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(RESEARCH ARTICLE)

Phylogenetic analysis on 16S rRNA of Pseudomonas Species isolated from clinical specimens in Nigeria and other Regions

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Abstract

This project was to study the clinical isolates of *Pseudomonas* organisms in Nigeria in comparison with those of other regions of the world, to understand their evolutional relationship, using phylogenetic analysis of 16SrRNA.

A total of 51 sequence data files of *Pseudomonas* species from different countries were selected based on 16S rRNA, using the National Centre for Biotechnology Information (NCBI) webpage. Geneious software (V9.0.5) was employed for sequence alignment. Tamura- Nei was used as the genetic distant model, while the tree build method was Neighbor-Joining with a bootstrap value of 1000 and 100 numbers of replicates to indicate the revolutionary process analyzed over time.

Majority of the strains from Nigeria (7) have very little or no mutations in the sequences. These strains with other strains from the other countries were clustered at the 2nd branch of the tree, with a bootstrap value of 96%. Generally, there is similarity in diversity of the *Pseudomonas* species across the globe. Much diversity was seen in all the strains from Saudi Arabia, as all were found on the 10th branch of the tree. Two *Pseudomonas* strains previously identified as belonging to other species than *aeruginosa* were seen to be *Pseudomonas aeruginosa* strains.

The low diversity shown by the strain isolates from Nigeria implies high fidelity to genetic stability with regard to the ancestral origin. The high mutation observed in all the strains from Saudi-Arabia indicates high diversity from the common ancestor and this may likely result to increased virulence and multi-drug resistance.

Keywords: *Pseudomonas aeruginosa*; Phylogenetic analysis; Virulence; Antibiotic resistance

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1. Introduction

Pseudomonas species are Gram negative aerobic, non-lactose fermenting bacteria. They are non-spore-forming and rod-shaped, belonging to the Phylum, Pseudomonadota; Class: Gamma proteobacteria; Order: Pseudomonadales and Family: Pseudomonadaceae. The genus, Pseudomonas is quite complex, comprising of more than 250 characterized and validated species [1]. The genus is divided into three phylogenetic lineages and 25 species are known to be of medical importance causing opportunistic infections in humans [2].

Pseudomonas are quite ubiquitous in nature being widely found in diverse habitats including soil, water bodies, vegetation, human bodies, and establishing themselves on moist surfaces with little or no supporting nutrients [3]. Many different species of *Pseudomonas* have been reported in clinical infections [4]. However, *Pseudomonas aeruginosa* is known to be the most isolated from clinical specimens of hospitalized patients especially patients with burns, respiratory diseases, catheterized, cancer and immune-compromised patients [5]. It has been isolated from various sites and specimens including but not limited to wounds, urine, blood, wounds, eye or ear swabs, and sputum. Though a known commensal in healthy humans, it is quite notorious and has been so much implicated in Urinary Tract Infections (UTI), microbial keratitis (MK), respiratory infections in patients suffering from cystic fibrosis (CF), Ventillator-Associated Pneumonia (VAP) in debilitated patients, bone and joint infections, dermatitis, otitis media, bacteremia and other numerous varieties of systemic infections [6, 7].

There have been many reports on the high incidence of multi-drug resistance by the *Pseudomonas* species, making their infections hard to treat. So many mechanisms of drug resistance have been reported to include acquired and intrinsic factors such as encoded genes, production of antibiotic inactivating enzymes, outer membrane proteins, efflux pumps, accessory genomes which are important for carrying virulence and acquired antibiotic resistance genes, mutation of chromosomal genes and lateral transfer of those genes between strains [8 – 10].

In recent times the use of molecular and bioinformatics tools has greatly facilitated the characterization of organisms. This is of high relevance clinically especially with regard to some bacterial superbugs whose pathogenicity and multidrug resistance have been outstandingly, great threats to treatment of infections, and more so as many organisms defy growing on artificial media. Bacteria typically have been genetically characterized based on the 16S rRNA which has been sequenced for all recognized species and is required when describing a new one [11]. The use of rRNA gene sequence and its importance to characterize and study evolution of bacteria had been described over 40 decades ago by Carl Woese.

16S rRNA is a component of the small unit (30 S) ribosomal RNA, found in bacteria and archaea. The small unit, 30S, together with the large unit, 50S and other ribosomal proteins make up the ribosomal molecule that has an overall coefficient sedimentation rate of 70S. The ribosomal rRNA plays vital roles in protein synthesis. While the small unit ensures detection of the right matching of codons on mRNA with that of tRNA, the large subunit is required in the peptide bond formation of the growing amino acid chain [12]. The small unit ribosomal RNA component, encoded by the 16S rRNA gene is possessed by all self -replicating systems and can be easily isolated [13]. Sequence analysis revealed 16S rRNA gene has multiple sub-regions, namely V1–V9 which can be used for the distinct identification of various prokaryotes; single or combination of different variable regions can be used for diversity analysis. It has both the hyper variable region and the conserved regions which allow the designing of universal primers for its amplification [14, 15]. And so, the 16s rRNA gene has been such a useful marker for taxonomical classification and separation, giving rise to various 16S identification tools and databases [13, 16]. Also, owing to its functional constancy and highly conserved nature, the 16S rRNA gene was used to define the three domains of Life [17].

This study was aimed at phylogenetic analysis of the *Pseudomonas* species isolated from clinical samples from Nigeria and from other countries based on the 16S rRNA gene, to match their relatedness and divergence from common ancestry. This may portray similarity in virulence and possession of those factors that enhance antibiotic resistance. This may also proffer remedy on antibiotic resistance which may be applicable to all the strains in different geographical regions.

2. Materials and Methods

2.1. Sequence Data Retrieval

Using the National Centre for Biotechnological Information (NCBI) webpage, a search was made for *Pseudomonas* species from clinical samples, and 51 sequence data files of *Pseudomonas* species from different countries were selected

based on 16S rRNA. The sequence data files were selected from the different Regions as follows: Carolina (1), Egypt (2), India (15), Iran (1), Iraq (5), Japan (2), Nigeria (8), Pakistan (1), Saudi Arabia (11), Spain (2), Thailand (2) and Washington (1).

2.2. Multiple Sequence Alignment and Phylogenetic analysis

The sequence data were uploaded in the Generous software for sequence alignment which was done using MUSCLE alignment to align sequences with 8 maximum number of Iterations.

Phylogenetic analysis was performed on the aligned sequences using Geneious Tree builder software (v9.0.5), to determine the evolutionary relatedness and diversities. To create a consensus Tree at the support threshold of 50%, Tamura- Nei was used as the genetic distant model, while the tree build method was Neighbor-Joining with a bootstrap value of 1000 and 100 numbers of replicates to indicate the revolutionary process analyzed over time.

Comparative analysis with a distance matrix of the tree was performed on Geneious (https://www.geneious.com) based on statistical analysis to determine positions of significant difference between the samples.

Table 1 shows the characteristics of the sequence data used in this study. Of all the retrieved sequence data, 44 sequenced *Pseudomonas* organisms isolated from clinical specimens were identified as purely *Pseudomonas aeruginosa*, 5 were *Pseudomonas* species not identified to species level while 3 belonged to other *Pseudomonas* species identified as *Pseudomonas taiwanensis*, *Pseudomonas otitidis* and *Pseudomonas tohonis.* This confirms the high predominance of *Pseudomonas aeruginosa* in clinical infections.

