

Evolutionary Insight into Global Multi-drug resistance *S. aureus* Strains isolated from blood: A 16S rRNA- Based Comparative Study of Resistance-Linked Divergence

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Abstract -

Background: The global burden of antimicrobial resistance (AMR) is rising, and *S.aureus* is a key pathogen implicated. *Staphylococcus aureus* (*S. aureus*), an opportunistic pathogen, is a serious human and livestock pathogen responsible for life-threatening infectious diseases. Understanding the evolutionary dynamics of resistance-related genes in *S. aureus* across regions can support better surveillance and intervention strategies that could help mitigate the risk of new strains emerging, reduce morbidity and mortality.

Objective: This study aims to investigate the evolutionary relationships and phylogeographic patterns of multidrug-resistant *S. aureus* strains using 16S rRNA gene sequences isolated from blood of human, other vertebrates and abiotic habitat (river) in different countries of the globe.

Materials and Methods: The steps involved in conducting a phylogenetic analysis of the 16S RNA gene include Sequence Data Retrieval, Sequence Alignment, Tree construction, and subsequent Evolutionary analysis of the resulting phylogenetic tree.

Results: Seventy-nine internal transcribed spacer sequence of *Staphylococcus aureus* strains from blood were retrieved from publicly available database NCBI. The Sequences were selected based on the representation in different parts of the world majorly from human blood. Phylo-evolutionary diversity tree between the *S.aueus* strains show some levels of divergence and similarities as shown from the bootstrap values and generational distance. The *S.aureus* strains isolated from clinical samples in Nigeria maintained a genetic integrity of the root ancestor of *S.aureus* spp.

Conclusion: There is a trend of diversity in the strains of *S.aureus* genetic sequence overtime which may result in enhanced or reduced pathogenicity and AMR

Keywords: Evolutionary Insight; antimicrobial resistance; *S. aureus*; 16S rRNA-Based Comparative Study; phylogenetics

I. INTRODUCTION

Staphylococcus aureus (*S. aureus*) , an opportunistic pathogen, is a serious human and livestock pathogen responsible for life-threatening septicemia, urinary tract infection, surgery and wound infections, respiratory, and gastrointestinal diseases. Sepsis particularly bactericaemia, is an overwhelming and life-threatening response to a spectrum of disorders that result from infection by bacteria, viruses, fungi, parasites or their toxic products[1]. It is a leading cause of morbidity and mortality in the neonatal intensive care unit globally particularly in low-medium-income countries with an estimated 3.9 million cases and 15% of all neonatal deaths each year [2-3]. *S.aureus* is one of the major causative agents of sepsis.

It also causes economically important mastitis in livestock [4]. *Staphylococcus aureus* is a bacterium that has evolved to become highly adaptable, displaying significant evolutionary trends across human, other vertebrates and even abiotic (such as River) hosts. These trends include host-jumping, the acquisition and loss of genes, particularly those on mobile genetic elements, and the development of

antibiotic resistance. *S. aureus* has demonstrated an ability to jump between various host species, including humans, livestock, and recreation centers such as rivers, swimming pools etc. These jumps are often linked to the acquisition or loss of host-specific genes, frequently found on mobile genetic elements. These evolutionary trends underscore the remarkable adaptability of *S. aureus* and its capacity to evolve in response to selective pressures from both host environments and antimicrobial interventions [5]. This compounds the problem leading to treatment failures, complications, increased morbidity and mortality [6]. Therefore, in order to have adequate information for treatment and control of *S. aureus* infections, it is crucial to understand the antibiotic-resistance gene, the occurrence and changes in types of *S. aureus* strains, and their geographic spread. This therefore, calls for an urgent need for an extensive and inclusive study to identify the evolutionary trends of this bacteria not just by geographical location, but also by different hosts and habitat specifics across the globe. Thus, the need for this study.

The global burden of antimicrobial resistance (AMR) is rising, and *S. aureus* is a key pathogen implicated [6]. Understanding the evolutionary dynamics of resistance-related genes in *S. aureus* across regions can support better surveillance and intervention strategies that could help mitigate the risk of new strains emerging, reduce morbidity and mortality [6]. Compared to other pathogens, *S. aureus* offers extensive genomic resources, making it a powerful model for evolutionary AMR studies. The 16S rRNA gene is universally conserved in bacteria and widely used for phylogenetic analysis[7]. In *S. aureus*, it enables strain-level resolution and can be used to trace evolutionary divergence, especially when complemented with metadata like resistance phenotypes or geographic origin.

This study investigated the evolutionary relationships and phylogeographic patterns of multidrug-resistant *S. aureus* strains using 16S rRNA gene sequences isolated from blood of human, other vertebrates and abiotic habitat (river) in different countries of the globe. These strains were grouped based on resistance profile, host and geographical source in order to evaluate evolutionary divergence and clustering patterns. Understanding these evolutionary trends is crucial for developing effective strategies to prevent and treat *S. aureus* infections.

II. RESEARCH ELABORATIONS/METHOD

MOLECULAR IDENTIFICATION AND CHARACTERIZATION MATERIALS AND METHOD

This section explains the methodological approaches, process, techniques, and tools used to conduct this study and show how all the stated key objectives of this study have been achieved. In accomplishing the research aim, this section also explains the different steps and activities that is involved in achieving the objective of the study. The steps involved in conducting a phylogenetic analysis of the RMG7 gene include Sequence Data Retrieval, Sequence Alignment, Tree construction, and subsequent Evolutionary analysis of the resulting phylogenetic tree.

Bacterial Strains

Seventy-nine *S. aureus* strains isolated from different biotic sources including human, bovine, ovine, avine and abiotic (river) were studied (Table 1). The strains were selected to represent the most abundant lineages identified by a population genetic study of 2,077 *S. aureus* isolates from worldwide sources [7].

The bovine and ovine strains were selected to represent the most abundant *S. aureus* lineages associated with intramammary infection in diverse localities [5]. Abiotic source (water) was selected to identify the most abundant water *S. aureus* lineages.

SEQUENCE DATA RETRIEVAL AND DATA PREPARATION

Seventy nine (79) ITS sequence of *Staphylococcus aureus* strains from blood were retrieved from publicly available database NCBI (<https://www.ncbi.nlm.nih.gov>)[10] on 20 May 2025 to investigate its evolution. The Sequences were selected based on the representation in different parts of the world majorly from human blood. 3 of these sequences were from Nigeria (KY290735; KY290730; KY290723) and were from humans, 14 were from Egypt starting from PP693439 followed by PP693432 and ended with MG966418 isolated from homo-sapiens. 17 were from Iraq (2 from sheep, goat, cow, 1 from buffalo and 10 from humans), 22 were from human blood in Saudi Arabia with accession number starting from PP064195 followed by PP064194 and lastly PP064175; 7 were from India -1 from Labeo rohita blood, Gomti River

water, *Prionailurus bengalensis* -heart blood each and 4 from humans). 6 were from humans in Pakistan, one was from China (JF746996,), Iran (PQ471556)

and Spain (PQ845015), 7 were from unknown locations (Table 1).

