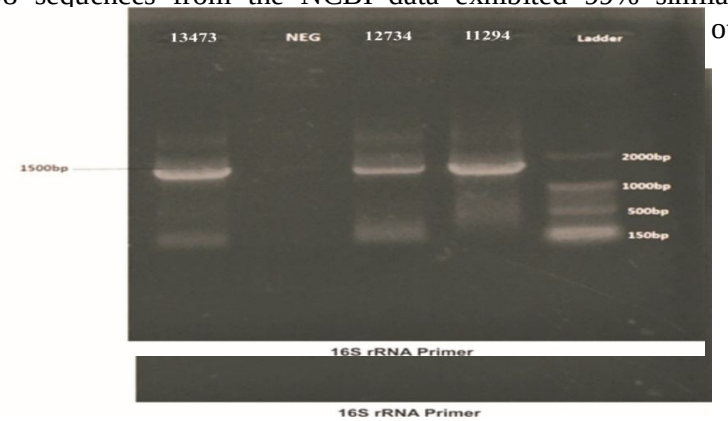
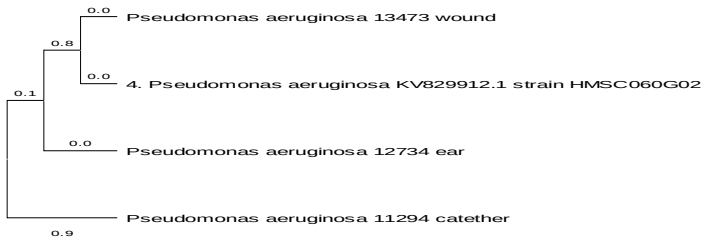


Plate1. An electrophoregram of 16S rRNA of *Pseudomonas aeruginosa* samples separated on agarose gel electrophoresis stained by ethidium bromide. The amplification of DNA appears as a lane-like pattern. Lane (ladder) DNA Marker (150bp), lane (13473, 12734, 11294) represent positive Isolates and lane (N) the negative control.

3.4 Figure 1: Phylogenetic tree of *Pseudomonas aeruginosa* from different sources using the Neighbor Joining method (Mega 11 software)



Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. (Next to the branches). The evolutionary distances were computed using the Poisson correction method and are in the units of the number of nucleotide substitutions per site. This analysis involved 4 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 282 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura, 2021).

3.5 Signaling Molecules of Isolate from urinary catheter.

N-octanoyl-L-homoserine lactone, N-decanoyl-DL-homoserine lactone, N-dodecanoyl-DL-homserine, and N-tetradecanoyl-L-homoserine, with corresponding retention times of 9.665, 10.884, 11.646, and 12.948 (minutes) respectively, were found to be four signaling molecules identified from urinary catheter (Table 4).

3.5 Signaling Molecules of Isolate from patient wound infections.

Three signaling molecules such as N-octanoyl-L-homoserine lactone, N-decanoyl-DL-homoserine lactone, and N-tetradecanoyl-L-homoserinewith retention durations of 9.672, 10.890, and 12.955 (minutes) respectively were identified from a wound swab (Table 5).

3.6 Signaling Molecules of Isolate from ear infections

One signaling molecule was identified from an ear swab isolate; N-Tetradecanoyl-L-Homoserinewith a retention time of 12.952 (minutes) (Table 6).

Table 4: Signaling Molecules of isolate from urinary catheter specimen obtained from UATH Abuja, Nigeria

Retention Time (min)	Name of Compound	Molecular Formula	Area (%)
9.665	Octanoyl-L-homoserine Lactone	$C_{22}H_{41}NO_3$	14.67
10.884	Decanoyl-DL-Homoserine Lactone	$C_{14}H_{29}NO_3$	14.40
11.646	Dodecanoyl-DL-Homoserine Lactone	$C_{16}H_{29}NO_3$	59.45
12.948	Tetradecanoyl-L-Homoserine Lactone	$C_{18}H_{33}NO_3$	11.48

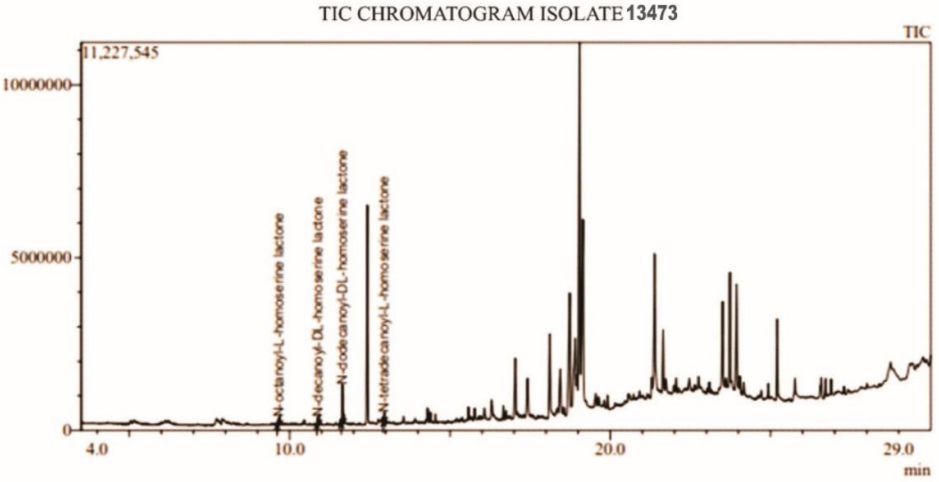


Figure 2: Chromatogram of *P. aeruginosa*Signaling Molecules from Catheter Isolate.