Table 1 Characteristics of the 51 Clinical *Pseudomonas* species used in this Study

S/N	SEQUENCE FILE ID NO	ACCESSION NO	NAME OF ORG	STRAN TYPE	REGION	SEQUENCE LENGTH	G-C CONTENT
1	Car_02-640	EF558702.1	*Pseudomonas sp.*	02-640	Carolina	894	54.00%
2	Egy_PS31	LC514698.1	*Pseudomonas aeruginosa*	PU15	Egypt	800	53.60%
3	Egy_PU15	LC619328.2	*Pseudomonas aeruginosa*	PS31		982	52.90%
4	Ind_ KU_NI04	KU743855.1	*Pseudomonas aeruginosa*	NRC47	India	827	53.20%
5	Ind_NRC47	OQ704181.1	*Pseudomonas aeruginosa*	A001		938	53.80%
6	Ind_Ps A001	MK598327.1	*Pseudomonas aeruginosa*	PsADMC02		1473	54.20%
7	Ind_Ps A007	MK598329.1	*Pseudomonas taiwanensis*	PsTW DMC 234		1481	53.80%
8	Ind_PsADMC01	MK598324.1	*Pseudomonas aeruginosa*	PsADMC01		1510	52.10%
9	Ind_PsADMC02	MK598330.1	*Pseudomonas aeruginosa*	PsADMC03		1517	52.20%
10	Ind_PsADMC03	MK598336.1	*Pseudomonas aeruginosa*	PsADMC09		1521	51.90%
11	Ind_PsADMC04	OQ363380.1	*Pseudomonas aeruginosa*	KU_NI04		857	53.80%
12	Ind_PsADMC07	OQ772264.1	*Pseudomonas aeruginosa*	A007		1102	54.20%

13	Ind_PsADMC08	MK598337.1	*Pseudomonas aeruginosa*	PsADMC10		1470	54.10%
14	Ind_PsADMC09	MK598332.1	*Pseudomonas aeruginosa*	PsADMC06		1474	54.20%
15	Ind_PsADMC10	MK598334.1	*Pseudomonas aeruginosa*	PsADMC08		1474	54.10%
16	Ind_PsOT DMC1231	MK595791.1	*Pseudomonas otitidis*	PsOT DMC1231		1476	53.90%
17	Ind_PsTW DMC 234	MK598331.1	*Pseudomonas aeruginosa*	PsADMC04		1485	54.30%
18	Ind-PsADMC06	MK598333.1	*Pseudomonas aeruginosa*	PsADMC07		1513	52.90%
19	Iran_IAUK1	MN103608.1	*Pseudomonas aeruginosa*	IAUK1	Iran	1400	54.70%
20	Iraq_ZHSAHD-1	ON808421.1	*Pseudomonas aeruginosa*	ZHSAHD-3	Iraq	409	51.80%
21	Iraq_ZHSAHD-2	ON808420.1	*Pseudomonas aeruginosa*	ZHSAHD-2		445	51.90%
22	Iraq_ZHSAHD-3	ON808422.1	*Pseudomonas aeruginosa*	ZHSAHD-4		622	52.40%
23	Iraq_ZHSAHD-4	ON808419.1	*Pseudomonas aeruginosa*	ZHSAHD-1		647	52.20%
24	Iraq_ZHSAHD-5	ON808423.1	*Pseudomonas aeruginosa*	ZHSAHD-5		655	52.10%
25	Jap_Hugh1066	AB247202.1	*Pseudomonas aeruginosa*	Hugh1066	Japan	520	54.00%
26	Jap_TUM18999	LC645211.1	*Pseudomonas tohonis*	TUM18999		1532	54.00%
27	Nig_AS11	ON853997.1	*Pseudomonas aeruginosa*	AS11	Nigeria	477	54.70%
28	Nig_SAUTHC7	OP209787.1	*Pseudomonas aeruginosa*	SAUTHC7		1402	54.10%
29	Nig_ZainJ1	OQ366428.1	*Pseudomonas aeruginosa*	Zain J3		1410	54.20%
30	Nig_ZainJ2	OQ366426.1	*Pseudomonas aeruginosa*	Zain J1		1408	54.30%
31	Nig_ZainJ3	OQ366427.1	*Pseudomonas aeruginosa*	Zain J2		1409	54.20%
32	Nig_ZainJ4	OQ366431.1	*Pseudomonas aeruginosa*	Zain J6		1409	54.20%
33	Nig_ZainJ5	OQ366429.1	*Pseudomonas aeruginosa*	Zain J4		1410	54.20%
34	Nig_ZainJ6	OQ366430.1	*Pseudomonas aeruginosa*	Zain 5		1410	54.20%
35	Par_SK4	LT545682.1	*Pseudomonas sp.*	SK4	Pakistan	1550	54.60%

36	Sau_BLA14	LC337983.1	*Pseudomonas aeruginosa*	WOF35	Saudi Arabia	700	55.10%
37	Sau_EAH3	LC337985.1	*Pseudomonas aeruginosa*	REH15		700	54.90%
38	Sau_MIH14	LC337986.1	*Pseudomonas aeruginosa*	REH4	Saudi Arabia	700	55.10%
39	sau_MIH20	LC337987.1	*Pseudomonas aeruginosa*	EAH3		700	55.40%
40	Sau_MIH21	LC337988.1	*Pseudomonas aeruginosa*	MIH14.		700	54.30%
41	Sau_MIH25	LC337989.1	*Pseudomonas aeruginosa*	MIH20		700	54.90%
42	Sau_MIH26	LC337990.1	*Pseudomonas aeruginosa*	MIH21		700	55.30%
43	Sau_MIH27	LC337991.1	*Pseudomonas aeruginosa*	MIH25		700	55.00%
44	Sau_REH4	LC337992.1	*Pseudomonas aeruginosa*	MIH26		700	55.10%
45	Sau_REH15	LC337993.1	*Pseudomonas aeruginosa*	MIH27		700	55.30%
46	Sau_WOF35	LC337976.1	*Pseudomonas aeruginosa*	BLA14		801	45.20%
47	Spa_CCUG 63225	LT601014.1	*Pseudomonas sp.*	SE1.	Spain	1382	54.00%
48	Spa_SE1	LT601010.1	*Pseudomonas sp.*	CCUG 63225		1425	54.00%
49	Tha_03-SYBR-2019	LC730905.1	*Pseudomonas aeruginosa*	03-SYBR-2019	Thailand	165	50.30%
50	Tha_ S7	OQ627392.1	*Pseudomonas aeruginosa*	S7		776	50.40%
51	WUSC-05_h02_1	KP844954.1	*Pseudomonas sp*	HMSC05H02 clone WUSC-05_h02_1	Washington	1347	54.20%

3. Results and Discussion

3.1. Comparative Sequence Alignments

Figure 1(a-d) shows the alignment views of the sequence data. Similarities as well as variations could be observed in some regions of the sequences at various points. The variations imply mutations which could be due to substitution, deletion, insertion or translocation of nucleic acid bases or sequences. These mutations are widely spread throughout the nucleotide sequences of strain 1 to strain 10, which include 7 strains from India, 2 from Spain and one from Japan. Much mutation could be observed in two other strains from India (strains no 31 and 32).

Observably, all the strains from Nigeria (7) and some strains from India (4), Iraq (3) Egypt (1), Washington (1) and Iran (1) had very little or no variations (no 11 to no. 27). This could translate to the ability to keep true to type or maintain high level of similarity to ancestral parents and high fidelity to genetic stability with regard to the ancestral origin. This would imply normal level of virulence or pathogenicity and antibiotic resistance expected of *Pseudomonas* species. A pronounced deletion showed in some strains: Iraq_ZHSAHD-1(no 15), Iraq_ZHSAHD-2 (no.25), Iraq_ZHSAHD-4(no.26), Iraq_ZHSAHD-3 (no. 28), Tha_03_SYBR_19 (no.30) and some others. In terms of genetic stability, the strains from

Nigeria were true to typical *Pseudomonas aeruginosa* species that have not acquired any or much resistance or virulent genes. Virulence and Antibiotic resistance two pathogenic armaments of *Pseudomonas* species. Virulence develops from the time of host colonization while antibiotic resistance developed in the course of antibiotics [18]. Both are controlled by many internal and acquired genetic factors.

