



Genetic Study for *icaAD* Gene to *Staphylococcus aureus* Isolated from Different Part of Computer

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Abstract: In this study to investigate the status of staphylococcus aureus that contamination of computer components (keyboard and mice). The samples were collected in two groups, first group (n= 50) from computer keyboards and mice were collected and second group (n=50) from staff and student that user computer daily after sterilization hands before using the computer show from first group 42(84%) were found to be contaminated with staphylococcus aureus and 1(2%) other staphylococcus ssp. ,and second group 37(74%) were found to be contaminated with staphylococcus aureus and 13(26%) other staphylococcus ssp. all of these bacteria were extracted DNA and subjected to amplified *icaAD* gene responsible on biofilm and adhere these bacteria on computer component appear 93 (100%) contained complete *IcaA* and *icaD* genes this guide to these bacteria forming biofilm and adhere onto computer keyboards and mice and hands. From herewe discover the level of knowledge among the computer about the possibility of microorganisms on the keyboard and mouse is very poor

Key Word: *icaAD* gene, *Staphylococcus aureus*, biofilm, computer compone

I. INTRODUCTION

Contamination occurs everywhere including environment and all its objects. Computer s keyboards and mice are the most open surface parts of computer which show 100% contamination. This study has demonstrated that microbial contamination of multiple-user computer keyboards may be a common mechanism of transfer of potentially pathogenic bacteria among users, computers continue to have an increased presence in almost every aspect of our occupational recreational and residential environments Pathogens are transmitted by carriage on hands from inanimate objects present in the university lab setting, including computer keyboards and mice, the increased availability of multiple-user computers in the university setting means that these items or equipment are handled by numerous users on a daily basis [18].

Students have indicated 100% access to computers, 92.1% regularly use internet and 73.3% regularly use e-mail ,there is need to recognize that computer equipment may act as a reservoir for the transmission of potential hazardous or pathogenic microorganisms, contamination of the office environment (including the computer keyboard and mouse) with bacteria is also recognized [18],[20]. From tests carried out, 95% of cultures from keyboards tested positive for though most were simple skin flora, the focus of research has been on pathogenic bacteria that pose threats to nosocomial infections.

Bacteria that are often found in a healthcare environment include coagulase negative *Staphylococcus*, *Bacillus* species, *Corynebacterium* species, streptococci, *Clostridium perfringens*, Enterococcus species, *Staphylococcus aureus*, gram negative bacteria, and fungi [21]. *Staphylococcus aureus* both a human commensal and a frequent cause of clinically important infections, including bacteremia, metastatic abscesses, septic arthritis, pneumonia, osteomyelitis and wound infections, *S. aureus* infections are frequently nosocomial and lead to increased hospital stay, antibiotic use, costs, and mortality , Certain *Staphylococcus* spp. strains are able to form biofilms on polymer surfaces and it is suggested that this property contributes significantly to the pathogenesis of staphylococcal infection. Biofilms are a population of multilayered cells growing on a surface and enclosed in the exopolysaccharide matrix [1].

The development of a biofilm is considered to be a two-step process, first, the bacteria adhere to a surface mediated by a capsular antigen, namely the capsular polysaccharide/adhesin (PS/A), then the bacteria multiply to form a multilayered biofilm, with production of polysaccharide intercellular adhesin (PIA) which mediates cell to cell adhesion and provides the protection against opsonophagocytosis and antimicrobial peptide activity [22].

The synthesis of PIA is encoded by the products of the chromosomal *ica*-genes (intercellular adhesion), which are organized in an operon structure. The operon contains the *icaADBC* genes, in addition to the *icaR* gene which exerts a regulatory function and is transcribed in the opposite direction, once this operon is activated, four proteins are transcribed, *IcaA*, *IcaD*, *IcaB* and *IcaC*, which are necessary for the synthesis of PIA [22],[23],[24], [25]. PIA is synthesized from UDP-N-acetylglucosamine by N-acetylglucosaminyl-transferase which is encoded by the *ica* locus, particularly *icaA*, the expression of this gene alone induces low enzymatic activity and production of low amount of polysaccharide. However, the simultaneous expressions of *icaA* and *icaD* promote a significant increase in N-acetylglucosaminyltransferase, with a consequent increase in the amount of polysaccharide, hence forming oligomers of 10-20 b-1,6-N-acetylglucosamine residues [26],[27], [29]. *IcaB* is the deacetylase responsible for the deacetylation of

mature PIA, in addition, the transmembrane protein *IcaC* seems to be involved in the externalization and elongation of the growing polysaccharide [30].

icaA and *icaD* responsible to formation of biofilms (slime layer) highly variable among staphylococci. Thus, slime layer formation is influenced by the environmental signals and can be induced in response to external stress and subinhibitory concentrations of certain antibiotics [3],[2],[1],[25]

