Genetic Diversity of Staphylococcus Saprophyticus Strains Causing Urinary Tract Infections in Lagos Metropolis

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Abstract

Background: *Staphylococcus saprophyticus* is a Gram-positive bacterium implicated in urinary tract infections in sexually active women along with other bacteria. There is a special need to accurately isolate and identify this organism in clinical specimens in order to prevent misidentification in the laboratory. The present study was carried out to evaluate the genetic diversity of one hundred different *Staphylococcus saprophyticus* strains isolated from women presenting with urinary tract infections reporting in four government hospitals in Lagos Metropolis using 16SrRNA gene sequence analysis.

Results: The PCR-amplified and sequenced 16SrRNA gene of 100 isolates and confirmed 88 strains of *S. saprophyticus* to subspecies level, while 8 belong to other genera and 4 could not be ascertained due to low significant similarity. Phylogenetic analysis showed the interrelationship between the isolates from different hospitals. The results showed the distribution of the isolates into two broad clusters and twenty-two sub-clusters.

Conclusion: The results revealed the genetic diversity of *Staphylococcus saprophyticus* isolated from women with urinary tract infections in Lagos Metropolis using 16SrRNA gene sequence.

Background

Urinary tract infection (UTI) is the presence of micro-organism in the urinary tract including urinary bladder, prostate, collecting system or kidney. It is the second most common encountered infection in clinical practice. The syndrome ranges from asymptomatic bacteriuria to perinephric abscess with sepsis [1]. Worldwide, about 150 million people are diagnosed with UTI each year costing the global economy in excess of 6 billion US dollars [2]. An estimated 40–50% of all women will experience at least one UTI in their lifetime, and one in three women would have had at least one clinically diagnosed UTI by the age of 24 [3].

Staphylococcus saprophyticus is implicated in UTI and it accounts for 5–10% of hospital visit globally [4]. It is estimated that *S. saprophyticus* causes up to one million UTI each year and it is the second most common cause of uncomplicated UTI in sexually active women [5, 6]. Over 40% of young, sexually active women are colonized with *S. saprophyticus* in the rectum, urethra, or cervix at any given time [7].

Staphylococcus belongs to the Gram-positive low GC content group of the Firmicutes division of bacteria. Three species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* are recognized as major human pathogens [8]. As they are widely spread in various niches such as clinical, environment and food manufacturing plants, it has become increasingly important to accurately identify the staphylococci at the genus and species levels. The conventional phenotypic tests used for the identification of *Staphylococcus* species have limitations [9, 10], thus several molecular methods have been developed [11]. These include DNA–DNA hybridization and 16S rRNA sequencing, as well as various PCR-based techniques [12, 13].

In Nigeria, *S. saprophyticus* is often misidentified as *S. aureus*. Ayeni and Odumosu [14] reported 85% misidentification rate of *S. aureus* in the Southern Nigerian States of Edo, Rivers, Bayelsa, Ekiti, Osun, Oyo, Ogun and Lagos.

In view of the emerging clinical importance of this organism, proper identification is of utmost importance for correct diagnosis.

The aim of the present study was to use 16SrRNA gene to determine the genetic diversity of *S. saprophyticus* strains isolated from women presenting with urinary tract infections in government hospitals in Lagos metropolis.

Results

Bacterial Isolates

The biochemical analyses showed all isolates to be Gram positive cocci, catalase positive, oxidase and coagulase negative and all were strictly resistant to 5µg novobiocin disc (Oxoid, UK), while 98 were urease positive, 66 showed lipase activity, 80 showed haemagglutination of sheep erythrocyte and 93 were hydrophobic (Table 1).