The 11(eleven) strains from Saudi Arabia (no 41 to 51), presented with much deletion of nucleotide bases from point 1 to point 380, after which regions of high mutation could be observed from point 381 to about point 550. The similarities in variation patterns suggest evolutionary relatedness in functional characteristics. The high mutations could be linked to much deviation from ancestral origin as the initial genetic makeup known for *Pseudomonas aeruginosa* changed overtime. The deletions on the other hand implied loss of genes and their functions. Since *Pseudomonas* sp are naturally known to possess many virulent factors which include but not limited to Lipopolysaccharide, Flagellum, Type IV Pili, Type III Secretion System, Exotoxin A, Proteases, Alginate, Quorum Sensing, Biofilm Formation, Type VI Secretion Systems [19], deletion may mean loss of genes controlling the production or formation of virulent proteins or loss of genes controlling the formation of virulent structures. Therefore, deletion might result in decreased pathogenicity of the *Pseudomonas aeruginosa* strains. The variations observed could translate to genetic diversity between species across the different geographical locations.

Figure 1b showed more clearly, with colouration, the regions of the mutated genes in the strains. The strains from Saudi Arabia had much elongated regions of nucleotide deletion which showed as dense regions of mutations. This might relate to high clinical virulence and antibiotic resistance.

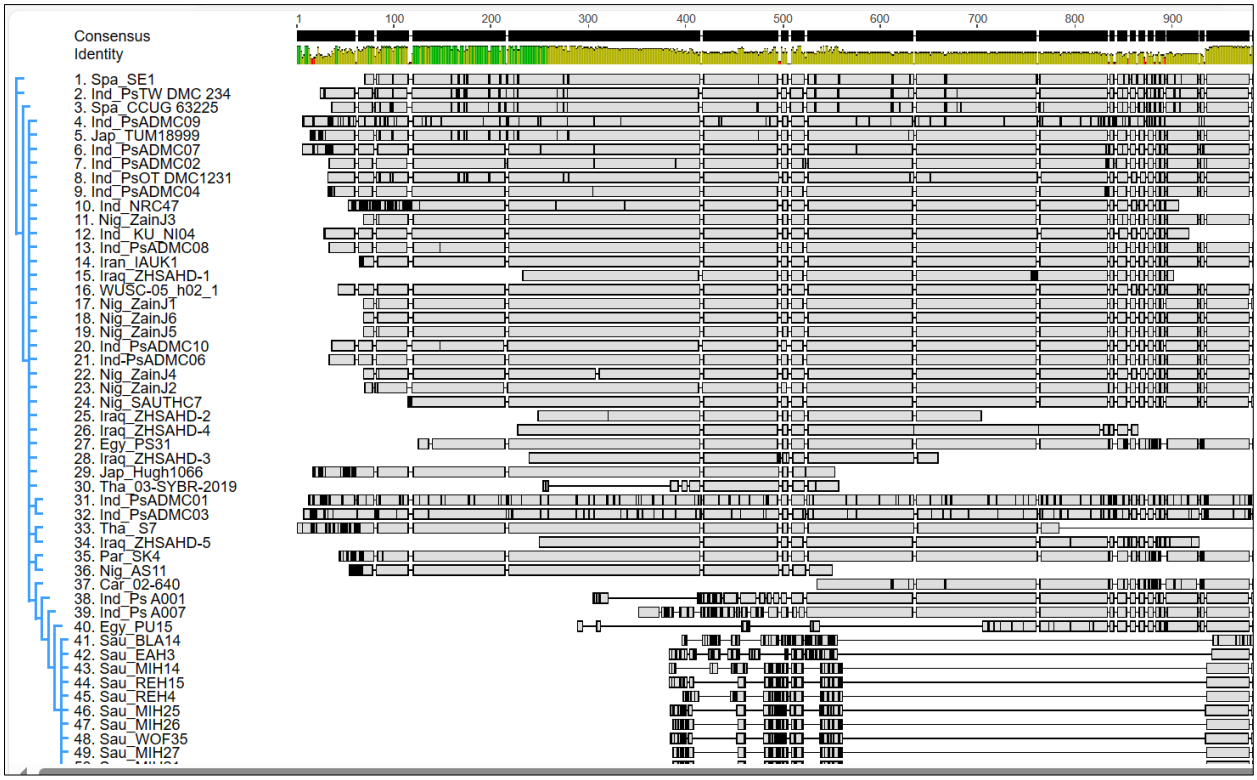


Figure 1a Alignment view of the Sequences (from the 1st to 900 nucleotides)

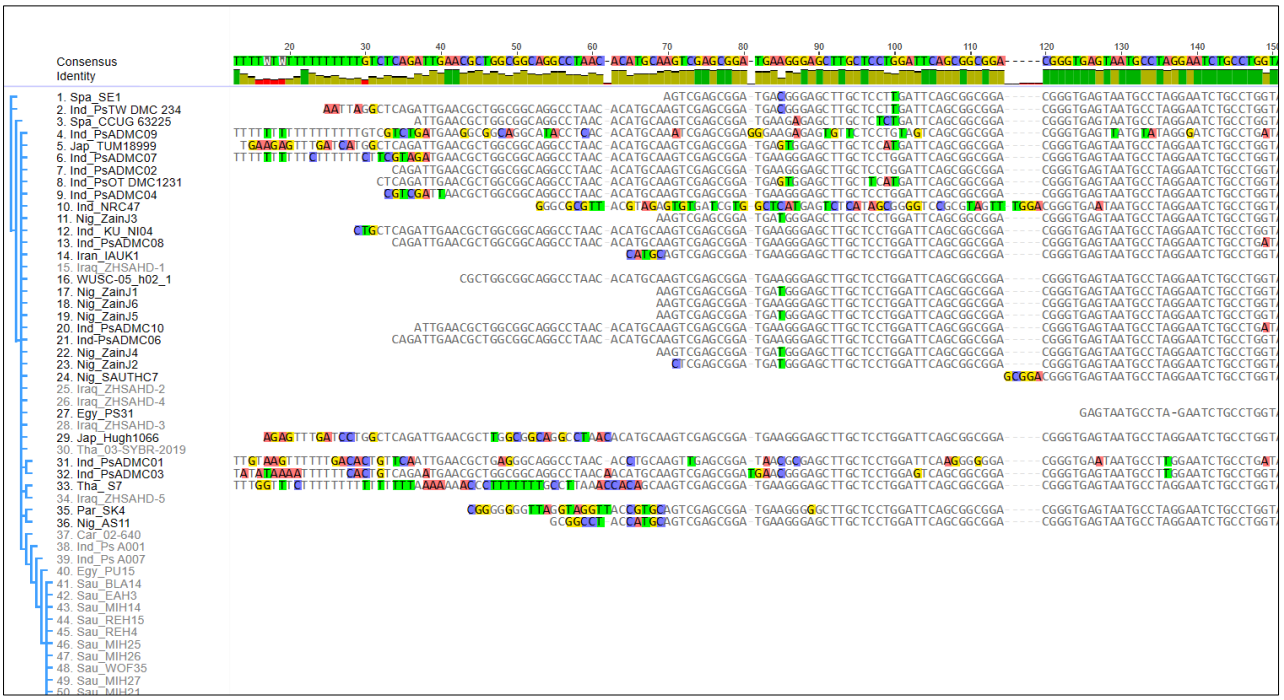


Figure 1b Alignment View of the sequences (nucleotide regions from1 to 150)