Table 1:Metadata of *S.aureus* strains from different parts of the globe

	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq.type
1	LC798346	<i>S. aureus</i>	HD5 gene	742 bp	Iraq (Nineveh)	blood	2024-01-02	Homo sapiens	Partial seq.
2	LC798345	<i>S.aureus</i>	DH5 gene	930 bp	Iraq: Mosul	blood	08-FEB-2024	Homo sapiens	Partial seq.
3	LC798344	<i>S.aureus</i>	HD4 gene	996 bp	Iraq: Mosul	blood	2024-01-02	Homo sapiens	Partial seq.
4	LC798343	<i>S.aureus</i>	DH4 gene	989 bp		Blood	2024-01-02	Buffalo	Partial seq.
5	LC798342	<i>S.aureus</i>	HD3 gene	779 bp	Iraq: Mosul	blood	2024-01-02	Sheep	Partial seq.
6	LC798341	<i>S.aureus</i>	DH3 gene	782 bp	Iraq: Mosul	Blood	2024-01-02	Sheep	Partial seq.
7	LC798340	<i>S.aureus</i>	HD2 gene	645 bp	Iraq: Mosul	Blood	2024-01-02	Cow	Partial seq.
8	LC798339	<i>S.aureus</i>	DH2	854 bp	Iraq: Mosul	blood	2024-01-02	Cow	Partial seq.
9	LC798338	<i>S.aureus</i>	HD1	500 bp	Iraq: Mosul	blood	2024-01-02	Goat	Partial seq.
10	LC798337	<i>S.aureus</i>	DH1	652 bp	Iraq: Mosul"	blood	2024-01-02	Goat	Partial seq.
	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq.type
1	JF7469196	<i>S.aureus</i>	PW2062	1276 bp	Hong Kong SAR, China	blood	30-MAR-2011	human	Partial seq.
1	PV639087	<i>S.aureus</i>	S2	667 bp	Pradesh 175021, India	BLOOD	01-Nov-2024	human	Partial seq.
1	PV173443	<i>S.aureus</i>	F21	311 bp	Iraq	blood	10-Oct-2015	Human	Partial seq.

14	PQ896951	S.aureus	OS	1552 bp	Iraq: Erbil	blood	Sep-2024	human	Partial seq.
15	PP693439	S.aureus	53Staph	809 bp	Egypt	blood	28-Dec-2022	Homo sapiens	Partial seq.
16	PP693432	S.aureus		809 bp	Egypt	blood	12-Nov-2022	Homo sapiens blood	Partial seq.
17	PP693430	S.aureus	2-Staph	809 bp	Egypt	blood	22-Oct-2022	Homo sapiens -	Partial seq.
18	PP494167	S.aureus	NT42	779 bp	Egypt	blood	20-Oct-2022	Homo sapiens -	Partial seq.
19	PP494166	S.aureus	NT41	858 bp	Egypt	blood	20-Oct-2022	Homo sapiens -	Partial seq.
20	PP494165	S.aureus	NT27	1413 bp	Egypt	blood	20-Oct-2022	Homo sapiens -	Partial seq.
21	OR458316	S.aureus	NT4	809 bp	Cairo	Blood	Nov-2021	Homo sapiens	Partial seq.
	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq. type
22	OR458313	S.aureus	NT1	809 bp	Cairo	Blood	Nov-2021	Homo sapiens -	Partial seq.
23	MZ144011	S.aureus	SA01	1555 bp		blood	12-MAY-2021	Homo sapiens -	Partial seq.
24	MN515405	S.aureus	NRLFFD288	1439	India	blood	01-Mar-2019	Labeo rohita -	Partial seq.
25	MK418963	S.aureus	KH05E	600 bp	India	blood	28-Nov-2018	Homo sapiens-	Partial seq.
26	MK346271	S.aureus	KH04D	715	India	blood	02-Oct-2018"	Homo sapiens-	Partial seq.
27	MH383083	S.aureus	YR1	1474	Pakistan	blood	01-Feb-2018	Homo sapiens-	Partial seq.

2		S.aure	Bz51	1463	India	River	17-	Gomti	Partia
8	KC538 823	us				water	MAR- 2013		l seq.
2	PQ845	S.aure	MBC073-	1490	Spain:				Partia
9	015	us	SAUneg-16S	bp	Granad a				l seq.
3	PQ471	S.aure	MS 24	1390	west of	blood	22-		Partia
0	556	us		bp	Iran		OCT- 2024		l seq.
3	PQ168	S.aure	NS2	589	India:	blood	Feb-		Partia
1	959	us		bp	Madura i		2024		l seq.
	Asserti on number	Organ ism type	Strains	Seque nce length	Region	speci men	Date of collect ion	Host	Seq.t ype
3	PP9078	S.aure	AH-2	1496	Egypt:	blood	15- Jan- 2024	Human	Partia
2	37	us		bp	Cairo				l seq.
3	PP9078	S.aure	AH-1	1422	Egypt:	blood	15- Jan- 2024	Human	Partia
3	35	us		bp					l seq.
3	PP0641	S.aure	Rhd-21	763	Saudi	blood	2021	Human	Partia
4	95	us		bp	Arabia				l seq.
3	PP0641	S.aure	Rhd-20	763	Saudi	blood	2021	Human	Partia
5	94	us		bp	Arabia				l seq.
3	PP0641	S.aure	Rhd-19	763	Saudi	blood	2021	Human	Partia
6	93	us		bp	Arabia				l seq.
3	PP0641	S.aure	Rhd-18	763	Saudi	blood	2020	Human	Partia
7	92	us		bp	Arabia				l seq.
3	PP0641	S.aure	Rhd-17	763	Saudi	blood	2020	Human	Partia
8	91	us		bp	Arabia				l seq.
3	PP0641	S.aure	Rhd-16	763	Saudi	blood	2019	Human	Partia
9	90	us		bp	Arabia				l seq.
4	PP0641	S.aure	Rhd-15	763	Saudi	blood	2019	Human	Partia
0	89	us		bp	Arabia				l seq.
4	PP0641	S.aure	Rhd-14	763	Saudi	blood	2019	Human	Partia
1	88	us		bp	Arabia				l seq.
4	PP0641	S.aure	Rhd-13	763	Saudi	blood	2019	Human	Partia
2	87	us		bp	Arabia				l seq.

	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq. type
43	PP064186	S.aureus	Rhd-12	763 bp	Saudi Arabia	blood	2019	Human	Partial seq.
44	PP064185	S.aureus	Rhd-11	763 bp	Saudi Arabia	blood	2018	Human	Partial seq.
45	PP064184	S.aureus	Rhd-10	763 bp	Saudi Arabia	blood	2018	Human	Partial seq.
46	PP064183	S.aureus	Rhd-09	763 bp	Saudi Arabia	blood	2018	human	Partial seq.
47	PP064182	S.aureus	Rhd-08	763 bp	Saudi Arabia	blood	2018	human	Partial seq.
48	PP064181	S.aureus	Rhd-07	763 bp	Saudi Arabia	blood	2018	human	Partial seq.
49	PP064180	S.aureus	Rhd-06	763 bp	Saudi Arabia	blood	2018	Human	Partial seq.
50	PP064179	S.aureus	Rhd-05	763 bp	Saudi Arabia	blood	2017	Human	Partial seq.
51	PP064178	S.aureus	Rhd-04	763 bp	Saudi Arabia	blood	2017	Human	Partial seq.
52	PP064177	S.aureus	Rhd-03	763 bp	Saudi Arabia	blood	2017	Human	Partial seq.
	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq. type
53	PP064176	S.aureus	Rhd-02		Saudi Arabia	blood	2017	Human	Partial seq.
54	PP064175	S.aureus	Rhd-01		Saudi Arabia	blood	2017	Human	Partial seq.
55	OR826672	S.aureus	B1	807 bp	Pakistan: Balochistan	blood		Human	Partial seq.
56	OR512168	S.aureus	Staph.TH	1149 bp	Iraq	blood	20-Sep-2022	Human	Partial seq.
57	OP809704	S.aureus	Abu_Elghait_Staph	1434 bp	Egypt	blood		Human	Partial seq.

