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Genetic Study for *ica*AD 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 discoverthe 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. INTRODUCATION

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 mechanismof transfer of potentially pathogenic bacteria among users, computers continueto have an increased presence in almost every aspect of our occupational recreational and residential environments Pathogens are transmitted by carriage on hands frominanimate 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]. Fromtests carried out, 95% of cultures fromkeyboards tested positive forthough most were simpleskin flora, the focus of research has been onpathogenic bacteria that pose threats tonosocomial infections.

Bacteria that are often found in ahealthcare environment include coagulasenegative*Staphylococcus, Bacillus* species,Corynebacterium species, streptococci, *Clostridiumperfringens*, Enterococcusspecies, *Staphylococcus aureus*, gramnegative bacteria, and fungi [21] .*Staphylococcus aureus*is both a human commensal and a frequent cause of clinically importantinfections, including bacteremia, metastatic abscesses, septic arthritis, pneumonia, osteomyelitis and wound infections,*S. aureus*infections are frequently nosocomial and lead to increasedhospital 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 polysac-charide intercellular adhesin (PIA) which mediates cell to cell adhesion and provides the protection against opsonophagocytosis and antimicrobial peptideactivity [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 con-tains the *ica*ADBC genes, in addition to the *ica*Rgene which exerts a regulatory function and is transcribed in the opposite direction, once this operon is activated, four proteins are transcribed, *Ica*A, *Ica*D, *Ica*B and *Ica*C, 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 *ica*A , the expression of this gene alone induces low enzymatic activity and production of low amount of poly-saccharide. However, the simultaneous expressions of *ica*A and *ica*D promote a significant increase in N-acetylglucosaminyltransferase, with a con-sequent increase in the amount of polysaccharide, hence forming oligomers of 10-20 b-1,6-Nacetylglucosamine residues [26],[27], [29]. *Ica*B is the deacetylase responsible for the deacetylation of

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

*ica*A and icaD responsible to formation of biofilms (slim 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 microbialstudies of pathogenic bacteria oncomputers mice and keyboards collectedfrom different computer labs and office ofCollege of Nursing,University of Basrah,Iraq.To test the presence of pathogenicbacteria.The samples were collectedin two groups,first group(n= 50) from computerkeyboards and mice were collected and second group(n=50) from staff and student that user computer daily aftersterilizationhandsbefore using thecomputer.The samples werecollected with the help of sterile cottonswabsandadded 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 ml of Tris-EDTA buffer, 30 µl of 10% Sodium DodecyleSulphate 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 slim formation) were according[10]used the primers*icaA* gene F-5'TCTCTTGCAGGAGCAATCAA3' 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 25µl containing 5.5µl Nuclease free water , 12.5µl master mixpromega ,10pmolof 1µl of Forward and1µlReverse primer(BIONEER ,Korea), 5µl DNA template .The PCR program involved initial denaturation at 94°C for 5 min, 50 cycle (denaturation at 94°C for 30sec., annealing at 55.5°C for 30 sec. and extension at 72°C for 30sec.) and final extension at 72°C for 1min, 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 µlethidium bromide in 100 ml agarose solution. Products were viewed under ultraviolet (UV) light system (VelberLourmat,EEC France). The band of 188bp and 198bp was indicative to for *icaA*and*icaD* gene respectively .

IV. RESULTS AND DISCUSSION

Dentification of staphylococci and biochemical testThe main objective of the presentmicrobial study was to isolate and identifythe pathogenic microorganisms on theexternal surface of computers mice andkeyboards to create public awarenessabout the health hazards resulting fromthese pathogenic microorganisms[6].[13].[10].[7].In this study, *staphylococcusaureus* and other *staphylococcus*spp.were isolated inall 100 specimen streaked on manitol salt agar show (n=50)from computerkeyboards and miceappear 42(84%)were found to be contaminated with*staphylococcusaureus* and 1(2%) other *staphylococcus ssp*. ,and (n=50) from students and staff hands user computers daily appear 37(74%) were found to be contaminated with*staphylococcusaureus* and 13(26%) other *staphylococcus* ssp.the identification of*S.aureus* was performed by the traditional biochemical tests including Grampositive bacteria , negative to oxidase test , positive to catalase test ,postive to coagulase test , and mannitol fermentation test [5].