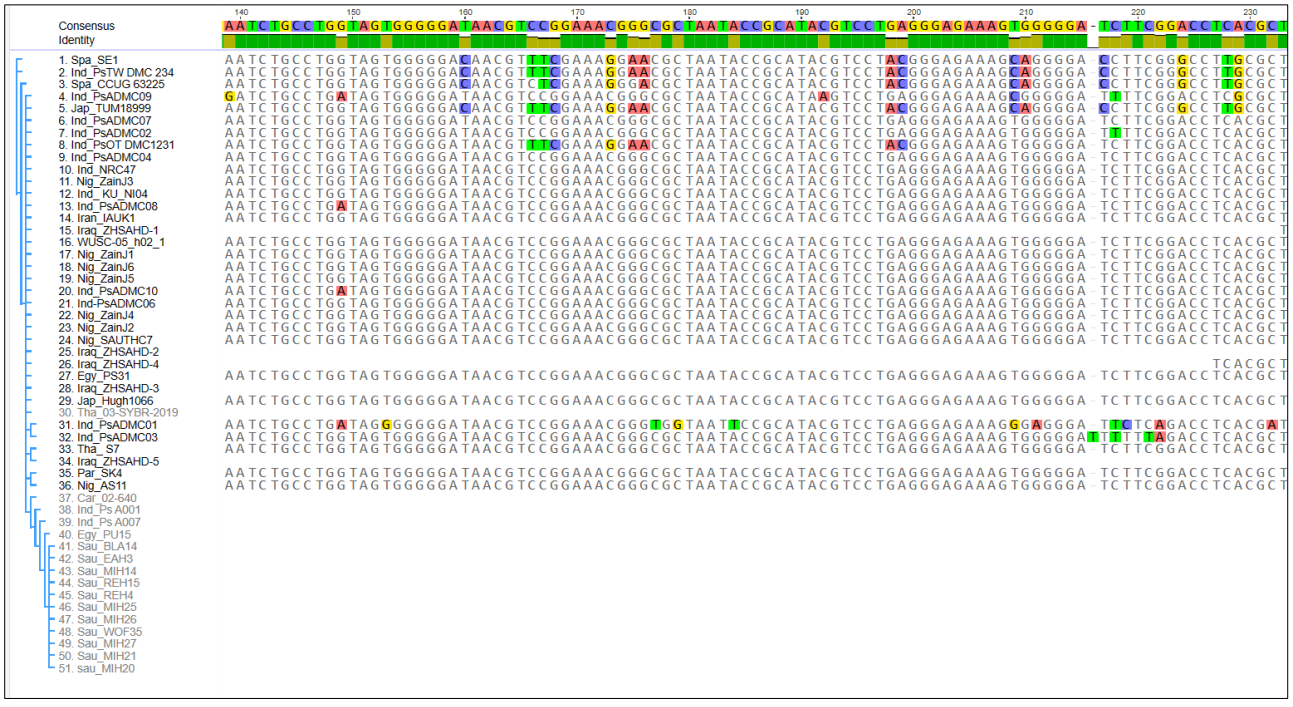


Figure 1c. Alignment View of the sequences (nucleotide regions from 140 to 230)

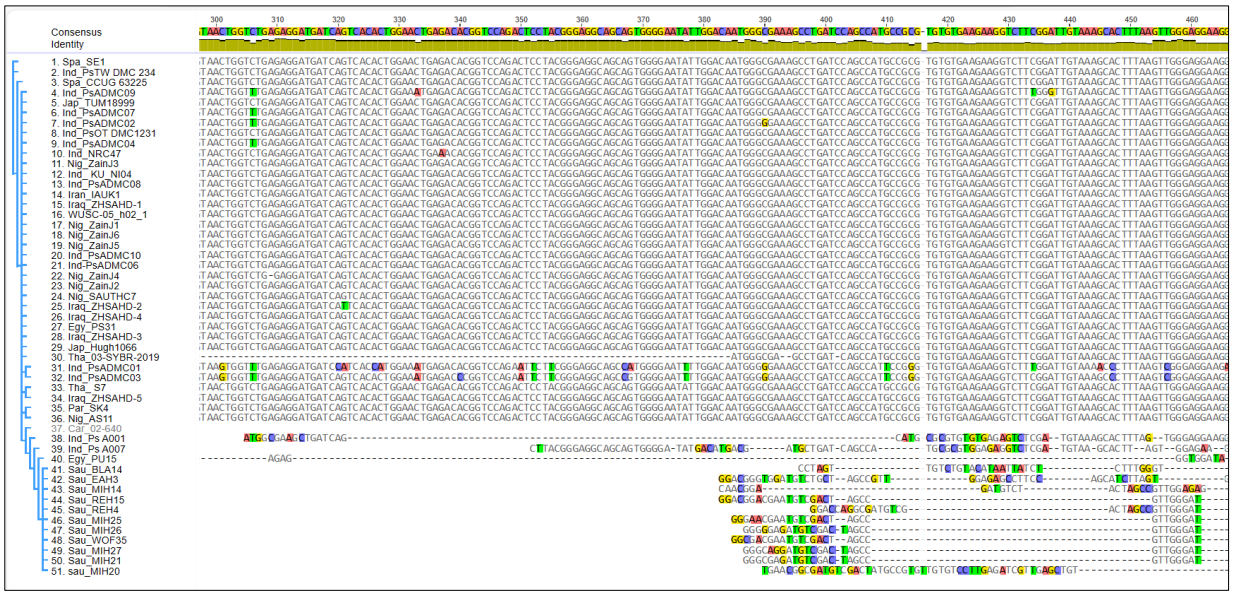
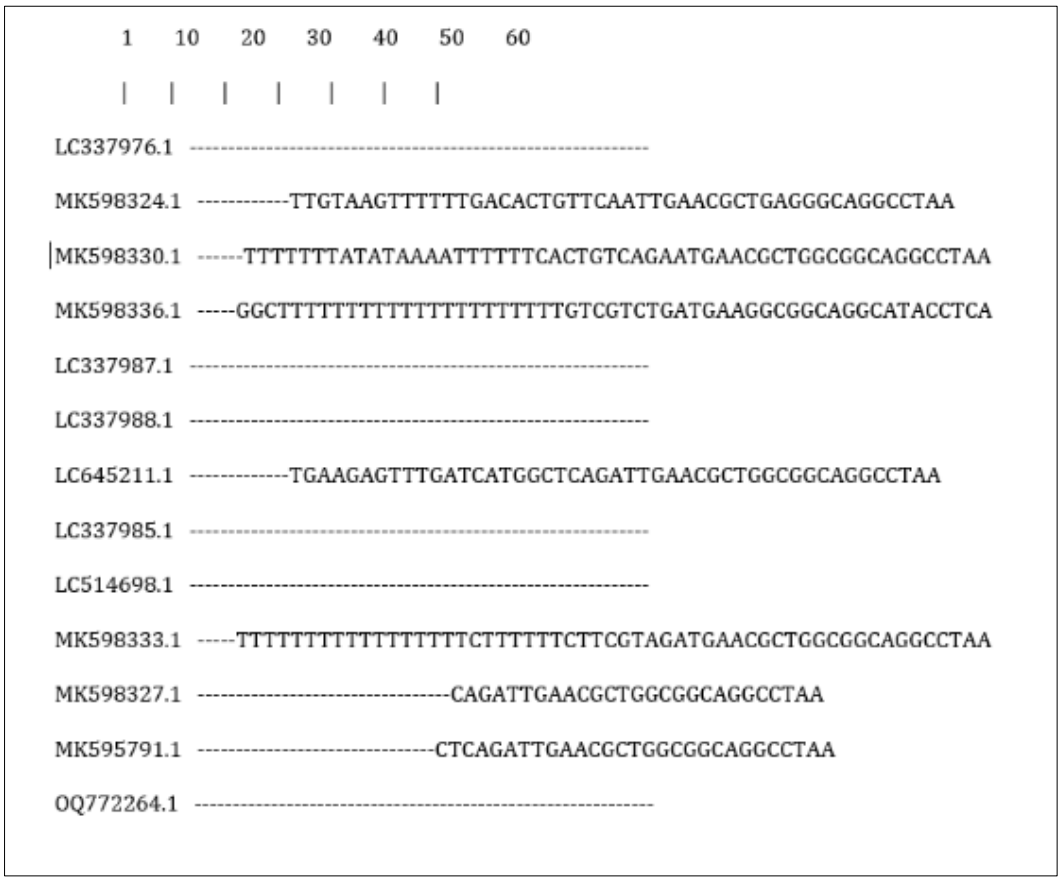


Figure 1d Alignment View of the sequences (nucleotide regions from 300 to 460)