Molecular identification of the isolates

In the present study, the 1500 bp partial 16SrRNA gene sequences of 100 bacterial isolates were PCR-amplified, sequenced (Figure 1) and the resulting data deposited in the GenBank database and accession numbers assigned (Table 2). In total, 88 (88%) out of 100 isolates sequenced were confirmed as *S. saprophyticus* species and subspecies whereas the biochemical tests identified all as *S. saprophyticus*. In this study, 4 (4%) isolates were confirmed as *Escherichia coli* earlier identified as *S. saprophyticus*; 2 (2%) as *Enterococcus faecalis*, 1 (1%) *Enterobacter cowanii* and 1 (1%) *Enterobacter cloacae*. While 4 (4%) isolates could not be adequately identified using 16SrRNA gene due to low significant similarity when it was compared with other sequences in the GENBANK. For comparison purposes, the submitted 16SrRNA gene sequences of *S. saprophyticus* from different parts of the world were downloaded from the GenBank database.

Phylogenetic analysis of the *S. saprophyticus* strains

The phylogenetic analysis confirmed the effectiveness of 16SrRNA gene as a means of isolates identification. The cladogram showed that the strains of *S. saprophyticus* based on their evolutionary closeness were broadly divided into two groups which comprises of 22 clusters with Group A having 16 clusters while Group B had 6 clusters (Figure 2). The tree showed that the strains FELA056 and 78 and FELA011 and 47 were the most closely related species sharing the same sister nodes. Strains FELA047

and 11 were obtained from different hospitals with 100% bootstrap value followed by strains FELAO36 and 86, 69 and 87 with 99% bootstrap value. Strains FELAO32 and 51 and FELAO93 and 94 had 98%.

The strain FELAO15 was phylogenetically closely related with the *S. saprophyticus* strain FVRI with accession number KP698299 isolated from India; likewise strain FELAO49 was related to strain JNS3 (KC503908) isolated from China. They belonged to the same cluster although the isolates were obtained from different hospitals. The other isolates obtained from different parts of the world were closely related forming a single cluster {16}.

Discussion

Staphylococci are opportunistic pathogens that can cause diverse range of infections. Therefore, appropriate identification method must be put in place in order to distinguish different groups of this organism with a view to ensuring better management of diseases caused by the organism.

Accurate identification at the species level may not only change the diagnosis but can also help to identify unusual antimicrobial resistance patterns. The ideal method should have high discriminatory power and allow the identification of closely related species while also being relatively simple, inexpensive, rapid and reproducible. Therefore, genetic methods based on PCR or sequencing are good candidates for identification purposes [15].

The 16S rRNA gene is widely distributed throughout the genome and useful for bacterial identification [16]. The 16S rRNA gene is a part of 30S ribosome and is around 1500 bp and is highly stable. Due to stability, it is widely used in molecular identification, taxonomic studies, and phylogenetic and evolutionary studies [17]. Das et al. [18], Egege et al. [19], Hardy et al. [20] and Syromyatnikov et al. [21] in their studies had also shown the effectiveness and precision in using 16S rRNA gene sequence-based assay for the identification of bacterial isolates obtained from different samples. In our study, we used PCR sequencing of 16SrRNA gene to effectively confirm *Staphylococcus saprophyticus* strains previously identified using biochemical and phenotypic characterisation. Mellmann et al. [22], Shukla et al. [23], Darmawati et al. [24], and Srinivasan et al. [25] had used the 16S rRNA gene sequence analysis for the identification of staphylococcal isolates from environmental and clinical samples. In this study, the identification of the 88 (88%) *S. saprophyticus* strains is in agreement with results of studies carried out by Kosecka-Strojek et al. [15], Woo et al. [26] and Bosshard et al. [27] that confirmed usefulness of 16SrRNA gene in the identification of bacterial isolates.

The phylogenetic analysis revealed that *S. saprophyticus* strains from Lagos Mainland are closely related while most of the strains from Lagos Island were found to be phylogenetically diverse from that of Mainland except strain FELAO11 (GHM) that was highly related to strain FELAO47 (GHS) with 100% bootstrap value.