58	OP809639	S.aureus	Abu_Elghait	1431 bp	Egypt	blood	16-NOV-2022	Human	Partial seq.
59	OP364883	S.aureus	SA1	1500 bp	Pakistan	blood	12-SEP-2022	Human	Partial seq.
60	OL344097	S.aureus	HB104	1476 bp	Egypt	blood	09-NOV-2021	Human	Partial seq.
61	OK090930	S.aureus	21-342MR	1149 bp	Pakistan	blood		human	Partial seq.
	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq. type
62	MN310030	S.aureus	CWH-19	502 bp	India	heart blood	04-Jun-2019	Prionailurus bengalensis	Partial seq.
63	MK690923	S.aureus	Blood-No.5	706 bp	Iraq	blood	31-MAR-2019	Human	Partial seq.
64	MK690922	S.aureus	Blood-No.4	707 bp	Iraq	blood	31-MAR-2019	Human	Partial seq.
65	MK690921	S.aureus	Blood-No.3	705 bp	Iraq	blood	31-MAR-2019	Human	Partial seq.
66	MK690920	S.aureus	Blood-No.2	704 bp	Iraq	blood	31-MAR-2019	Human	Partial seq.
67	MK690919	S.aureus	Blood-No.1	707	Iraq	blood	31-MAR-2019	Human	Partial seq.
68	MG966418	S.aureus	AS2	1428	Egypt	blood	08-Nov-2017	Human	Partial seq.
69	KY290735	S.aureus	BAC_C22	361 bp	Nigeria	blood	2016	Human	Partial seq.
70	KY290730	S.aureus	BAC_C15 BAC_C6	449 bp	Nigeria	blood	27-Aug-2016	Human	Partial seq.
71	KY290723	S.aureus	BAC_C6	598 bp	Nigeria	blood	30-Jul-2016	Human	Partial seq.

	Assertion number	Organism type	Strains	Sequence length	Region	specimen	Date of collection	Host	Seq. type
7	JQ8632	S.aureus	IARS-4	447 bp	Pakistan	blood culture	29-Aug-2011	Human	Partial seq.
7	JQ3222	S.aureus	DSPV 004PSA	1410 bp		blood		slaughterhouse avian	Partial seq.
7	HM061	S.aureus	KIBGE-MB04	791 bp		blood		Human	Partial seq.
7	HM061	S.aureus	KIBGE-MB03	429 bp		blood		Human	Partial seq.
7	HM061	S.aureus	KIBGE-MB02	410 bp		blood		Human	Partial seq.
7	HM061	S.aureus	KIBGE-MB01	802 bp		blood		Human	Partial seq.
7	EU603	S.aureus	XJTUMS25	1366 bp		blood		Human	Partial seq.
7	EU603	S.aureus	XJTUMS2	1362 bp		blood		Human	Partial seq.

MULTIPLE SEQUENCE ALIGNMENT

Sequence alignment of the 16s rRNA gene involves comparing the nucleotide or amino acid sequence of the gene from different *Staphylococcus aureus* strains or accessions to identify similarities and differences. There are multiple processes involved in performing a sequence alignment of the 16s rRNA gene from different *Staphylococcus aureus* strains. By comparing and contrasting DNA sequences, sequence alignment can reveal information about the evolutionary links or functional conservation of the gene. The retrieved data was then uploaded to the Geneious software (<https://www.geneious.com>) for sequence alignment and a sequence alignment tool (such as muscle, geneious aligner, or cluster) was used for the alignment. An appropriate sequence alignment method was selected based on the type of sequence (DNA) and the expected level of conservation in the 16s rRNA gene. The sequence alignment was done using MUSCLE alignment at Re-align sequences with 8 maximum number of Iterations(<https://www.ebi.ac.uk/Tools/services/rest/muscle>). The alignment algorithm was employed to compare the sequences and identify regions of similarity or conservation.

The generated alignment was examined to identify conserved regions, insertions, deletions and variations among the sequences. Conserved regions indicate important functional elements of the genes, while variations may represent potential differences in disease resistance.

III. RESULT AND DISCUSSIONS

Comparative Sequence Analysis

Sequence Alignment

The output of the alignment was a multiple sequence alignment (MSA), which shows the aligned sequences with gaps introduced to maximize the similarity. The consensus identity view is shown in Fig. 1. Comparative Sequence Alignments Figure 1 shows the alignment views of the sequence data. Gaps (represented by dashes) were displayed where sequences have been modified to improve alignment). 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.

KY290730 and KY290735 from Nigeria, have the same nucleotide sequence with gap at base 10. Both have 361 and 446 base pairs respectively and 10 gaps. They have points of similarities from nucleotide base 80 to 514 with strains : PP064182.(Rhd-08), PP064183 (Rhd-09) , PP064190 (Rhd-16) , PP064177 (Rhd-03), PP064178 (Rhd-04) PP064181, (Rhd-07) PP064185,(Rhd-11) PP064184 (Rhd-10) PP064189 (Rhd-15), PP064191,(Rhd-17) PP064188, (Rhd-14)PP064175,(Rhd-01) PP064192,(Rhd-18) all from human blood samples from Saudi Arabia and MG966418, (AS2) from Egypt, EU603705 (XJTUMS25)), EU603682 (XJTUMS2) both from blood samples of an undisclosed location (Figure 1 & 2).

The sample KY290723, from Nigeria, has similarities with OP809704 (Abu_Elghait_Staph), OP809639,(Abu_Elghait) from human blood samples from Egypt, LC798338, (HD1) goat blood sample from Iraq and dissimilarities with the other strains (Figure 1 & 2).

This shows a spread of this organism from country to country and from zoonotic source . This can be the reason for antimicrobial resistance that have been reported as on the increase in Nigeria[8].

LC798337 (DH1) a strain from goat blood in Iraq expressed high level of difference with other strains though it expressed a relatively slight similarity with LC798344 (HD4) which is a strain from human sample from Iraq (figures 1 and 2).

These were further confirmed using the phylogenetic tree (figure 3).