3.2. Multiple Sequence Alignment Text Format by Muscle

The alignment Text format below (Figure 2) highlights clearly the nucleotides with deletions and variations in the base sequences of the data, from point 1 to point > (greater than) 60 nucleotide sequence length. The differences in the sequences show varied characteristics that can be of clinical relevance.



```
LC337986.1  ----------
MK598331.1  ----------CGTCGATTAACGCTGGCGGCAGGCCTAA
KU743855.1  ----------GGGCGCGT
OQ366428.1  ----------
LC337991.1  ----------
LC337992.1  ----------
OQ363380.1  ----------CTGCTCAGATTGAACGCTGGCGGCAGGCCTAA
MK598334.1  ----------CAGATTGAACGCTGGCGGCAGGCCTAA
LC337983.1  ----------
OQ704181.1  ----------
LC337993.1  ----------
MN103608.1  ----------
ON808419.1  ----------
KP844954.1  ----------CGCTGGCGGCAGGCCTAA
OQ366426.1  ----------
OQ366431.1  ----------
OQ366430.1  ----------
MK598337.1  ----------ATTGAACGCTGGCGGCAGGCCTAA
MK598332.1  ----------CAGATTGAACGCTGGCGGCAGGCCTAA
LT545682.1  ----------CGGGGGGGTTAGGTAGG
OQ366429.1  ----------
OQ366427.1  ----------
OP209787.1  ----------
ON808420.1  ----------
ON808422.1  ----------
OQ627392.1  TTTTTTGTTTTTTTTGGTTTCTTTTTTTTTTTTTTTTAAAAAAACCCTTTTTTTGCCTTAA
LC619328.2  ----------
LC337990.1  ----------
ON853997.1  ----------GCGGCC
```

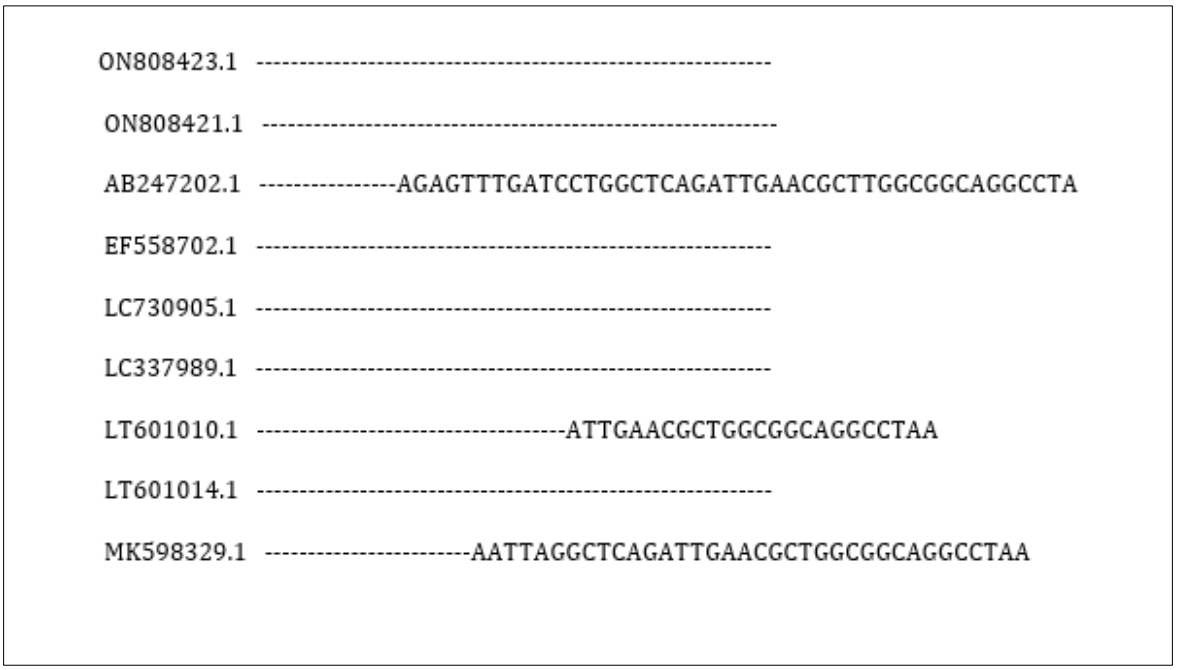
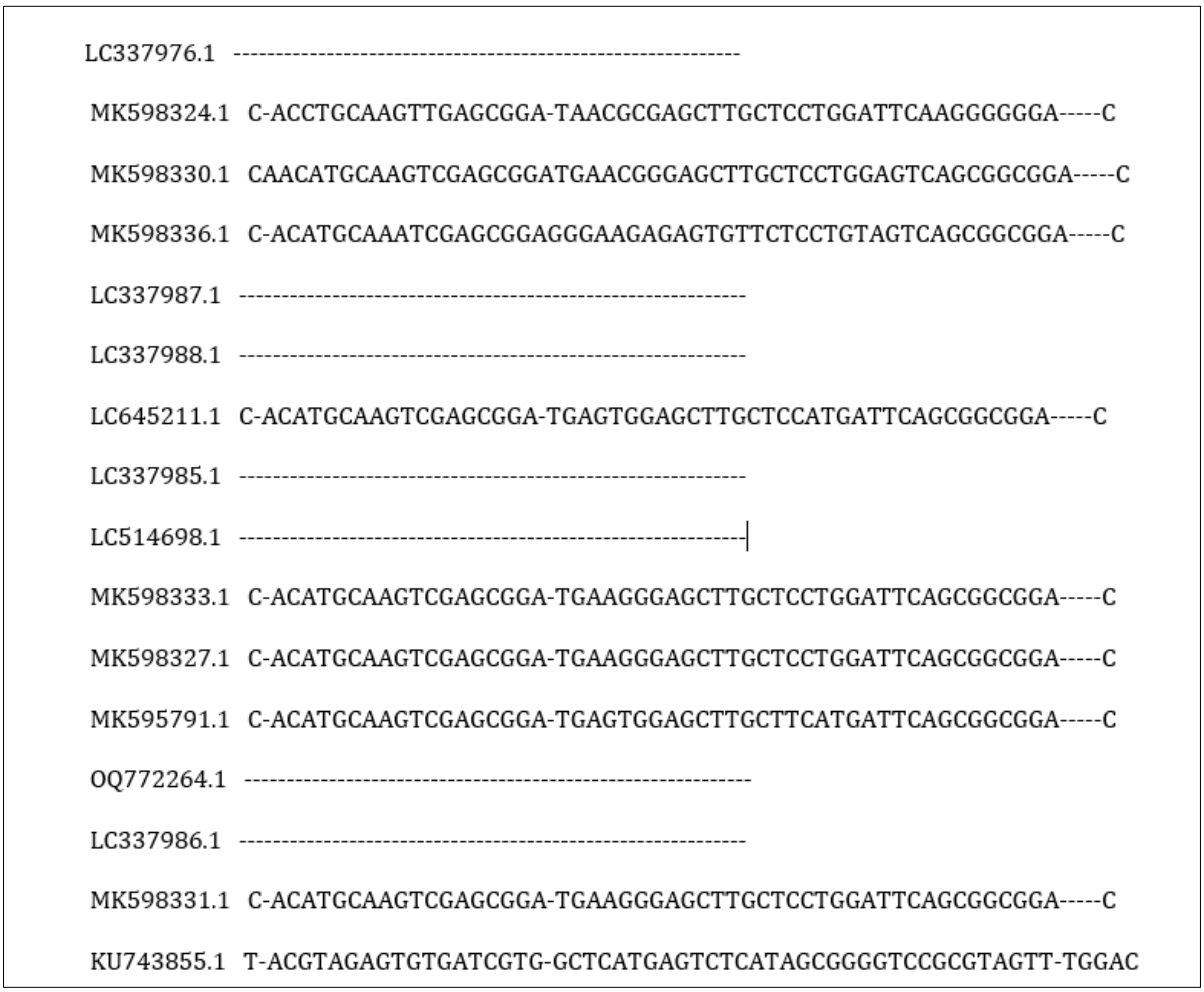