Table 5: Signaling Molecules of isolate from patient wound specimen from the UATH Abuja, Nigeria

	Retention Time (min)	Name of Compound	Molecular Formula	Area (%)
	9.672	Octanoyl-L-homoserine Lactone	$C_{22}H_{41}NO_3$	36.94
	10.890	Decanoyl-DL-Homoserine Lactone	$C_{14}H_{29}NO_3$	28.72
	12.955	Tetradecanoyl-L-Homoserine Lactone	$C_{18}H_{33}NO_3$	34.34

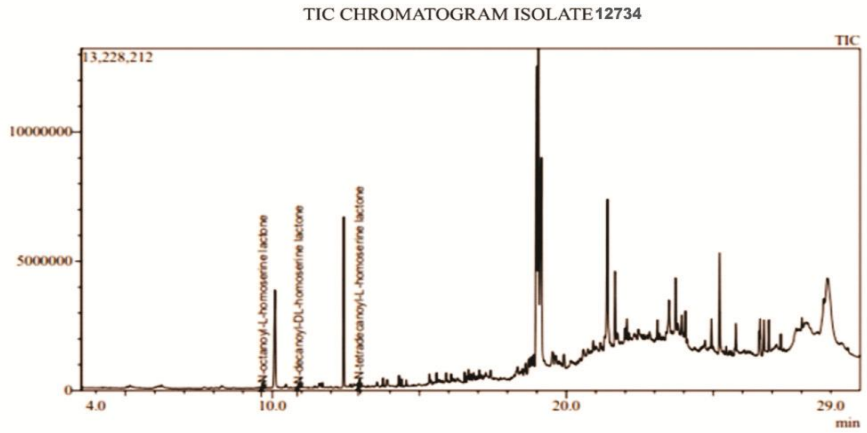


Figure 3: Chromatogram of *P. aeruginosa* signaling molecules from wound isolate

Table 6: Signaling molecules of isolate from ear infection specimen obtained from UATH Abuja, Nigeria

Retention Time (min)	Name of Compound	Molecular Formula	Area (%)

	12.952	Tetradecanoyl-L-Homoserine Lactone	$C_{18}H_{33}NO_3$	100.00

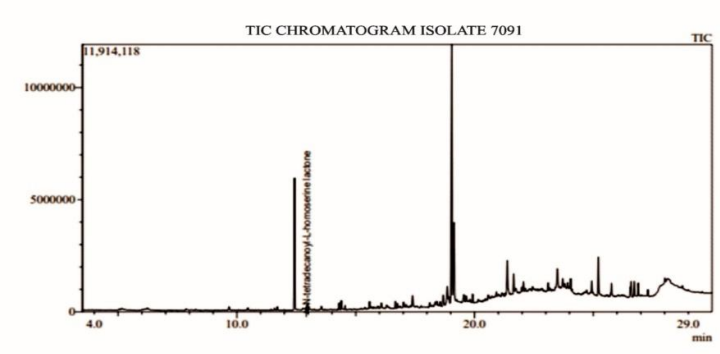


Figure 4: Chromatogram of *P. aeruginosa* Signaling Molecules from Ear isolate

Table 7: The Percentage of antibiotics resistance with Relationship to the number of Signaling Molecules identified from each isolate of different specimen.

Percentage (%) of Antibiotics Resistance												
	Piperacillin tazobactem	Cefoperazone	Ofloxacin	Tetracycline	Amikacin	Gentamycin	Bacitracin	Clarithromycin	Cefalotin	Levofloxacin	Cefpirome	No. of Signaling Molecules identified
Isolates from Catheter	88.9	80.9	91.3	66.7	89.5	94.7	100	70	62	72	85.7	4
Isolates from Wound	66.7	59	60.9	66.7	63.2	63.2	40	100	100	56	71.2	3
Isolates from Ear	44.4	50	47.8	66.7	47.4	43.1	60	30	38	52	42.9	1

4. DISCUSION

The results of the isolates obtained from the clinical specimens showed that wound specimens have a greater number of isolates in comparison with other specimens. Garbaet al. (2012) stated that this is not unexpected given that wounds, particularly those caused by thermal burns, have vast exposed areas of dead tissues devoid of any defenses, making them prime locations for infection by bacteria from the environment or natural microbiota.