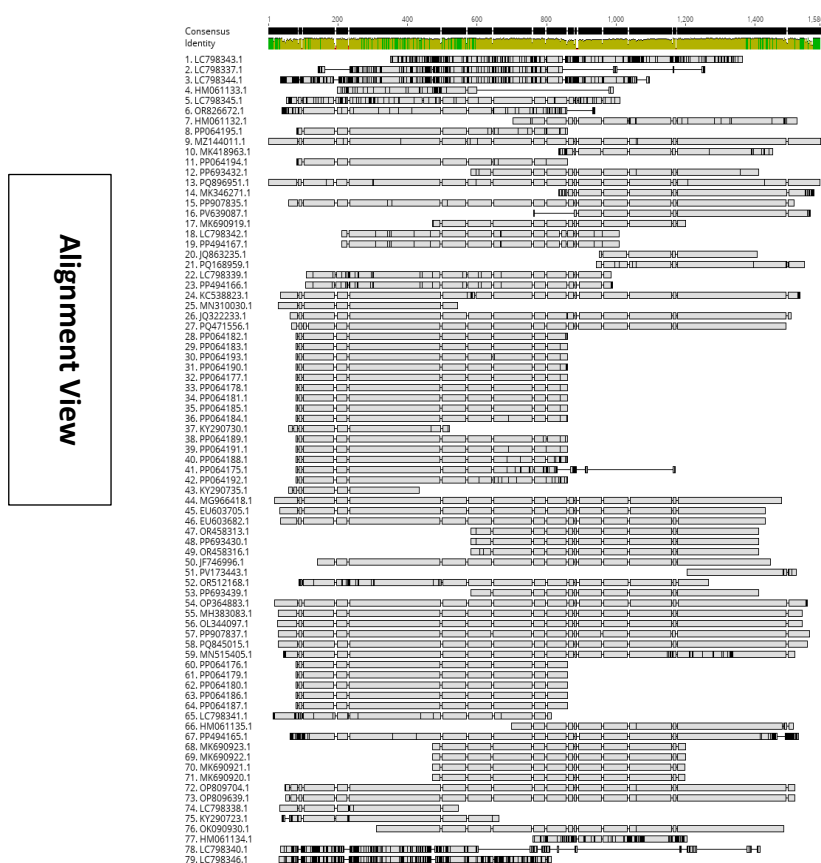


Figure1 ... show the sequence alignment view, the areas of green colouration represent sites with 100% identity, green-brown represents sites with at $\geq 30 < 100\%$, and red represents sites with less than 30%

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 and epidemiological relevance.

1	10	20	30	40	50	60

LC798343.1 -----
LC798337.1 -----
LC798344.1 -----AAACGGGCGGCAGGCTTACACATGCA
HM061133.1 -----
LC798345.1 -----ACAATGCA
OR826672.1 -----GGCATGGGGCGGCCTACAATGC
HM061132.1 -----
PP064195.1 -----
MZ144011.1 -----
TTTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA
MK418963.1 -----
PP064194.1 -----
PP693432.1 -----
PQ896951.1 -----
TTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA
MK346271.1 -----
PP907835.1 -----GCA
PV639087.1 -----
MK690919.1 -----
LC798342.1 -----
PP494167.1 -----
JQ863235.1 -----
PQ168959.1 -----
LC798339.1 -----
PP494166.1 -----
KC538823.1 -----ACGCTGGCGGCGTGCCTAATACATGCA
MN310030.1 -----GGATGAACGCTGGCGGCGTGCCTAATACATGCA
JQ322233.1 -----
PQ471556.1 -----
PP064182.1 -----
PP064183.1 -----
PP064193.1 -----
PP064190.1 -----
PP064177.1 -----
PP064178.1 -----
PP064181.1 -----
PP064185.1 -----
PP064184.1 -----
KY290730.1 -----TGC
PP064189.1 -----
PP064191.1 -----
PP064188.1 -----
PP064175.1 -----
PP064192.1 -----
KY290735.1 -----GC
MG966418.1 -----ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA
EU603705.1 -----GAACGCTGGCGGCGTGCCTAATACATGCA
EU603682.1 -----GCTGGCGGCGTGCCTAATACATGCA
OR458313.1 -----
PP693430.1 -----
OR458316.1 -----
JF746996.1 -----

PV173443.1 -----
OR512168.1 -----
PP693439.1 -----
OP364883.1 -----ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA
MH383083.1 -----GATGAACGCTGGCGGCGTGCCTAATACATGCA
OL344097.1 -----AGGATGAACGCTGGCGGCGTGCCTAATACATGCA
PP907837.1 -----GATGAACGCTGGCGGCGTGCCTAATACATGCA
PQ845015.1 -----GGATGAACGCTGGCGGCGTGCCTAATACATGCA
MN515405.1 -----CGGCGTGCTATACATGCA
PP064176.1 -----
PP064179.1 -----
PP064180.1 -----
PP064186.1 -----
PP064187.1 -----
LC798341.1 -----TTTAATTCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA
HM061135.1 -----
PP494165.1 -----
MK690923.1 -----
MK690922.1 -----
MK690921.1 -----
MK690920.1 -----
OP809704.1 -----CTGCTATACATGCA
OP809639.1 -----CTATACATGCA
LC798338.1 -----GAACGCTGGCGGCGTGCCTAATACATGCA
KY290723.1 -----GCAATCTGC-----
OK090930.1 -----
HM061134.1 -----
LC798340.1 -----ACGCTGGCGGCATGCCTTACACATGCA
LC798346.1 -----TTGAACGCTGGCGGCATGCCTTACACATGCA

LC798343.1 -----
LC798337.1 -----
LC798344.1 AGTCGAGCGGTAGCACAGGGGAGCTTGCTCC-
CTGGGTGACGAGCGGCGGACGGGTGAGT
HM061133.1 -----
LC798345.1 GTCGAGCGGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTACGCGGCGGAGAGGTGAGT
OR826672.1 AGTCGAGCG-ACGGACGATAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
HM061132.1 -----
PP064195.1 -----CAACTTGCTTCTCTGA--TGTTAGCGGCGGACGGGTGAGT
MZ144011.1 AGTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
MK418963.1 -----
PP064194.1 -----AAACTTGCTTCTCTGA--TGTTAGCGGCGGACGGGTGAGT
PP693432.1 -----
PQ896951.1 AGTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
MK346271.1 -----
PP907835.1 AGTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
PV639087.1 -----

MK690919.1 -----
LC798342.1 -----
PP494167.1 -----
JQ863235.1 -----
PQ168959.1 -----
LC798339.1 -----GGACGGGTGAGT
PP494166.1 -----CGGACGGGTGAGT
KC538823.1 AGTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
MN310030.1 AGTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
JQ322233.1 -GTCGAGCGAACGGACGAGAAGCTT-GCTTCTCTGA--
TGTTAGCGGCGGACGGGTGAGT
PQ471556.1 -----GCGAACGGACGAGAAGCTT-GCTTCTCTGA--TGTTAGCGGCGGA-GGGTGAGT
PP064182.1 -----AAGCTT-GCTTCTCTGA--TGTTAGCGGCGGACGGGTGAGT
PP064183.1 -----AAGCTT-GCTTCTCTGA--TGTTAGCGGCGGACGGGTGAGT
PP064193.1 -----AAGCTT-GCTTCTCTGA--TGTTAGCGGCGGACGGGTGAGT
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OR458316.1 -----
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PP064188.1
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PP064175.1
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PP064192.1
GCAGCCGCGGTAATACGTAGGTGGCAAGCG-TTATCCGGAATTATTGGGCGTAAAGC...
KY290735.1 -----...
MG966418.1
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EU603705.1
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PP064187.1
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LC798341.1
GCAGCCGCGGTAATACGTAGGTGGCAAGCG-TTATCCGGAATTATTGGGCGTAAAGC...
HM061135.1 -----...
PP494165.1
GCAGCCGCGGTAATACGTAGGTGGCAAGCG-TTATCCGGAATTATTGGGCGTAAAGC...
MK690923.1
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MK690922.1
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MK690920.1
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OP809639.1
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LC798338.1 GCA-----...
KY290723.1
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OK090930.1
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HM061134.1 -----...
LC798340.1
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LC798346.1
GCAGCCGCGGTAATACGTAGGGCGCGAGCG-TTGTCCGGAATTATTGGGCGTAAAGG...

This alignment is very long (1,586 x 79). Only first first 597 columns are shown.

Trees are too large to display in text format. If you want the Newick representation of these trees, use File->Export.