The present study showed that microbial contamination occur on computerkeyboards 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*andother *staphylococcus*ssp.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.isolateswere extracted and electrophoreses (Figure 1), then subjected to PCR for amplifying their*icaA and icaD*gene(Figure 2 and 3) respectively

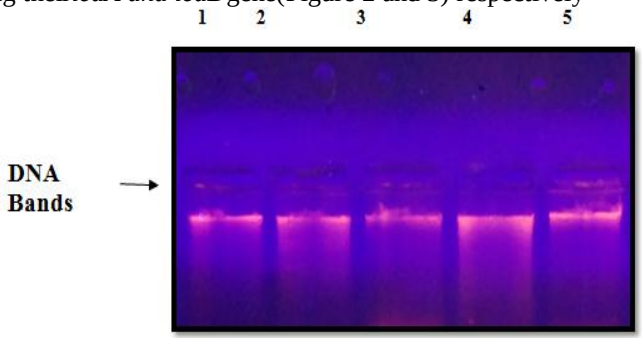

Figure (1): Agarose (0.8%) gel electrophoresis for DNA bands (1-5) of random bacterial isolates from dentures and orthodontic under UV transilluminator.

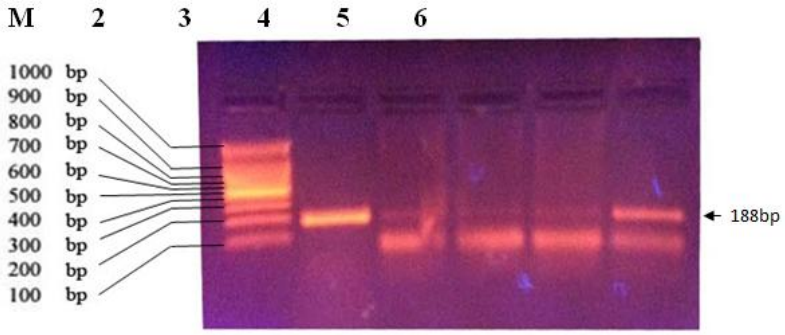

Figure (2): Agarose (1%)gel electrophoresis showed PCR product of *icaA* gene for denture and orthodontic isolates.Lane 1: (100bp-1000bp DNA marker),Lane 2 to 6: *icaA* bands (188bp) of different isolates.

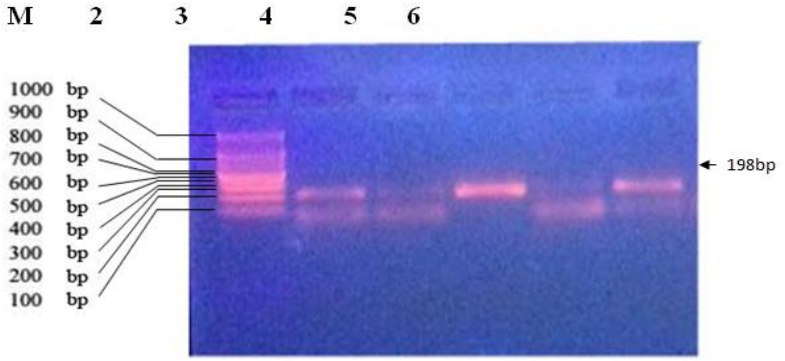

Figure (3): Agarose (1%) gel electrophoresis showed PCR product of *icaD* gene to denture and orthodontic isolates.Lane 1: 1Kb (100bp-1000bp DNA marker).Lane 2 to 6: *icaD*bands(198bp) of different isolates.

All staphylococcus aureus and other staphylococcus spp. 93 (100%)contained complete *IcaA* and *icaD* genesthis 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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