Figure 2(i) The alignment Text format (1st set)



```
OQ366428.1  --------AAGTCGAGCGGA-TGATGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
LC337991.1  ------------------------------------------------------------
LC337992.1  ------------------------------------------------------------
OQ363380.1  C-ACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
MK598334.1  C-ACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
LC337983.1  ------------------------------------------------------------
OQ704181.1  ------------------------------------------------------------
LC337993.1  ------------------------------------------------------------
MN103608.1  ----CATGCAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
ON808419.1  ------------------------------------------------------------
KP844954.1  C-ACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
OQ366426.1  --------AAGTCGAGCGGA-TGATGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
OQ366431.1  --------AAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
OQ366430.1  --------AAGTCGAGCGGA-TGATGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
MK598337.1  C-ACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
MK598332.1  C-ACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
LT545682.1  TTACCGTGCAGTCGAGCGGA-TGAAGGGGGCTTGCTCCTGGATTCAGCGGCGGA-----C
OQ366429.1  --------AAGTCGAGCGGA-TGATGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
OQ366427.1  ----------CTCGAGCGGA-TGATGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
OP209787.1  ----------------------------------------------------GCGGAC
ON808420.1  ------------------------------------------------------------
ON808422.1  ------------------------------------------------------------
OQ627392.1  ACCACAGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
LC619328.2  ------------------------------------------------------------
LC337990.1  ------------------------------------------------------------
ON853997.1  T-ACCATGCAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
ON808423.1  ------------------------------------------------------------
ON808421.1  ------------------------------------------------------------
AB247202.1  ACACATGCAAGTCGAGCGGA-TGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGA-----C
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```
EF558702.1  ----------
LC730905.1  ----------
LC337989.1  ----------
LT601010.1  C-ACATGCAAGTCGAGCGGA-TGAAGAGAGCTTGCTCTCTGATTCAGCGGCGGA-----C
LT601014.1  ---------AGTCGAGCGGA-TGACGGGAGCTTGCTCCTTGATTCAGCGGCGGA-----C
MK598329.1  C-ACATGCAAGTCGAGCGGA-TGACGGGAGCTTGCTCCTTGATTCAGCGGCGGA-----C
```

Figure 2(ii) The alignment Text format (2nd set)

```
LC337976.1  ----------
MK598324.1  GGGTGAATAATGCCTTGGAATCTGCCTGATAGGGGGGGATAACGTCCGGAAACGGGTGGT
MK598330.1  GGGTGAGTAATGCCTTGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK598336.1  GGGTGAGTTATGTATAGGGATCTGCCTGATAGTGGGGGATAACGTCCGGAAACGGGCGCT
LC337987.1  ----------
LC337988.1  ----------
LC645211.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCT
LC337985.1  ----------
LC514698.1  ----------
MK598333.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK598327.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK595791.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAAGGAACGCT
OQ772264.1  ----------
LC337986.1  ----------
MK598331.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
KU743855.1  GGGTGAATAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366428.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
LC337991.1  ----------
LC337992.1  ----------
```

```
OQ363380.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK598334.1  GGGTGAGTAATGCCTAGGAATCTGCCTGATAGTGGGGGATAACGTCCGGAAACGGGCGCT
LC337983.1  ----------------------------------------------------------
OQ704181.1  ----------------------------------------------------------
LC337993.1  ----------------------------------------------------------
MN103608.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
ON808419.1  ----------------------------------------------------------
KP844954.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366426.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366431.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366430.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK598337.1  GGGTGAGTAATGCCTAGGAATCTGCCTGATAGTGGGGGATAACGTCCGGAAACGGGCGCT
MK598332.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
LT545682.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366429.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OQ366427.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
OP209787.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
ON808420.1  ----------------------------------------------------------
ON808422.1  ----------------------------------------------------------
OQ627392.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
LC619328.2  ----GAGTAATGCCTA-GAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
LC337990.1  ----------------------------------------------------------
ON853997.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
ON808423.1  ----------------------------------------------------------
ON808421.1  ----------------------------------------------------------
AB247202.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT
EF558702.1  ----------------------------------------------------------
LC730905.1  ----------------------------------------------------------
LC337989.1  ----------------------------------------------------------
```

```
LT601010.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTCTCGAAAGGGACGCT

LT601014.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCT

MK598329.1  GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCT
```

Figure 2(iii) The alignment Text format (3rd set)

3.3. Phylogenetic Tree and Evolution

The tree (Figure 3) is an illustration of phylo-evolutionary diversity between the *Pseudomonas* species and strains. The tree has 10 branches. Each of the branches signifies diversity from parent, which may be little or much as is shown by the bootstrap values. The first branch has 59% bootstrap value, indicating 59% relatedness to the immediate ancestor. The first parent has two daughters; Spa_ SE1 from Spain which is unbranched and the other branch from where emanates a cluster of branches which show 96% relatedness to the ancestral parent. Within this cluster are found majority of the *Pseudomonas aeruginosa* strains (27) from the different geographical regions: India (10), Japan (2), Nigeria (7), Iran (1), Iraq (4), and Washington (1). Egypt (1) and Thailand1). These strains are therefore geo-evolutionary related. This is quite interesting, showing that *Pseudomonas aeruginosa* species in clinical samples can have identical genetic characteristics.

It is quite remarkable to observe that the two strains thought to be of different species of *Pseudomonas: Pseudomonas otitidis* PsOT DMC1231 from India and *Pseudomonas tohonis,* TUM18999 from Japan are found within this 2nd cluster of branches showing 96% resemblance to the successive parents. This shows they are not of different species but true *Pseudomonas aeruginosa* strains in clinical samples. However, *Pseudomonas taiwanensis* (PsTW DMC 234) from India and *Pseudomonas sp.* (SE1) from Spain are truly not *Pseudomonas aeruginosa* species; they belong to other species. Apart from these two, which fall as outliers, all the strains in this study including the other named *Pseudomonas* sp are all *Pseudomonas aeruginosa*.

The 3rd, 4th and 5th branches emanate from the 2nd branch and have bootstrap values of 93%, 52% and 52% respectively showing the percentage of their relatedness to their parents. The 3rd branch is a parent to 2 species of India; the 4th branch begot two daughter strains, 1 from Thailand and the other from Iraq; the 5th branch also begot two daughter strains, 1 from Pakistan and the other from Nigeria. The 6th branch developed from the 2nd branch as well but has developed into 4 generations as depicted by the 4 branches, the 7th, 8th, 9th and 10th generations depicting evolution over time. These branches are each 80%, 65%, 100% and 99% to the respective successive ancestral parents. The 11 strains from Saudi Arabia are from the 10th branch or generation and represent pronounced diversity overtime.

Across the tree branches from ancestors to later generations, there is a trend of less genetic diversity between species, such that there are more evolutionary similarities than differences.

Each cluster of strains emanating from the parent have same percentage of similarity to immediate parent, and that may indicate same or very similar pattern of pathogenicity. Diversity overtime may translate evolution of clinically relevant attributes of the *Pseudomonas aeruginosa* strains, such as virulence, antibiotic resistance, and ability to transfer resistance laterally or vertically, owing to acquisition of some genetic elements. Such genetic elements include plasmids which are auto- replicative, independent of the cell's normal chromosomal replicative mechanism, and can enable large transfer of factors between or across species of different organisms.