II. MATERIALS AND METHODS

Source of isolation

The research was focused on the microbial studies of pathogenic bacteria on computers mice and keyboards collected from different computer labs and office of College of Nursing, University of Basrah, Iraq. To test the presence of pathogenic bacteria. The samples were collected in two groups, first group (n= 50) from computer keyboards and mice were collected and second group (n=50) from staff and student that use computer daily after sterilization hands before using the computer. The samples were collected with the help of sterile cotton swabs and added in sterile tubes of Brain Heart Infusion Broth (HIMEDIA) and streaked on Mannitol Salt Agar (ALPHA). Colonies grown after incubation were Gram stained and cultured into Nutrient Agar (ALPHA) for testing [14].

Biochemical test

- 1- Streaking onto mannitol salt agar (MSA) [14].
- 2- Gram stain slides were investigated [16].
- 2- Free coagulase Test [15].
- 3- Catalase Test [15].
- 4- Oxidase Test [15].

III. MOLECULAR GENETIC STUDY

Genomic DNA extraction

Deoxyribonucleic acid (DNA) extraction was done according to [13],[11], [12]. 5 ml of Tryptic Soy Broth (ALPHA) was inoculated with tested bacteria and incubated at 37°C for 18 h. The grown bacteria were re-washed three times by Phosphate Buffer Saline (Oxoid). The washed bacteria was resuspended in 500 µl of Tris-EDTA buffer, 30 µl of 10% Sodium Dodecyl Sulphate and 30 µl of 25 mg/ml solution of Proteinase K (Promega) and then incubated for 1 to 3 h at 37°C. 100 µl of 5 M NaCl solution was added and incubated at 65°C for 10 min. DNA was purified by two extraction with phenol: chloroform: isoamyl alcohol (24:25:1) and precipitated with 70% chilled ethanol. The DNA was resuspended in 50 µl of Tris-EDTA buffer as stock. To check for DNA, the samples were loaded in 0.8% agarose gel 1 × TBE (54 g Tris-base, 0.5 M EDTA, 1- L distilled water, PH = 8, then diluted with 400 ml of distilled water) and electrophoresed at 60V for 30 min.

Detection of *icaA* and *icaD* gene(s) by PCR:

The PCR method for amplification of *icaA* and *icaD* by thermocycler apparatus (BioRAD Co.) to detect the biofilm (as slime formation) were according [10] used the primers *icaA* gene F-5'TCTCTTGAGGAGCAATCAA3' R-5'TCAGGCACTAACATCCAGCA3' and *icaD* gene F-5'ATGGTCAAGCCCAGACAGAG-3' R-5'CGTGTTTTCAACATTTAATGCAA3'. The polymerase chain reaction (PCR) is a mixture of the final volume of 25 µl containing 5.5 µl Nuclease free water, 12.5 µl master mix promega, 10 pmol of 1 µl of Forward and 1 µl Reverse primer (BIONEER, Korea), 5 µl DNA template. The PCR program involved initial denaturation at 94°C for 5 min, 50 cycle (denaturation at 94°C for 30 sec., annealing at 55.5°C for 30 sec. and extension at 72°C for 30 sec.) and final extension at 72°C for 1 min, then soaking at 4°C to indefinite. The amplified PCR mixtures were resolved by electrophoresis through 1% agarose gel at 60V for 1.5 h prepared in 1 × TBE buffer containing 1 µl ethidium bromide in 100 ml agarose solution. Products were viewed under ultraviolet (UV) light system (Velber Lourmat, EEC France). The band of 188 bp and 198 bp was indicative to for *icaA* and *icaD* gene respectively.

IV. RESULTS AND DISCUSSION

Identification of staphylococci and biochemical test The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms on the external surface of computers mice and keyboards to create public awareness about the health hazards resulting from these pathogenic microorganisms [6],[13],[10],[7]. In this study, *Staphylococcus aureus* and other *Staphylococcus* spp. were isolated in all 100 specimens streaked on mannitol salt agar show (n=50) from computer keyboards and mice appear 42 (84%) were found to be contaminated with *Staphylococcus aureus* and 1 (2%) other *Staphylococcus* spp., and (n=50) from students and staff hands user computers daily appear 37 (74%) were found to be contaminated with *Staphylococcus aureus* and 13 (26%) other *Staphylococcus* spp. the identification of *S. aureus* was performed by the traditional biochemical tests including Gram positive bacteria, negative to oxidase test, positive to catalase test, positive to coagulase test, and mannitol fermentation test [5].