S.aureus XJTUMS2

1	10	20	30	40	50
GCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTT					
GCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTAC					
CTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATAT					
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CACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGG					
GCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATC					
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CTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCG					
CGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCG					
CGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTG					
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GCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGAC					
TACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAA					
TACGTTCCCGGG					

S. aureus XJTUMS25

1	10	20	30	40	50
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CTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATA					
ATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTT					
ATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA					
AGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAAGT					
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA					
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GATCGTAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGC					
ACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCA					
GCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAA					
AGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAAC					
CGTGGAGGGTCATTGGAAGTGGAAAACCTTGAGTGCAGAAGAGGAAAGTG					
GAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGT					

GGCGAAGGCGACTTTCTGGTCTGTAAGTACGCTGATGTGCGAAAGCGTG
GGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
TGCTAAGTGTTAGGGGGTTCCGCCCTTAGTGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTG
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GAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGC
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TCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACT
CGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGG
TGAATACGTTCCCGGG

S. aureus KIBGE-MB01

1	10	20	30	40	50

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CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
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ACGTGCTACAATGGATAATACAAAGGGCAGCGAACCCGCGAGGTCAAGCA
AATCCCATAAAATTATTCTCAGTTCGGATTGTAGTCTGCAACTCGACTAC
ATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATAC
GTTCCCGGGTCTTGTAACACCCGCCGTCACCCACGAGAGTTTGTAACA
CCCGAAGCCGGTGGCGTTCCTTTTATTAGGAGCTAGCCGTCGAAGGTGGG
AC

S. aureus KIBGE-MB02

1	10	20	30	40	50

ACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGG
ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG
GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
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AACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTT
GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG
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GCAGGTGGTT

S. aureus KIBGE-MB03

1	10	20	30	40	50

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CAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGTTCTCGTTCGCCGTAGAGATACGGTTTCCCCTTTGGG
GCGGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGT
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GTGATGGTGGGACTCATGGGAGACTGCC

S. aureus KIBGE-MB04

1 10 20 30 40 50
| | | | | |
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GAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCTTT
CCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
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S. aureus PW2062

1 10 20 30 40 50
| | | | | |
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GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCG
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CACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGC
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CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATA
CGTTCCCGGTCTTGTACACACCGCC

Tree construction and evolutionary analysis

After sequence alignment, we carried out phylogenetic analysis using the Tamura-Nei using MEGA 5.2 [(molecular evolutionary genetic analysis -12) [9-11] as the genetic distant model and neighbor joining with a bootstrap value of 1000 and 100 number of replicates to indicate the evolutionary process analyzed over time. It was then edited with the geneious tree builder version 9.0.5 to determine the evolutionary relatedness and divergence. Comparative analysis with a distance matrix of the tree was performed on Geneious (<https://www.geneious.com>) based on the statistical analysis to determine positions of significant differences between the samples.

The tree (Figure 3a & b) is an illustration of phylo-evolutionary diversity between the *S.aueus* strains. The tree has 15 branches. There were 4 major clusters (clades) identified by their most recent ancestor, divergence and bootstrap value. Cluster one consist of 2 strains (BAC C15 & BAC C22) from Nigeria with bootstrap values of 100% similarity with the root ancestor (node 156, figure 2a).

This denotes maintained genetic integrity among *S. aureus* strains in Nigeria. Thus, in terms of genetic stability, the strains from Nigeria were true to typical *S. aureus* species that have not acquired any or much resistance or virulent genes. Virulence and antibiotic resistance are two pathogenic armaments of *S.aureus*. Virulence develops from the time of host colonization while antibiotic resistance developed in the course of antibiotics [12]. Both are controlled by many internal and acquired genetic factors. However, this is contrary to the recent report on the increasing trends of antibiotic resistance strains of *S.aureus* in Nigeria [8, 13]. This discrepancy may be due to time difference. The samples downloaded fr[8]om NCBI [10] for this study with regards to Nigeria were collected and submitted between July and August 2016 [12]. Thus, there is a need to carry out a more recent wet laboratory study on the molecular and genetic characteristics of *S. aureus* in Nigeria. This

will provide an up to date information on the evolutionary trend in Nigeria. This is much more important in the face of the global rise in AMR strains. This can be applied in epidemiology study and surveillance to track antibiotic resistance or susceptibility genes in *S.aureus* infections and may influence accurate emperic choice of antibiotics for the treatment particularly in neonates, thereby helping to reduce morbidity and mortality due to *S. aureus*.

Cluster 2 consist of strains starting from KBGE MB102 F isolated from human blood from an undisclosed location to Bz51 isolated from Gomti River in India which was closely followed by NRLFFD288 isolated from Labeo rohita in India with 100% similarity to the most recent ancestor of that generation (node 154, figure 3a). This depicts similarity in the ancestral origin of these strains though they were from different host and habitat. The much closeness of Bz51 (distance, 0.0032) isolated from Gomti River and NRLFFD288 (distance=0.0056) isolated from Labeo rohita both in India and from human blood in Egypt (distance 0.0025) could suggest preservation of genetic material and cross-infection of this pathogen from other vertebrate and inanimate (River) to humans and vice versa. Also, their closeness with strains isolated from human blood from Saudi Arabia, Pakistan, Iraq (distance value /branch length = 0.0000) and sheep blood from Iraq further confirms preservation of genetic material and cross-infection of this pathogen from animals and abiotic habitat (River) to humans. The values indicate strong statistical support for high similarity and suggests closely related strains or low divergence. The similarities in variation patterns suggest evolutionary relatedness in functional characteristics[12].

Cluster 3 contains majority of the strains. It consist of strains DH5 (LC798345) (node 82, figure 3a) isolated from human samples in Iraq to strain OS (PQ896951) (node 152 , figure 3a)isolated from human blood in Iraq with 69% (bootstrap value,

figure 3b) similarity to the most recent ancestor (node 153, figure 3a). This depicts significant level of differences between the strains and connote significant deviation from the original genetic make-up of *S. aureus*. This signifies mutation in the genetic 16srRNA genes of this pathogen, which ordinarily is a conserved gene. The branch length ranged from 0.000 to 39.769. This could suggest evolution of new sequences. The high mutations could be linked to much deviation from ancestral origin as the initial genetic makeup known for *S. aureus* changed overtime. This could be the genetic reason for antibiotic resistance in *S. aureus*. Several studies have reported on the increasing emergence of antibiotic resistance strains of *S. aureus*.

The 4th cluster is a relatively minor cluster with 5 generations and 5 branches and node 144 as the most recent ancestor with its first generation having a

bootstrap value of 64% . This implies that there was a further mutation in this clade. This clade show high level of diversity and loss of genetic integrity and identity with time elapse. This generational genetic integrity loss suggests the possible reason for antimicrobial resistance. The strains include strain KIBGE-MB01 (HM061132), from human blood samples from an undisclosed location. This strain had no offspring, but had a sister branch that had offspring. This strain had 3 generational offsprings that were closely related to their respective most recent ancestors with bootstrap values >80%. This suggests that though the original genetic make-up of these strains were lost at node 144, the subsequent offsprings starting from KIBGE-MB04 (HM061135) isolated from human samples from an undisclosed location to F21 (PV173443) isolated from human blood samples from Iraq maintained the genetic identity of their subsequent ancestors.