The sequence similarity of 16SrRNA gene ranged from 76 to 95% in all isolates. To the best of our knowledge, this is the first time that the PCR amplicons of the *S. saprophyticus* strains from this

environment will be sequenced and the resulting data deposited in the GenBank data.

Conclusion

The sequence-based identification of isolates has tremendous advantage over biochemical and phenotypic characterizations in that it can identify isolates to subspecies level. Therefore, clinical laboratories should be mandated to include *S. saprophyticus* in the list of possible pathogens in the case of bacteriuria and UTI cases. This would prevent misdiagnosis, help the clinicians to give a better diagnosis and treatment regimen. The findings have shown the occurrence and diversity of *Staphylococcus saprophyticus* strains in this environment.

Methods

Study design

A total of 384 urine samples were collected once from women reporting for gynaecological care and those presenting with urinary tract infections in government hospitals in Lagos State from February 2014 to April 2016. Pregnant women were excluded from this study, while only women in the age group from 20 to 50 years were included. The participants in this study were instructed on how to collect first morning urine in a sterile universal bottle through the clean-catch midstream method.

Bacterial strains and culture conditions

The isolates were collected from four different hospitals with three located in the mainland (General Hospital, Gbagada {GHG}, General Hospital, Somolu {GHS} and Lagos University Teaching Hospital {LUTH}) and one in island part of the state (General Hospital, Marina {GHM}). One hundred and twenty-six isolates were obtained from the Department of Medical Microbiology, LUTH; eighty-one isolates from GHM; one hundred and sixteen isolates from GHG and sixty-one isolates from GHS. The reference strain (*Staphylococcus saprophyticus* subsp. *bovis* DSM 18669) was obtained from Leibniz-Institut DSMZ, Germany.

One millilitre of the urine sample was inoculated into 9 mL of brain heart infusion broth (Himedia, India) containing 5 µg/mL of novobiocin (Oxoid, UK). The samples were then transported to the Microbiology Laboratory of the Bells University of Technology, Ota; where further analyses were carried out. After 24 h of incubation at 37 °C, a loopful from broth medium was streaked on to brain heart infusion agar (Himedia, India) plate containing 5 µg/mL of novobiocin. The organism was incubated overnight at 37 °C for 24 h [7]. The isolates were frozen at -80 °C in brain heart infusion broth containing 10% glycerol until the study.

Biochemical identification / phenotypic characterisation

The isolates were Gram stained and tested for production of oxidase, catalase, coagulase, urease, resistance to 5 µg novobiocin disc (Oxoid, UK) [28], lipase activity [29, 30], haemagglutination assay, and hydrophobicity test [30] for the preliminary identification. A total of 100 novobiocin resistant isolates were processed and analysed genotypically.

Molecular analyses of *Staphylococcus saprophyticus* isolates

DNA Isolation

Genomic DNA of the *S. saprophyticus* isolates was extracted using QIAGEN DNA extraction kit (QIAGEN, Germany) in accordance to manufacturer instruction with minor modification that 180 µL of lysozyme (10 µg/mL) was added at the cell lysis stage. The crude DNA extract was kept at -80 °C until it was used for PCR amplification.

PCR Amplification

The molecular confirmation of the isolates was carried out using 16S rRNA primers: 27F: 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R: 5'-GGTTACCTTGTTACGACTT-3' [31]. For all *S. saprophyticus* strains, amplification was performed from purified genomic DNA. The PCR amplification was performed in a 25 µL reaction mixture containing 2.5 µL of 10x *EasyTaq*® Buffer, 2 µL of 2.5mM dNTPs, 0.5 µL of *EasyTaq*® DNA Polymerase (Transgen Biotech, China), 1 µL (5 µM) for each of the primers and 3 µL of template DNA and final volume was brought to 15 µL with distilled water. PCR reaction was carried out in accordance with the modified method of Ferreira da Silva et al. [32] with initial denaturation of 5 min at 95 °C, 30 cycles of final denaturation at 94 °C for 30 s, annealing of 30 s at 52 °C, initial elongation of 1 min 25 s at 72 °C and final elongation step at 72°C for 10 min was applied in a BIO-RAD C1000 touch thermal cycler.