The present study showed that microbial contamination occur on computer keyboards surfaces located in a university setting and may reflect the multiple-user environment where the possibility of contamination by individuals who are carriers of bacteria such *Staphylococcus aureus* and other *Staphylococcus* spp. is greater and the isolation of viable microorganisms suggest that the species present are able to persist for a period of time on these surfaces, It is suggested that computer keyboards and mice in institutions may act as a vehicle for the transmission of pathogenic organisms [4].

Detection of *icaA* and *icaD* gene for adherence:-

In the present study, DNA of all *staphylococcus* spp. isolates were extracted and electrophoresed (Figure 1), then subjected to PCR for amplifying their *icaA* and *icaD* gene (Figure 2 and 3) respectively

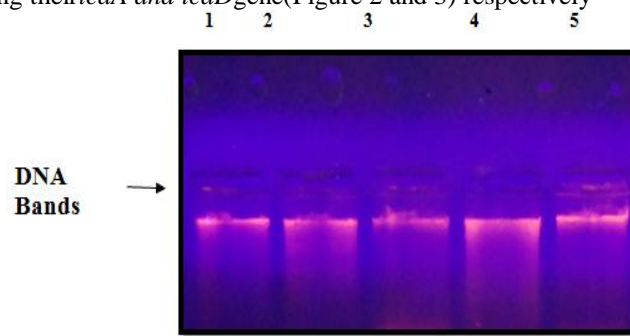


Figure (1): Agarose (0.8%) gel electrophoresis for DNA bands (1-5) of random bacterial isolates from dentures and orthodontic under UV transillumination.

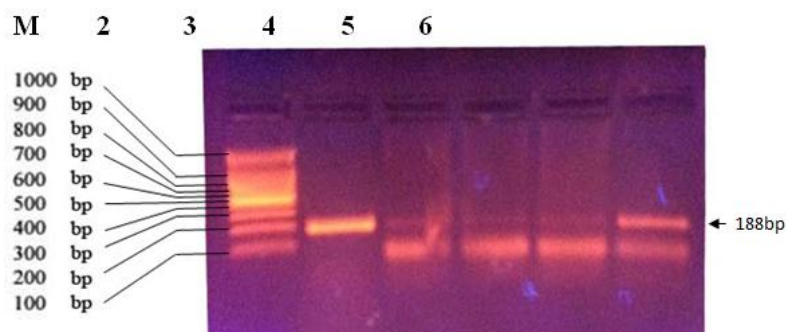


Figure (2): Agarose (1%) gel electrophoresis showed PCR product of *icaA* gene for denture and orthodontic isolates. Lane 1: (100bp-1000bp DNA marker), Lane 2 to 6: *icaA* bands (188bp) of different isolates.

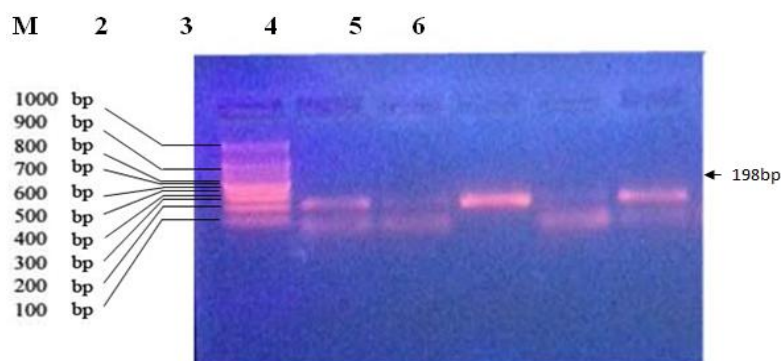


Figure (3): Agarose (1%) gel electrophoresis showed PCR product of *icaD* gene to denture and orthodontic isolates. Lane 1: 1Kb (100bp-1000bp DNA marker). Lane 2 to 6: *icaD* bands (198bp) of different isolates.

All *staphylococcus aureus* and other *staphylococcus* spp. 93 (100%) contained complete *IcaA* and *icaD* genes. This guide to these bacteria forming biofilm and adhere onto computer keyboards and mice and hands. Moreover, the expression of the *ica* operon and therefore the formation of biofilms seems to be highly variable among *staphylococci* [3]. [2]. Thus, the biofilm expression is influenced by environmental signals [1].

We observe that the level of knowledge among the computer about the possibility of microorganisms on the keyboard and mouse is very poor. Eating should be avoided while using the computers and hand washing hygiene practices should be encouraged and maintained and keyboard and mice should be cleaned with disinfectant at least weekly and should be covered where necessary. The process of disinfection is to reduce microbial load on the solid surfaces. Microbes are everywhere, including the air around us, it is therefore greatly recommended that hand-washing hygiene should be adopted before and after using the computers to reduce the microbial transmission.

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