Phylogenetic tree

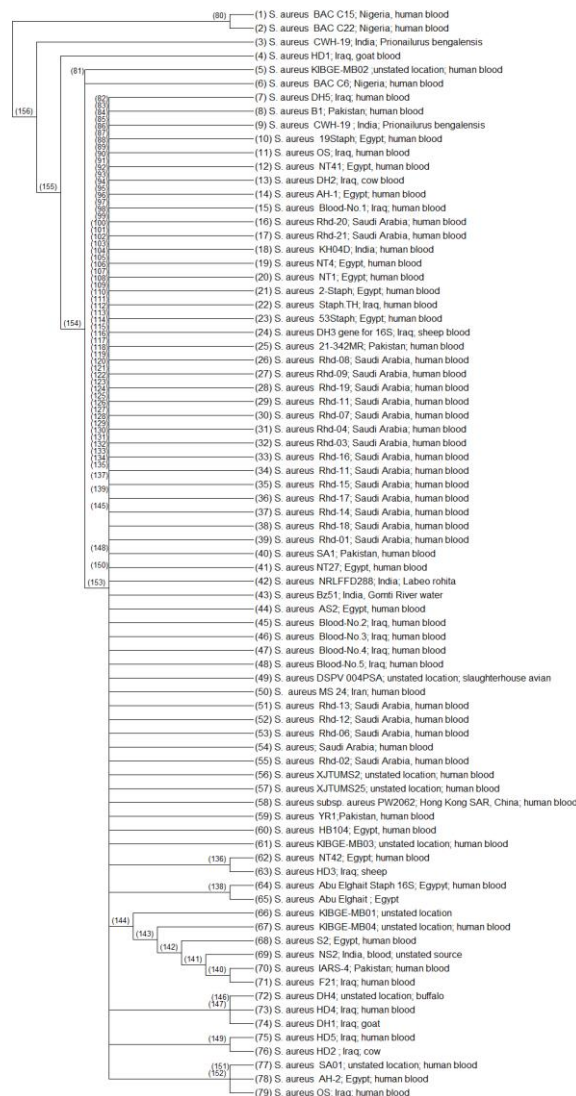


Figure 3a: Phylogenetic tree showing node ID

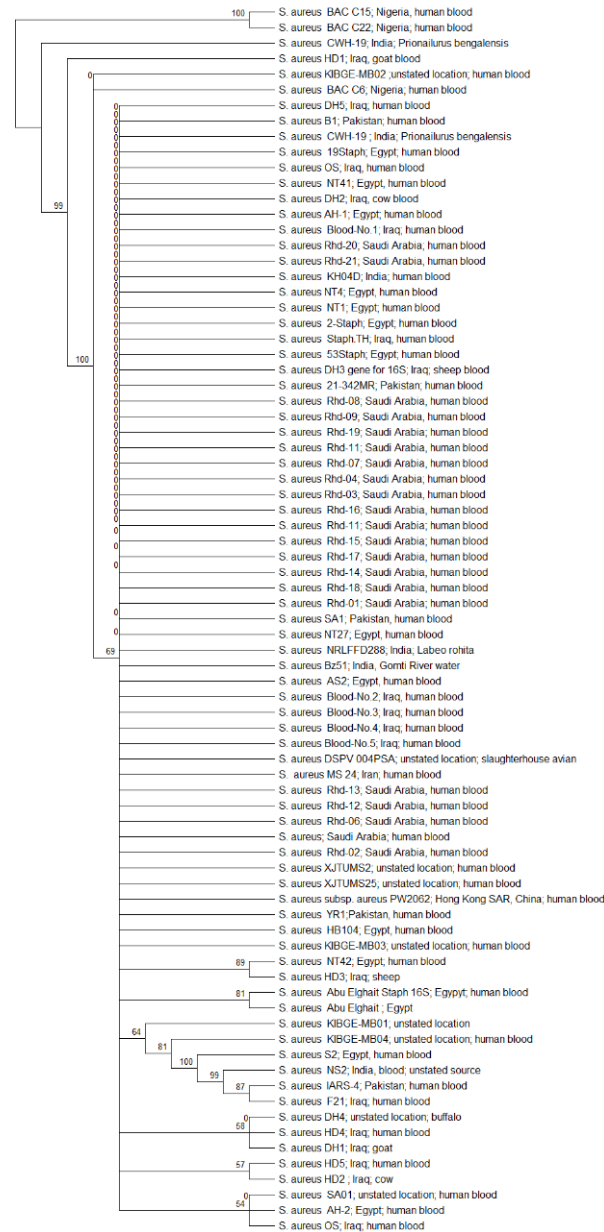


Figure 3b: Phylogenetic tree showing bootstrap values

Statistical analyses and Distance Matrix

For comparative analysis and to determine the position of crucial importance among sequences, a distance matrix of the constructed phylogenetic tree was performed using Geneious software v9.0.5, and other statistical analyses including Excel models. The distance matrix was carried out to identify divergence among analyzed strains while neighbor-joining clustering was used to access the genetic distance and evolutionary relationship between sequences.

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 the distance between two matched pairs increases, the figures increase (figures 4a & b). The highest value got in this analysis was 100% and the lest was 0%. The analysis shows high level of divergence among the various strains as most of them had a distance >50%. The diagonal line across the rectangle dividing the rectangle into two equal triangles indicates no distance between two matched same strains.

