

Detect of Phylogenetic Relationships by RAPD_PCR among *Staphylococcus aureus* Isolated from Different Sources in Hilla City

Amal Raqib Shamran¹, Zainab A. Tolaifeh²

¹University of Babylon/Collage of Science for women, Iraq,

²University of Babylon/Collage of Science for Women, Iraq

Abstract

Staphylococcus aureus (brilliant staphylococcus) is a Gram-positive, cocci – shape, it is an individual from the typical verdure of the body, oftentimes found in the nose, respiratory tract, and on the skin. Usually positive for catalase and nitrate decrease. We can portrayed *S. aureus* strains that are across the board in hospitals in Hilla city, 60 clinical and condition tests were gathered from numerous parts of patients like injuries, skin, nails and urinary tract disease taken from general doctor's facilities of Hilla city. Strategies for segregation and distinguishing *S. aureus* dependent on culture strategies with biochemical tests, A sum of 17 enhanced DNA sections from 250 to 1K base match) were watched utilizing the 2 groundworks, and every one of preliminary that fruitful giving intensification groups uncovered distinctive hereditary example. End: RAPD-Polymerase Chain Reaction investigation it used to discover an incentive in structuring an assortment of sub-atomic DNA unique finger impression dependent on epidemiological examinations that centers around the recognizable proof and portrayal of *S. aureus*.

Keywords: *Phylogenetic, RAPD_PCR, Staphylococcus aureus*

Introduction

Staphylococcus aureus is an opportunistic pathogen that causes a nosocomial infections ranging from self-limiting to lifethreatening in both developing and developed countries^{13,14}. It is critical in the study of disease transmission and nature to have the capacity to distinguish bacterial species and strains precisely. Fast distinguishing proof and order of microscopic organisms is ordinarily completed by morphology, healthful prerequisites, anti-microbial obstruction, isoenzyme examinations, phage affectability^{3,4,2,5} Meanwhile, a few strains of *S. aureus* have *mecA* quality which presents protection from methicillin and the greater part of the regularly utilized antimicrobial operators including

b-lactams and cephalosporins. These strains are called methicillin-safe *Staphylococcus aureus* (MRSA) and they thought to be more destructive than methicillin-powerless *Staphylococcus aureus* (MSSA) strains¹⁶. The expanding rates of nosocomial and network related MRSA diseases and their capacity to exchange between people, ox-like, and nourishment of creature birthplaces have turned into a worldwide hazard¹⁵. Recognition and distinguishing proof techniques utilizing the PCR to enhance DNA have been utilized for different creatures⁹, yet these require sequence information for particular preliminaries. In any case, PCR utilizing discretionary groundworks (AP-PCR) requiring no earlier arrangement data has uncovered DNA polymorphisms that might be valuable for fingerprint (Welsh and McClelland, 1990; Williams et al., 1990). Haphazardly Amplified Polymorphic DNA (RAPD), a straightforward PCR based procedure, has been broadly utilized for epidemiological examination. In addition, RAPD preliminaries can successfully examines the entire chromosomal DNA for the nearness of little reversed rehashes and intensifies the

Corresponding Author:

Amal Raqib Shamran

University of Babylon/Collage of Science for Women,
Iraq

interceding DNA portions of variable length that can be utilized for distinguishing hereditary variety and setting up strain-particular fingerprints. Likewise the test can be performed with low convergence of DNA utilizing short engineered oligonucleotide groundworks long¹⁰.

Methodology

Sampling: Sixty samples were collected from clinical and environmental cases. Samples were taken from out and inpatients who admitted to AL-Hilla General Teaching Hospital and Babylon Hospital for Maternity and Pediatric Hospital. Between October 2016 and May 2017.

Bacterial Isolation: Around 250 mil *S. aureus* detach was enraptured into a 100 mil of supplement soup (pH seven.5) and command underneath consistent shaking at thirty seven C for twenty-four h. The microorganism cell was expelled o by action, washed with zero.1mM Tris EDTA and unbroken at - 20oC for DNA extraction.

Genotypic identification:

DNA Extraction: DNA of *staphylococcus aureus* isolates was extracted and purified using Extraction and purification Kit from Geneaid company (UK).

Primers: Two arbitrary or random primers (OPB-10,OPX-01) obtained from Bioneer, IDTDNA (USA). Bacterial isolates were tested for single primers for RAPD-PCR technique (table 1).