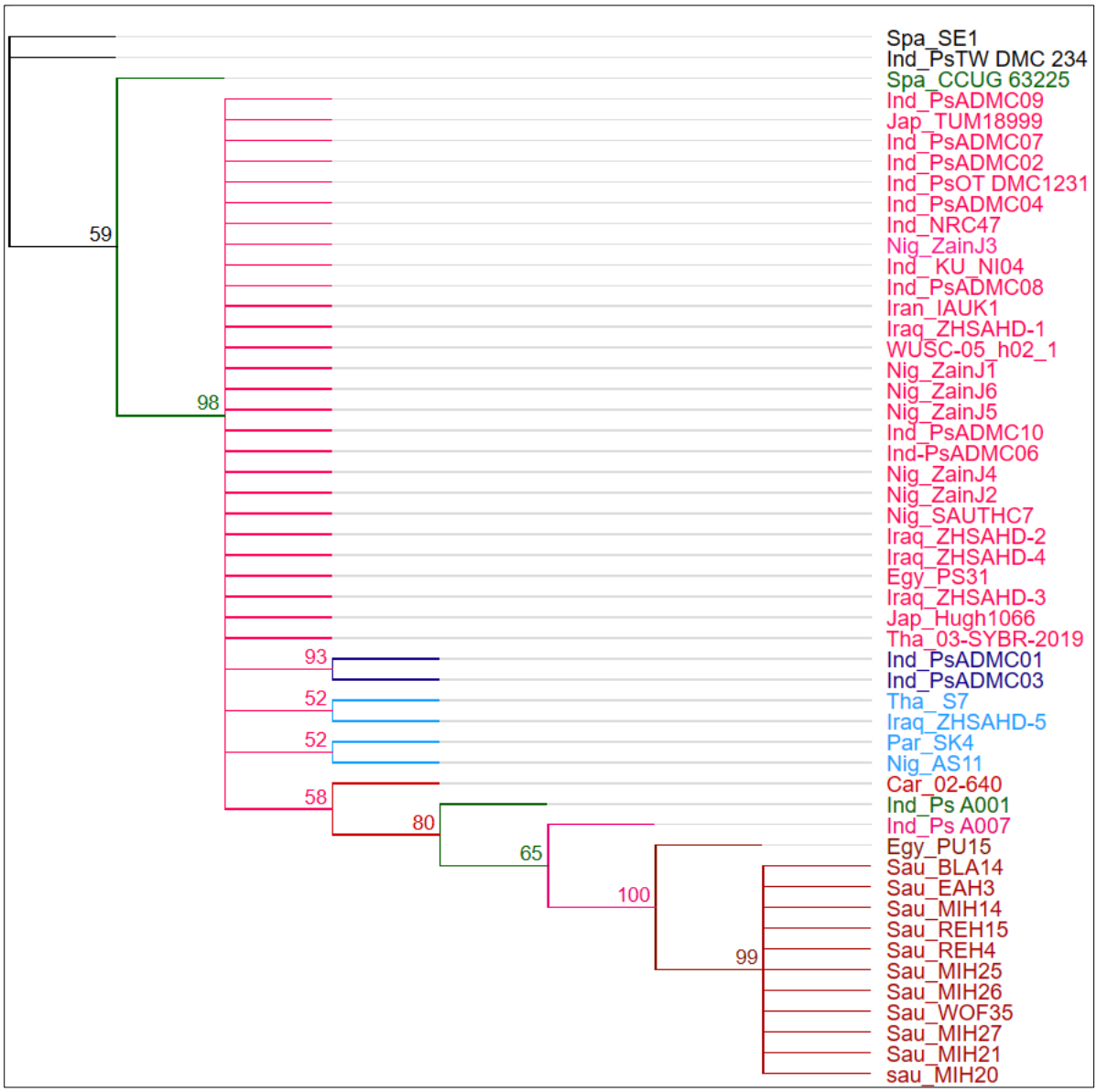


Figure 3 Phylogenetic Tree of the Sequence Data

3.4. Distance Matric

The distance matric could indicate the relationship between strains, how close they are on the evolutionary scale. The strains are matched against themselves and the relative distance between any pair is shown by numeral figures. As relatedness increases, the numbers approximate to zero (0). On the other hand, as can be seen in figure 4a (ii), as the distance between two matched pairs increases, the figures increase. The highest figure got in this analysis is 0.46 which was obtained between Sau_Bla14 (a strain from Saudi-Arabia) and Ind_PsADMC01 (a strain from India), indicating that they are farther in similarity when compared to the other ones. In order words, diversity is expected.

The diagonal line across the rectangle dividing the rectangle into two equal triangles indicates no distance between two matched same strains.

	Spa_...	Ind_...	Spa_...	Ind_...	Jap_...	Ind_...	Ind_...	Ind_...	Ind_...	Ind_...	Nig_...	Ind_ ...	Ind_...	Iran_...	Iraq_...	WUS...	Nig_...	Nig_...
Spa_SE1		0.01	0.02	0.06	0.02	0.03	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ind_PsTW DMC 2...	0.01		0.02	0.07	0.03	0.03	0.03	0.03	0.03	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02
Spa_CCUG 63225	0.02	0.02		0.07	0.03	0.04	0.04	0.03	0.03	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ind_PsADMC09	0.06	0.07	0.07		0.05	0.05	0.05	0.05	0.05	0.06	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04
Jap_TUM18999	0.02	0.03	0.03	0.05		0.01	0.01	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ind_PsADMC07	0.03	0.03	0.04	0.05	0.01		0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ind_PsADMC02	0.02	0.03	0.04	0.05	0.01	0.01		0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ind_PsOT DMC12...	0.02	0.03	0.03	0.05	0.01	0.01	0.01		0.01	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Ind_PsADMC04	0.02	0.03	0.03	0.05	0.01	0.01	0.01	0.01		0.02	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ind_NRC47	0.04	0.04	0.05	0.06	0.02	0.03	0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Nig_ZainJ3	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ind_ KU_NI04	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00		0.00	0.00	0.00	0.00	0.00	0.00
Ind_PsADMC08	0.02	0.02	0.03	0.04	0.01	0.01	0.01	0.00	0.01	0.02	0.00	0.00		0.00	0.00	0.00	0.00	0.00
Iran_IAUK1	0.02	0.03	0.03	0.05	0.01	0.01	0.01	0.01	0.01	0.02	0.00	0.00	0.00		0.00	0.00	0.00	0.00
Iraq_ZHSAHD-1	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00		0.00	0.00	0.00
WUSC-05_h02_1	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00		0.00	0.00
Nig_ZainJ1	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00		0.00
Nig_ZainJ6	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Nig_ZainJ5	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ind_PsADMC10	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ind-PsADMC06	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nig_ZainJ4	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nig_ZainJ2	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nig_SAUTHC7	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Iraq_ZHSAHD-2	0.02	0.02	0.03	0.04	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

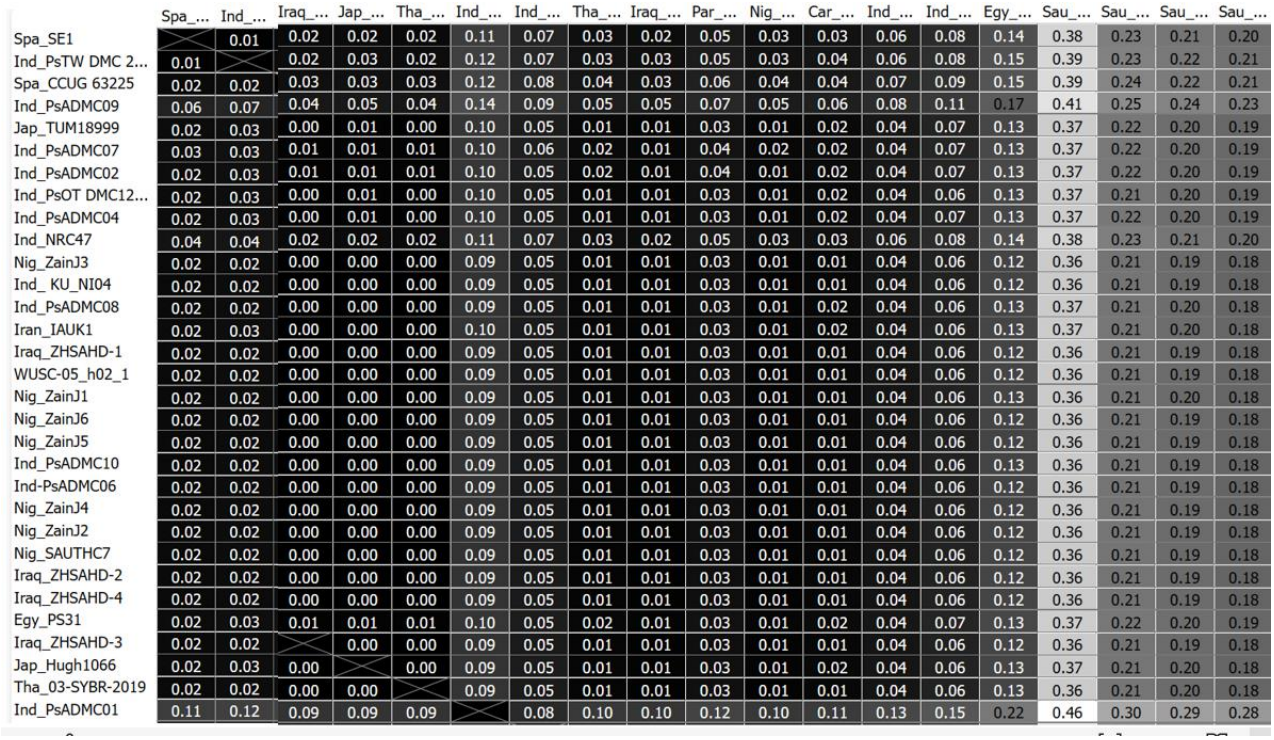
Figure 4a (i) Distance Matric of the Sequence Data