Figure 4a: Percentage Identity

	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...
Staphylococcus aureus str...		84.95%	72.80%	89.56%	72.08%	70.05%	0%	90.05%	1.85%	89.66%	90.29%	0%	89.81%	89.81%	87.68%	90.53%	90.53%	0%
Staphylococcus aureus DH...	84.95%		74.65%	77.36%	70.89%	56.86%	94.58%	92.70%	24.83%	95.89%	92.80%	92.31%	88.96%	91.45%	92.77%	95.97%	93.18%	92.01%
Staphylococcus aureus DH...	72.80%	74.65%		36.95%	36.95%	91.46%	55.51%	79.57%	77.83%	42.11%	79.22%	77.80%	81.16%	77.90%	77.48%	75.75%	74.75%	74.00%
Staphylococcus aureus str...	89.56%	77.36%	36.95%		56.59%	65.52%	20.85%	58.16%	2.28%	32.52%	58.08%	1.16%	80.33%	80.58%	58.24%	50.42%	95.68%	94.90%
Staphylococcus aureus HD...	72.08%	70.89%	91.46%	56.59%		69.78%	74.61%	72.81%	24.60%	74.46%	72.81%	67.79%	68.74%	75.06%	73.05%	74.43%	74.00%	73.55%
Staphylococcus aureus DH...	70.05%	56.86%	55.51%	65.52%	69.78%		26.73%	47.31%	3.10%	35.47%	47.33%	5.25%	58.93%	60.22%	47.33%	43.16%	70.07%	69.36%
Staphylococcus aureus str...	0%	94.58%	79.57%	20.85%	74.61%	26.73%		97.44%	78.28%	98.12%	97.26%	95.80%	58.91%	99.26%	97.48%	98.34%	98.05%	90.03%
Staphylococcus aureus str...	90.05%	92.70%	77.83%	58.16%	72.81%	47.31%	97.44%		56.65%	99.35%	99.32%	98.56%	79.79%	97.19%	99.47%	99.43%	99.08%	97.90%
Staphylococcus aureus str...	1.85%	24.83%	42.11%	2.28%	24.60%	3.10%	78.28%	56.65%		67.28%	56.61%	97.60%	18.15%	23.74%	56.80%	48.31%	4.36%	91.81%
Staphylococcus aureus str...	89.66%	95.89%	79.22%	32.52%	74.46%	35.47%	98.12%	99.35%	67.28%		99.26%	98.70%	66.84%	98.72%	99.51%	99.50%	98.17%	90.88%
Staphylococcus aureus str...	90.29%	92.80%	77.80%	58.08%	72.81%	47.33%	97.48%	99.32%	56.61%	99.26%		98.31%	80.08%	97.54%	99.37%	99.72%	98.95%	97.77%
Staphylococcus aureus str...	0%	92.31%	81.16%	1.16%	67.79%	5.25%	95.80%	98.56%	97.60%	98.70%	98.31%		18.70%	100%	98.57%	98.01%	0%	98.64%
Staphylococcus aureus str...	89.81%	88.96%	58.40%	80.33%	68.74%	58.93%	58.91%	79.79%	18.15%	66.84%	80.08%	18.70%		99.88%	79.79%	71.87%	96.75%	95.66%
Staphylococcus aureus DH...	89.81%	91.45%	75.92%	80.58%	75.06%	60.22%	99.26%	97.19%	23.74%	98.72%	97.54%	100%	99.88%		97.19%	98.79%	96.74%	95.66%
Staphylococcus aureus str...	89.81%	92.77%	77.90%	58.24%	73.05%	47.33%	97.48%	99.47%	56.80%	99.51%	99.37%	98.57%	79.79%	97.19%		99.72%	99.08%	97.90%
Staphylococcus aureus str...	87.68%	95.97%	77.48%	50.42%	74.43%	43.16%	98.34%	99.43%	48.31%	99.50%	99.72%	98.01%	71.87%	98.79%	99.72%		98.95%	96.84%
Staphylococcus aureus str...	90.53%	93.18%	75.75%	95.68%	74.00%	70.07%	98.05%	99.08%	4.36%	98.17%	98.95%	0%	96.75%	96.74%	99.08%	98.95%		11.76%
Staphylococcus aureus str...	90.53%	92.01%	74.75%	94.90%	73.35%	69.36%	93.51%	97.90%	4.36%	95.24%	97.77%	0%	95.66%	95.66%	97.90%	96.84%	98.82%	11.76%
Staphylococcus aureus str...	0%	64.57%	74.00%	1.35%	59.30%	5.76%	92.40%	92.40%	91.81%	90.88%	92.33%	98.64%	30.29%	66.89%	92.55%	86.15%	11.76%	
Staphylococcus aureus str...	8.14%	31.42%	48.67%	2.63%	30.12%	3.52%	80.05%	68.28%	96.52%	67.49%	68.23%	98.64%	21.70%	30.43%	67.84%	52.66%	5.76%	94.00%
Staphylococcus aureus str...	89.22%	92.56%	76.19%	77.21%	75.50%	61.76%	97.97%	97.95%	26.33%	98.07%	97.95%	96.92%	93.33%	96.30%	98.46%	98.66%	97.97%	70.69%
Staphylococcus aureus HD...	89.22%	92.56%	76.19%	77.21%	75.50%	61.76%	97.97%	97.95%	26.33%	98.07%	97.95%	96.92%	93.33%	96.30%	98.46%	98.66%	97.97%	70.69%
Staphylococcus aureus str...	0%	94.55%	81.44%	1.19%	68.35%	6.10%	97.54%	99.66%	99.11%	99.55%	99.78%	98.66%	15.00%	100%	100%	0%	0%	100%
Staphylococcus aureus str...	89.66%	95.89%	79.22%	32.52%	74.46%	35.47%	98.12%	99.47%	67.53%	99.26%	99.51%	98.70%	66.84%	98.72%	99.75%	99.67%	98.17%	91.23%
Staphylococcus aureus str...	89.66%	96.14%	79.35%	32.65%	74.65%	35.63%	98.12%	99.60%	67.53%	99.63%	99.63%	98.70%	67.01%	98.97%	99.88%	99.83%	98.53%	95.60%
Staphylococcus aureus str...	89.66%	96.14%	79.35%	32.65%	74.65%	35.63%	98.12%	99.60%	67.53%	99.63%	99.63%	98.70%	67.01%	98.97%	99.88%	99.83%	98.53%	95.60%
Staphylococcus aureus str...	0%	0%	82.17%	2.86%	0%	14.29%	94.89%	98.39%	96.73%	99.51%	97.75%	97.43%	0%	0%	98.36%	0%	0%	98.39%
Staphylococcus aureus str...	89.56%	91.76%	77.85%	61.91%	73.47%	47.61%	98.35%	97.95%	48.36%	99.55%	98.26%	98.42%	80.65%	98.24%	98.09%	99.43%	97.09%	88.26%
Staphylococcus aureus str...	93.10%	96.38%	79.48%	32.78%	74.85%	35.78%	98.12%	99.72%	67.53%	99.51%	99.75%	98.70%	67.18%	99.23%	100%	100%	98.90%	95.97%

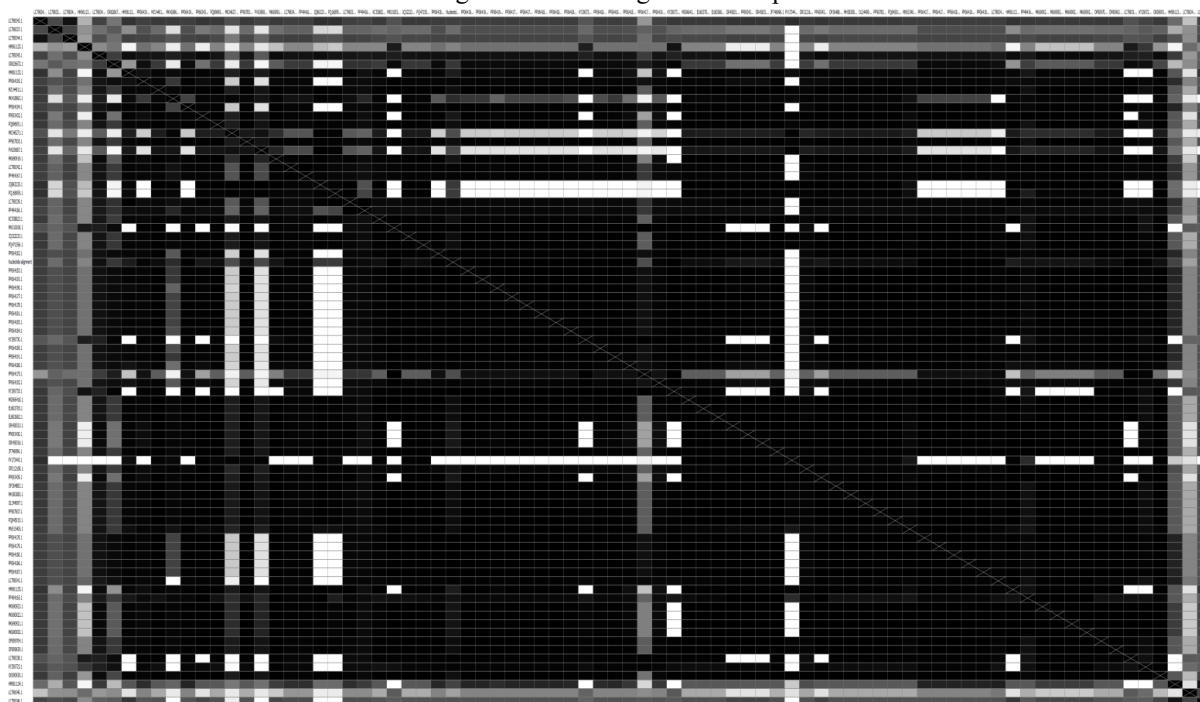
Figure 4b: Total differences in nucleotide bases