RAPD-PCR amplification: Final product of 30µl reaction volumes containing ten ul of single primer,12.5 ul of inexperienced Master combine, 5 ul of Genomic DNA and therefore the volume of reaction was completed up to thirty ul by adding a pair of.5 ul of enzyme free water Amplification was applied during a thermo-cycler (Eppendorf) programmed for 2 minutes at 94°C; for 45cycles one minute at 94°C, one minute at 35°C and 2 minutes at 72°C; and a final extension of 5 minutes at 72°C. Amplification product were electrophoresed in one.8% agarose gels so pictured by staining with ethidium bromide commonplace molecular markers were conjointly enclosed in every action run. Ultraviolet trans-illuminated gels were photographed.

Phylogenetic Analysis:: Positions of unequivocally scorable RAPD bands were transformed into a binary characters matrix ("1" for the presence and "0" for the absence of a band at a particular position). Phylogenetic

tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis.

Results and Discussion

RAPD analysis of staphylococcus aureus: Polymorphism assay for *staphylococcus aureus* isolates was carried out using two primers.. Random amplification of the DNA of *S. aureus* isolates reveals the efficacy of these selected nucleotides sequences in determination the similarity or variations among all isolates.

***S.aureus* isolates by RAPD:** A total of 9 amplified DNA fragments ranging in size from 250 to 1 Kpb were observed using two random amplified polymorphic DNA (RAPD) primers (opX-10,opB-01) where as 17 polymorphic amplified fragments were commonly detected among the 9 *S.aureus* isolates (table 1) and each of primer give different genetic profiles. UPGMA analysis for the dendrogram made based on the RAPD data generated by primer OPX-10 were performed and shown in figure 3. Analysis showed that the 9 *s. aureus* strains were grouped into two and clustered into three classes. The large cluster comprised the Stap4, Stap5, Stap9,Stap7, and Stap8; a second cluster included strains of the S2, S8 and S9; and the third remaining clusters corresponded to the Stap1, Stap3,Stap2 and Stap6. While primer OPX-01 were performed and shown in figure 4. Analysis showed that the 9 *S. aureus* strains were grouped into two and clustered into three classes also. Genetic fingerprint and process diversity between *Staphylococcus aureus* isolates were determined by ever-changing RAPD data into a Jaccard similarity analysed by UPGMA to produce a organic process tree. The compound band pattern obtained is equivalent to a Universal Product Code, allowing the identification of each individual.as Associate in Nursing example, isolate Sa1 presents distinctive bands once its compound amplified with most of the primers tested (Figure 1. It's well documented that RAPD-PCR is one of the most widely used method to investigate the genetic variability of any given nosocomial pathogen, moreover in RAPD the power of designated and discriminatory primers can be been easily assessed ¹⁷. phenotypes consist of isolates that less related and such identification isolates using cultural and morphological techniques often lack consistency and precision ¹¹. In the current examination, we have discovered that distinguishing proof of hereditary assorted variety in *S. aureus* relies upon wellsprings of segregates, diverse host cells and event of freaks. RAPD markers uncovered conceivable

association between host start line, amendment and hereditary selection among *S. aureus* separates, and this shown its process and symptomatic potential. Clearly, for these deoxyribonucleic acid teams examples to possess a helpful significance within the zones of prescription, people science and also the study of illness transmission, explicit deoxyribonucleic acid teams should be known with host beginning points, transformation and quality qualities, Rapd-pcr is important to possess fast and solid epidemiologic composing method to screen the bury or intra-spread of multidrug safe MRSA strains¹⁸. this might be practiced by a organized correlation of deoxyribonucleic acid band styles among microorganisms differentiating for the varied host birthplaces, transformation and

quality qualities gift. Comparative methodology has been utilised to separate forceful from non-forceful disengages of the seed assault microorganism *Phoma* symbol⁽¹²⁾. The DNA mark characterized for each race of *S. aureus* ought to be valuable for epidemiological reviews, medicinal analyses, and in the distinguishing proof of new destructive strains and detaches and their birthplace.