	Nig_...	Nig_...	Nig_...	Iraq_...	Iraq_...	Egy_...	Iraq_...	Jap_...	Tha_...	Ind_...	Ind_...	Tha_...	Iraq_...	Par_...	Nig_...	Car_...	Ind_...	Ind_...	Egy_...	Sau_...
Spa_SE1	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.11	0.07	0.03	0.02	0.05	0.03	0.03	0.06	0.08	0.14	0.38
Ind_PsTW DMC 2...	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.02	0.12	0.07	0.03	0.03	0.05	0.03	0.04	0.06	0.08	0.15	0.39
Spa_CCUG 63225	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.12	0.08	0.04	0.03	0.06	0.04	0.04	0.07	0.09	0.15	0.39
Ind_PsADMC09	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.04	0.14	0.09	0.05	0.05	0.07	0.05	0.06	0.08	0.11	0.17	0.41
Jap_TUM18999	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.07	0.13	0.37
Ind_PsADMC07	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.10	0.06	0.02	0.01	0.04	0.02	0.02	0.04	0.07	0.13	0.37
Ind_PsADMC02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.10	0.05	0.02	0.01	0.04	0.01	0.02	0.04	0.07	0.13	0.37
Ind_PsOT DMC12...	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37
Ind_PsADMC04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.07	0.13	0.37
Ind_NRC47	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.11	0.07	0.03	0.02	0.05	0.03	0.03	0.06	0.08	0.14	0.38
Nig_ZainJ3	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Ind_ KU_NI04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Ind_PsADMC08	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37
Iran_IAUK1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37
Iraq_ZHSAHD-1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
WUSC-05_h02_1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Nig_ZainJ1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.13	0.36
Nig_ZainJ6	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Nig_ZainJ5	0.00	0.00	0.00	0.00	0	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Ind_PsADMC10	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.13	0.36
Ind-PsADMC06	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Nig_ZainJ4		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Nig_ZainJ2	0.00		0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36
Nig_SAUTHC7	0.00	0.00		0.00	0.00	0.01	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36

Figure 4a(ii) Distance matric

3.5. Distance Matric / Heatmap

The darker the areas, the smaller the distance between the organisms matched and the closer their identity (figure 4b).



	Spa_...	Ind_...	Iraq_...	Jap_...	Tha_...	Ind_...	Ind_...	Tha_...	Iraq_...	Par_...	Nig_...	Car_...	Ind_...	Ind_...	Egy_...	Sau_...	Sau_...	Sau_...	Sau_...
Spa_SE1		0.01	0.02	0.02	0.02	0.11	0.07	0.03	0.02	0.05	0.03	0.03	0.06	0.08	0.14	0.38	0.23	0.21	0.20
Ind_PsTW DMC 2...	0.01		0.02	0.03	0.02	0.12	0.07	0.03	0.03	0.05	0.03	0.04	0.06	0.08	0.15	0.39	0.23	0.22	0.21
Spa_CCUG 63225	0.02	0.02	0.03	0.03	0.03	0.12	0.08	0.04	0.03	0.06	0.04	0.04	0.07	0.09	0.15	0.39	0.24	0.22	0.21
Ind_PsADMC09	0.06	0.07	0.04	0.05	0.04	0.14	0.09	0.05	0.05	0.07	0.05	0.06	0.08	0.11	0.17	0.41	0.25	0.24	0.23
Jap_TUM18999	0.02	0.03	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.07	0.13	0.37	0.22	0.20	0.19
Ind_PsADMC07	0.03	0.03	0.01	0.01	0.01	0.10	0.06	0.02	0.01	0.04	0.02	0.02	0.04	0.07	0.13	0.37	0.22	0.20	0.19
Ind_PsADMC02	0.02	0.03	0.01	0.01	0.01	0.10	0.05	0.02	0.01	0.04	0.01	0.02	0.04	0.07	0.13	0.37	0.22	0.20	0.19
Ind_PsOT DMC12...	0.02	0.03	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37	0.21	0.20	0.19
Ind_PsADMC04	0.02	0.03	0.00	0.01	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.07	0.13	0.37	0.22	0.20	0.19
Ind_NRC47	0.04	0.04	0.02	0.02	0.02	0.11	0.07	0.03	0.02	0.05	0.03	0.03	0.06	0.08	0.14	0.38	0.23	0.21	0.20
Nig_ZainJ3	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Ind_ KU_NI04	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Ind_PsADMC08	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37	0.21	0.20	0.18
Iran_IAUK1	0.02	0.03	0.00	0.00	0.00	0.10	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37	0.21	0.20	0.18
Iraq_ZHSAHD-1	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
WUSC-05_h02_1	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Nig_ZainJ1	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.13	0.36	0.21	0.20	0.18
Nig_ZainJ6	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Nig_ZainJ5	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Ind_PsADMC10	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.13	0.36	0.21	0.19	0.18
Ind-PsADMC06	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Nig_ZainJ4	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Nig_ZainJ2	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Nig_SAUTHC7	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Iraq_ZHSAHD-2	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Iraq_ZHSAHD-4	0.02	0.02	0.00	0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Egy_PS31	0.02	0.03	0.01	0.01	0.01	0.10	0.05	0.02	0.01	0.03	0.01	0.02	0.04	0.07	0.13	0.37	0.22	0.20	0.19
Iraq_ZHSAHD-3	0.02	0.02		0.00	0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.12	0.36	0.21	0.19	0.18
Jap_Hugh1066	0.02	0.03	0.00		0.00	0.09	0.05	0.01	0.01	0.03	0.01	0.02	0.04	0.06	0.13	0.37	0.21	0.20	0.18
Tha_03-SYBR-2019	0.02	0.02	0.00	0.00		0.09	0.05	0.01	0.01	0.03	0.01	0.01	0.04	0.06	0.13	0.36	0.21	0.20	0.18
Ind_PsADMC01	0.11	0.12	0.09	0.09	0.09		0.08	0.10	0.10	0.12	0.10	0.11	0.13	0.15	0.22	0.46	0.30	0.29	0.28

Figure 4b Distance Matric/ Heatmap

4. Conclusion

Pseudomonas aeruginosa is a highly clinically relevant bacteria which is quite notorious globally possessing much virulent genes, as well as known for multi-drug resistance.

There is a trend of diversity in the strains of *Pseudomonas aeruginosa*, such that while some are at the ancestral level maintaining the virulent and other pathogenicity capabilities, most strains have shown a lot of diversity over time owing to mutations which result in change in nucleic acid bases (deletion, insertion, etc.). This change of genetic type overtime results in enhanced pathogenicity due to acquisition of new genetic factors or multi-drug resistance. It may also result in reduced pathogenicity.

From this study so far, it could be seen that *Pseudomonas aeruginosa* strains isolated from clinical samples in Nigeria are not so much different from those from India, Iraq, Iran, Egypt, Washington, and Japan in terms of evolution. This implies that similar trends of diversity are obtainable.

However, when compared to the strains from Saudi Arabia, those in Nigeria have closer similarity to their ancestor (2nd parent) while those from Saudi Arabia have so diversified from their ancestor, being found in the 10th generation. This could imply much developed pathogenicity capabilities or /and multi-drug resistance.

Recommendation

There is need to explore the pathogenicity genes by these organisms using more robust bioinformatics techniques to unravel the mutations and pathogenic genes, as well as the mechanism will enable adequate research for pharmaceutical interventions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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