	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...	Staphylo...
Staphylococcus aureus str...	100%	99.61%	97.39%	99.61%	98.95%	99.61%	99.21%	99.21%	99.21%	99.21%	99.21%	99.21%	100%	99.61%	99.48%	99.61%	99.61%	99.61%
Staphylococcus aureus str...	100%	99.61%	97.39%	99.61%	98.95%	99.61%	99.21%	99.21%	99.21%	99.21%	99.21%	99.21%	100%	99.61%	99.48%	99.61%	99.61%	99.61%
Staphylococcus aureus str...	100%	98.69%	96.48%	98.69%	98.04%	98.69%	98.69%	97.37%	97.37%	97.37%	97.37%	97.37%	100%	98.69%	98.56%	98.69%	98.69%	98.69%
Staphylococcus aureus str...	100%	98.17%	95.95%	98.17%	97.51%	98.17%	98.17%	96.32%	96.32%	96.32%	96.32%	96.32%	100%	98.17%	98.03%	98.17%	98.17%	98.17%
Staphylococcus aureus str...	100%	68.70%	67.20%	68.61%	68.26%	68.70%	68.70%	51.10%	51.10%	51.10%	51.10%	51.10%	100%	68.64%	66.61%	68.70%	93.18%	93.18%
Staphylococcus aureus str...		98.90%	93.11%	98.90%	98.90%	98.90%	98.90%	0%	0%	0%	0%	0%	98.90%	99.44%	99.15%	98.90%	100%	100%
Staphylococcus aureus str...	98.90%		94.22%	98.06%	99.52%	99.80%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.93%	99.80%	100%	100%
Staphylococcus aureus str...	93.11%	94.22%		92.78%	93.88%	94.22%	96.40%	100%	100%	100%	100%	100%	94.92%	94.98%	95.70%	94.22%	97.78%	97.78%
Staphylococcus aureus str...	98.90%	98.06%	92.78%		97.71%	98.06%	98.01%	99.43%	99.43%	99.43%	99.43%	99.39%	98.16%	98.13%	98.06%	100%	100%	100%
Staphylococcus aureus str...	98.90%	99.52%	93.88%	97.71%		99.52%	99.65%	99.29%	99.29%	99.29%	99.29%	100%	99.57%	99.57%	99.52%	99.35%	99.35%	99.35%
Staphylococcus aureus str...	98.90%	99.80%	94.22%	98.06%	99.52%		100%	100%	100%	100%	100%	100%	100%	99.93%	99.93%	100%	100%	100%
Staphylococcus aureus str...	98.90%	100%	96.40%	98.01%	99.65%	100%		100%	100%	100%	100%	100%	100%	99.93%	99.93%	100%	100%	100%
Staphylococcus aureus str...	0%	100%	100%	99.43%	99.29%	100%	100%		100%	100%	100%	100%	100%	99.86%	100%	100%	100%	100%
Staphylococcus aureus str...	0%	100%	100%	99.43%	99.29%	100%	100%	100%		100%	100%	100%	100%	99.86%	100%	100%	100%	100%
Staphylococcus aureus str...	0%	100%	100%	99.43%	99.29%	100%	100%	100%	100%		100%	100%	100%	99.86%	100%	100%	100%	100%
Staphylococcus aureus str...	98.90%	100%	94.92%	99.39%	100%	100%	100%	100%	100%	100%	100%	100%		100%	99.78%	100%	100%	100%
Staphylococcus aureus str...	99.44%	99.93%	94.98%	98.16%	99.57%	99.93%	99.93%	99.86%	99.86%	99.86%	99.86%	100%	100%		99.86%	99.93%	100%	100%
Staphylococcus aureus str...	99.15%	99.93%	95.70%	98.13%	99.57%	99.93%	99.93%	100%	100%	100%	100%	100%	99.78%	99.86%		99.93%	99.87%	99.87%
Staphylococcus aureus str...	98.90%	99.80%	94.22%	98.06%	99.52%	100%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.93%	100%	100%	100%
Staphylococcus aureus str...	100%	100%	97.78%	100%	99.35%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99.87%	100%	100%	100%
Staphylococcus aureus str...	100%	100%	97.78%	100%	99.35%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99.87%	100%	100%	100%
Staphylococcus aureus str...	100%	100%	97.78%	100%	99.35%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99.87%	100%	100%	100%
Staphylococcus aureus str...	100%	100%	97.78%	100%	99.35%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99.87%	100%	100%	100%
Staphylococcus aureus str...	100%	100%	97.78%	100%	99.35%	100%	100%	100%	100%	100%	100%	100%	100%	100%	99.87%	100%	100%	100%
Staphylococcus aureus str...	98.90%	100%	98.06%	97.94%	99.63%	100%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.92%	100%	100%	100%
S. aureus strain XJTM25...	98.90%	100%	98.06%	97.94%	99.63%	100%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.92%	100%	100%	100%
S. aureus subsp. aureus st...	100%	100%	99.69%	98.04%	99.61%	100%	100%	100%	100%	100%	100%	100%	100%	99.92%	100%	100%	100%	100%
Staphylococcus aureus str...	98.90%	100%	94.22%	98.06%	99.52%	100%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.93%	100%	100%	100%
Staphylococcus aureus str...	98.90%	100%	94.22%	98.06%	99.52%	100%	100%	100%	100%	100%	100%	100%	100%	99.93%	99.93%	100%	100%	100%
Staphylococcus aureus str...	0%	79.55%	79.55%	79.09%	79.55%	79.55%	79.55%	79.26%	79.31%	79.41%	79.36%	0%	79.37%	79.55%	79.55%	86.60%	86.60%	86.60%
Staphylococcus aureus HD...	65.78%	69.11%	66.84%	68.54%	68.48%	69.11%	71.99%	71.99%	71.99%	71.99%	71.99%	65.43%	68.32%	67.97%	69.11%	68.36%	68.36%	68.36%
Staphylococcus aureus HD...	65.24%	67.75%	65.14%	67.11%	67.06%	67.75%	67.75%	70.17%	70.17%	70.17%	70.17%	64.96%	66.82%	66.41%	67.75%	66.82%	66.82%	66.82%

Distance Matric / Heatmap

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

Figure 4C: Showing the heatmap



IV. CONCLUSION

S.aureus is a highly clinically relevant bacteria possessing much virulent genes, as well as known for multi-drug resistance. From this study, there is a trend of diversity in the strains of *S.aureus*. This may imply divergence in some characteristics such as virulence, AMR pathogenicity etc. Some were noted to be at the ancestral level, thus possibly maintaining the virulent and other pathogenicity capabilities. Most strains showed a lot of diversity over time owing to mutations which resulted from change in nucleic acid bases (deletion, insertion, etc.).

The *S.aureus* strains isolated from clinical samples in Nigeria maintained a genetic integrity of the root ancestor of *S.aureus* spp. One of the strains (BAC C6) from Nigeria have a standalone 100% (bootstrap value) genetic identity with the third generation ancestor (node 154) and in close proximity with strain of zoonotic origin.

Most of the strains from Saudi Arabia, Egypt, Iraq, Pakistan, Iran, Spain and India show much diversity from the most recent ancestor in terms of evolution. This implies that similar trends of diversity are obtainable. This may imply much developed pathogenicity capabilities or /and multi-drug resistance.

V. 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. Secondly, there is a need to carry out a wet laboratory analysis of *S.aureus* strains in Nigeria and having them submitted in the databases for recent trends in *S. aureus* evolutionary trend as this is scarce.

VI. ACKNOWLEDGMENTS

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Authors' contribution

CAO conceived the study, drafted the proposal, was involved in data analysis and interpretation and writing of the final manuscript. BB, NNP and EA were involved in drafting of the proposal, reviewing and editing of the manuscript.

Conflict of interest

The authors have no conflict of interest whatever

Founding

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