PCR products were analysed by electrophoresis in 1% (w/v) agarose gel in 1X TBE buffer. The PCR reaction mixture was stained with 1% solution of ethidium bromide (CDH, India) and visualized using UVP Benchtop UV Transilluminator (BioDoc-It). The sizes of the amplicon were compared with 100 bp DNA ladder (New England Biolabs, USA).

16S rRNA Sequencing

The purification and sequencing of the amplified PCR products were carried out using the Sanger sequencing method. It was carried out in MacroGen Laboratory in Maryland USA. The samples were first purified using Zymo PCR cleanup Kit. Purified samples were sequenced using Applied Biosystems

Seqstudio Genetic Analyzer. Sequencing analysis was performed on the 1500bp PCR product. The 100 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank by using the NCBI Basic Local Alignment Search Tools (BLAST-n) programme. (<http://www.ncbi.nlm.nih.gov/BLAST>).

Phylogenetic Analysis

Phylogenetic construction of 16S rRNA gene sequences

In this study, phylogenetic tree was constructed to show interrelationship between all the 88 strains of *S. saprophyticus* obtained in this study and 11 strains from different parts of the world. The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 4.24945984 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 99 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1827 positions in the final dataset.

Pairwise and multiple alignments of 16S rRNA gene sequences of *S. saprophyticus* were carried out using Clustal W [33]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 [34].

Abbreviations

PCR: Polymerase chain reaction; %: Percentage; UTI: Urinary tract infection; GC: Guanine Cytosine; DNA: Deoxyribonucleic acid; UK: United Kingdom; bp: Base pairs; GHM: General Hospital, Marina; GHS: General Hospital, Somolu; GHG: General Hospital, Gbagada; LUTH: Lagos University Teaching Hospital; DSM: *Deutsche Sammlung von Mikroorganismen*; SrRNA: Ribosomal ribonucleic acid; mL: Millilitre; µg/mL: Microgramme per milliliter; °C: Degree Celsius; h: Hour; µg: Microgramme; µL: Microlitre; µM: Micromolar; dNTPs: Deoxyribonucleotide triphosphates; s: Second; min: Minute; w/v: Weight per volume ; CDH: Central Drug House; UV: Ultraviolet; USA: United States of America; NCBI: National Centre for Biotechnology Information; MEGA: Molecular evolutionary genetics analysis

Declarations

Ethics approval and consent to participate

This study formed part of a thesis for Doctor of Philosophy (PhD) degree in Microbiology and was approved by the Department of Microbiology, Faculty of Science, University of Lagos, the Health Research and Ethics Committee (ADM/DCST/HREC/879) of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos and the Institutional Review Board of Nigerian Institute for Medical Research, Lagos. The written informed consent was obtained from each of the study participants before enrolment.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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None to declare

Author's contributions

FA carried out the experimental work and wrote the manuscript, SS coordinated the work, EO assisted in experimental work, IA coordinated the work and edited the manuscript. All authors contributed to the writing of the manuscript and approved the final manuscript.

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Tables

Due to technical limitations, table 1 and table 2 are only available as a download in the Supplemental Files section.

Figures

Figure 1

PCR amplification of 16SrRNA gene universal primer (27F/1492R) of *Staphylococcus saprophyticus* from urine samples. Lane M: 100 bp DNA Ladder.

Figure 2

Neighbour-joining tree showing the phylogenetic relationships among *S. saprophyticus* isolates based on a comparison of 1500bp 16SrRNA gene sequences. Bootstrap values based on 1000 replicates are given at the branching points when they are above 50%. NCBI accession numbers are in parentheses followed by sampling site.

Supplementary Files

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