Table 1. Type and sequence of RAPD primers used for pcr analysis

Primer	Sequence 5-----3
OPB-10	5- TCGCATCCCT-3
OPX-01	5- GGTGGCATCT-3

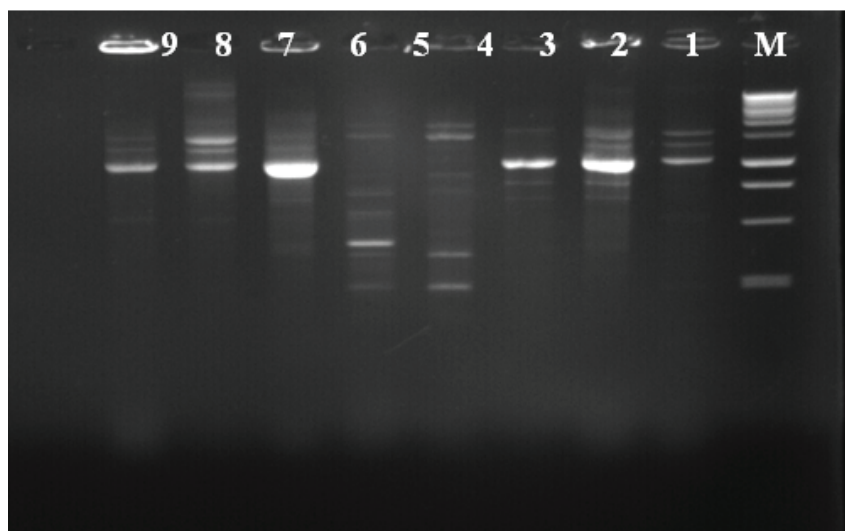


Figure 1. RAPD –PCR using the primer OPX-10. {M Line (ladder), the isolates numbered (1,2,3,4,5,6,7,8,9) were positive for OPX10 primer}

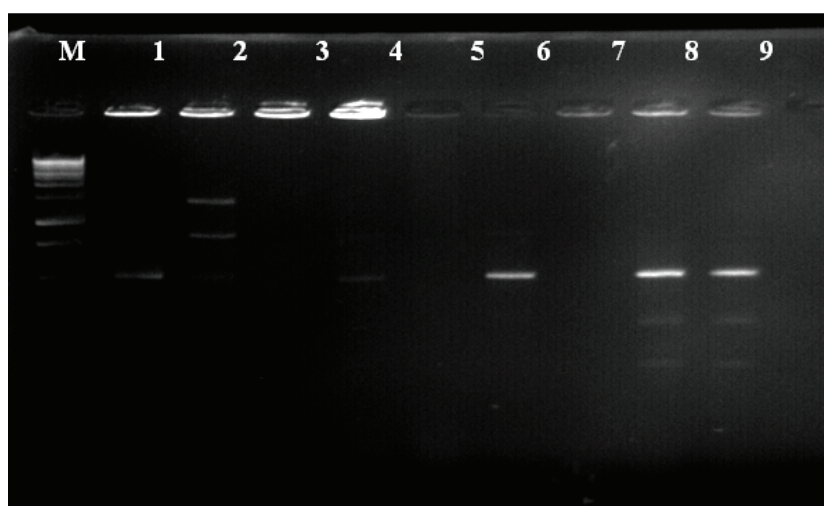


Figure 2. RAPD –PCR using the primer OPX-01 {M Line (ladder), the isolates numbered (1,2, 4, 6, 8,9) were positive for OPX-01 primer, while3,5,7 were negative}

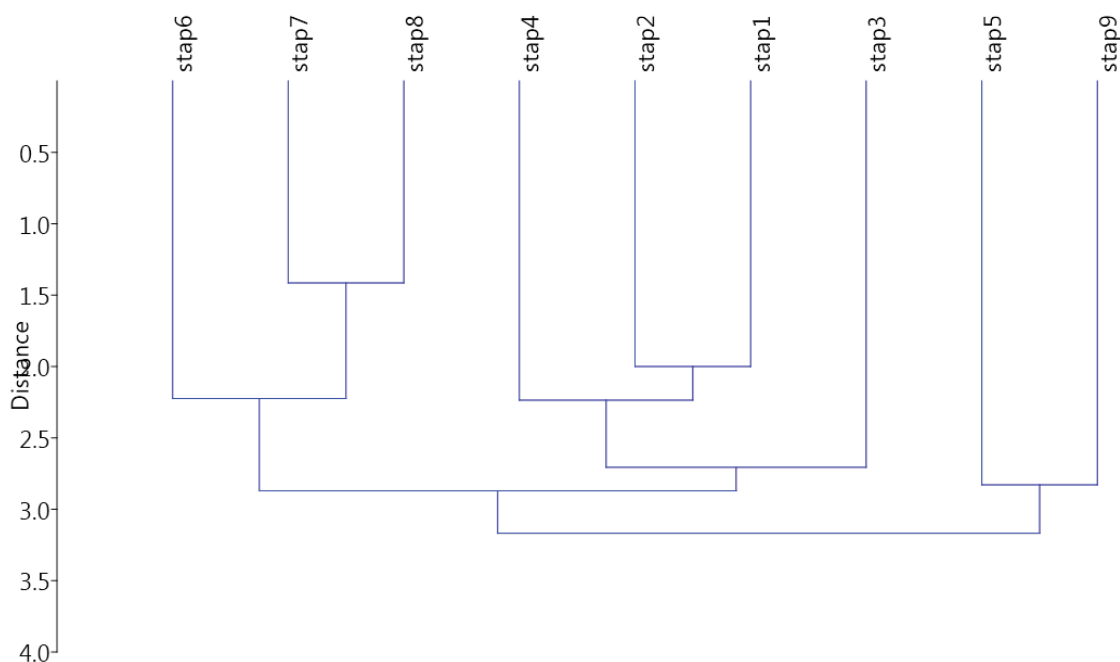


Fig. 5: Dendrogram analysis showing phylogenetic diversity of 9 *Staphylococcus* isolates identified by RAPD markers using OPX-10, OPX-01