Pseudomonas aeruginosa isolates are more prevalent in adults than in children, according to the demographic profile results of the clinical isolates. The result obtained was in line with the study of Iregbu and Eze (2014), who stated that Pseudomonas aeruginosa isolates were 185(70%) in adults in comparison with children. Due to their weakened immune systems and higher frequency of comorbid conditions like diabetes and cardiovascular disease, older people are typically at a higher risk of contracting nosocomial infections, especially MDR infections (Augustine and Bonomo, 2011).

Additionally, more isolates of *P*seudomonas aeruginosa were found in males than in females. This is in line with findings from international research that demonstrated that males are more likely than females to be infected with gram-negative bacteria and to develop antibiotic resistance to those infections, including *P. aeruginosa* (Ibrahim, 2018). Due to physiological reasons relating to sex chromosomes and hormones, men are typically more prone to bacterial infections. Additionally, behavioral variations between men and women, such as smoking rates or treatment compliance, may contribute to the discrepancy between men and women (Dias et al., 2022).

Pseudomonas aeruginosa was identified using both morphological and biochemical traits; however, biochemical traits are shared by morphologically related species. The 16S rRNA sequence provided data that was helpful in matching the correct taxonomy, and 99% similarity was found in the NCBI BLAST study, which confirms Pseudomonas aeruginosa asa molecular-level organism. This is supported by the international study, which stated that species with comparable morphologies share biochemical characteristics, and the 16S rRNA gene sequence, which is extremely conserved, aids in our ability to anticipate the correct classification (Amutha and Kokila, 2014).

Acyl-homoserine lactones (AHLs) with various molecular structures were found in the clinical specimens, such as urinary catheters, wounds, and ear swabs, respectively. This is supported by Cataldi*et al. (2007)*, who stated that distinguishable compounds with autoinducer activity occurred in culture supernatants of common Gram-negative bacteria. These signaling molecules are synthesized in response to the antibiotic susceptibility pattern at sub-inhibitory concentrations. These signaling molecules are P. aeruginosa cells' communication system and, at a certain threshold, form the quorum system, which controls the expression of genes unique to the bacterial population. Other research has shown that Pseudomonas aeruginosa manufactures acyl homoserine lactones, which are determinants of quorum systems that govern a number of bacterial activities, and that acyl homoserine synthases, RhII and Las I, produce a wide variety of AHLs in P. aeruginosa. The average percentage of resistance of the *P.a*. isolates varies depending on the sources of the specimens, and the higher the signal molecules identified from each isolate, the higher the resistance.

4.1 CONCLUSION

Theantimicrobial susceptibility has provided an answer to the questions of how low concentrations of antimicrobials affect bacterial viability and, more critically, whether or not the molecules might have other activities at sub-inhibitory concentrations (SIC).At antibiotic sub-inhibitory concentrations, P. aeruginosasecretes signaling molecules (N-AHLs). The amount of signaling molecules released by P. aeruginosa from various clinical specimens varies, and it has been noted that this variation is influenced by the typical percentage of antibiotic resistance. Thus, it may be said that P. aeruginosa had a higher resistance capacity due to the presence of additional signaling molecules. An important concern for this research is how isolates of the same strain that were taken from different clinical specimens varied in their capacity to resist antimicrobials, as well as how many signaling molecules (N-AHLs) they produced. Bacterial communication based on quorum sensing binds the individual bacterial cells to function as multicellular organisms by utilizing signal molecules and to support their population survival in the environment or hosts. Inhibiting the

signaling molecules of Pseudomonas aeruginosa and its mechanisms can be a successful strategy to control the refractory character of this bacterium. Identification of P. aeruginosa sensing signals (AHLs) in clinical laboratory samples can be useful for prognosis, diagnosis, and treatment. The secretion of more N-AHLs by *P. aeruginosa* confined high resistance ability; therefore, more effort should be directed towards developing AHL inhibitors that will be effective in weakening the *P.a* resistant ability to antibiotics. Treatment of *Pseudomonas aeruginosa* infections in a clinical setting should be a combination of AHL inhibitors and antibiotics for effective control and eradication of the infection of the infection or disease.

Ethical Approval

The National Health Research Ethics UATH gave approval for the research sample collections.

4.2 RECOMMENDATIONS

- Further research should be done to develop rapid tests that could be possible to detect acyl homoserine specific to *Pseudomonas aeruginosa* present to human samples and deliver results in real time.
- Longer functional-life compounds that block quorum sensing have received relatively little attention in therapeutic studies. Therefore further investigation into the signaling molecules inhibitors that might produce the subsequent class of antibacterial drugs.
- The fundamental role of each signal molecule in the production of biofilms should also be investigated, as well as whether *Pseudomonas aeruginosa* needs one or more signaling molecules to form quorum sensing.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.No AI technologies (ChatGPT, COPILOT and test-to –image generator) usage were applied in the research work.

2.

3.

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