Conclusion

Strategies for segregation and distinguishing *S. aureus* dependent on culture strategies with biochemical tests, A sum of 17 enhanced DNA sections from 250 to 1K base match) were watched utilizing the 2 groundworks, and every one of preliminary that fruitful giving intensification groups uncovered distinctive hereditary example. End: RAPD-Polymerase Chain Reaction investigation it used to discover an incentive in structuring an assortment of sub-atomic DNA unique finger impression dependent on epidemiological examinations that centers around the recognizable proof and portrayal of *S. aureus*.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon/collage of science for women, Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. Onasanya A, Mignouna H, Thottappilly G. Genetic fingerprinting and phylogenetic diversity of *Staphylococcus aureus* isolates from Nigeria African Journal of Biotechnology. 2003; 2 (8): 246-250.
2. Aber RC, DC Mackel. Epidemiological typing of nosocomial microorganisms. Am J Me. 1981; 70: 899-905.
3. Eisenstein BI. New molecular techniques for microbial epidemiology and the diagnosis of infectious diseases. J Infect Dis. 1990; 160: 595-602.
4. Selander RK, DA Caugant and TS Whittam. *Escherichia coli* and *Samonella typhimurium*. Cellular and Mol Biology. Neidhardt FX (Ed. In Chief) ASM. 1987: 1625-1648.
5. Milkman R. Electrophretic variation in *Escherichia coli* from natural sources. Science. 1973; 182: 1024-1026.
6. Amann RI, Krimholz L. Fluorescent-oligonucleotide probing of whole cells for

- determinative, phylogenetic and environmental studies in microbiology. *J Bacteriol.* 1990; 172: 762-770.
7. Smith NH, RK Selander. Sequence invariance of the antigencoding central region of the phase 1 flagella filament gene (fliC) among strains of *Salmonella typhimurium*. *J Bacteriol.* 1990; 172: 603-609.
 8. McCabe PC. PCR Protocols: a guide to method and applications. Academic Press. 1990: 76-83.
 9. Hartskeerl RA, De Wit MYL, Klatser PR. Polymerase chain reaction for the detection of *Mycobacterium leprae*. *J. Gen. Microbiol.* (1989). 135: 2357-2364.
 10. Idil N, Bilkay IS. Application of RAPD-PCR for determining the clonality of methicillin resistant *Staphylococcus aureus* isolated from different hospitals. *Braz. Arch. Biol. Technol.* 2014; 57(4): 548–553.
 11. Coltman K. Urinary tract infections: New thoughts on an old subject. *Practitioner.* 1979; 223: 351-355.
 12. Schafer E, J Wostmeyer. Random primer dependent PCR differentiates aggressive and nonaggressive isolates of the oilseed rape pathogen *Phoma lingam* (*Leptosphaeria maculans*). *J Phytopathol.* 1992; 136: 124-136.
 13. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community associated methicillin-resistant *Staphylococcus aureus*. *Lancet.* 2010; 375: 1557–68.
 14. Kennedy AD, Deleo FR. Epidemiology and Virulence of Community-Associated MRSA. *Clin. Microbiol. Newslett.* 2009; 31: 153–60.
 15. Nnachi AU, Emele FE, Ukaegbu CO, Agah MV, Udu-Ibiam OE, Chukwu O. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meat and meat handlers in Onitsha, Nigeria. *Eur J Prev Med.* 2014; 2:9–15.
 16. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Di.* 2003; 36:53–59.
 17. Nikbakht M, Nahaei MR, AkhMT, Asgharzadeh M, Nikvash S. Molecular fingerprinting of methicillin-resistant *Staphylococcus aureus* strains isolated from patients and staff of two Iranian hospitals. *J. Hosp. Infect.* 2008; 69: 46–55.
 18. Debnath A, Chikkaswamy B. Randomly Amplified Polymorphic DNA Assay of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples from Bengaluru, India *Int.J.Curr. Microbiol. App.Sci.* 2015; 4(11): 342-355.