

***Genotypic Characterization of *Staphylococcus* spp. Isolated from the Bodies of Workers in Units of MRI, CAT, X-Ray, Restaurants', and Testing Their Ability to Biofilms Formation**

Asst. Prof. Ali Abd Raheem Al-Nashe
ali.alnashe@qu.edu.iq

Saif Lateef Shakir
saif.lateef@gmail.com

Department of Biology/ College of Education/ University of Al-Qadisiyah/ Iraq

Abstract

The present study aimed to isolating and diagnosing of *Staphylococcus* spp., which forms the biofilm from different areas of males and females workers bodies in units of: magnetic resonance imaging (MRI), computerized axial tomography (CAT) and x-ray as well as restaurants, using some phenotypic and genotypic characterization methods as well as testing their ability to phenotypically and genetically of biofilms formation. Isolates were collected within the range of hospitals in Al-Diwaniyah Governorate at the period from 1/11/2016 till 1/4/2017 and a total of 200 samples distributed equally on predefined isolation units. The results showed that superior the bacterial isolation percent of male workers in these units exceeded the females at all sources of isolation.

Staphylococcus isolates were diagnosed by using the API-20 Staph system and the species of genus (*Staph. epidermidis*, *Staph. haemolyticus* and *Staph. aureus*) have been affiliated a higher frequency of isolates number (57 isolates), which were divided between 23, 21 and 13 isolates, respectively. The isolates consisted of biofilm formation were 13 isolates from *Staph. epidermidis*, 16 isolates from *Staph. haemolyticus* and 10 isolates of *Staph. aureus*. Genetic diagnosis of all isolates was done by using the PCR technique in terms of having a 16S rRNA gene as well as investigating the ability of *Staphylococcus* isolates to biofilms formation in terms of having the *icaA* gene. The results of phenotypic investigation were identical to the results of genetic investigation.

Keywords: Genotypic characterization; *Staphylococcus*; Biofilms formations.

Introduction

Staphylococci of positive Gram stain were represents the bulk of the normal flora on the skin surface, the mucous surfaces of respiratory tract, the upper part of the digestive system and the urogenital tract ⁽¹⁾. The human pathogen is the most adaptive and capable of causing a wide range of human diseases, ranging from abscesses and impetigo to more invasive and serious infections such as: osteomyelitis, septic arthritis, pneumonia and endocarditis ⁽²⁾. The ability of *Staphylococcus* to cause disease has been attributed to a range of virulence factors had a wide spectrum effects, which are associated with cell walls such as protein A, clumping factors, fibronectin binding proteins, and other cellular adhesives, as well as exotoxins: coagulase, haemolysin, Shock syndrome toxin-1, exfoliative toxins, and leucocidin toxins as the determinants of virulence ⁽³⁾. The evolution of the disease in *Staphylococcus aureus* is the result of interaction between a variety of host factors and bacterial virulence determinants ⁽⁴⁾.

Biofilms represent the binding points of bacteria to the surfaces of living and non-living materials, these membranes are well arranged in clusters of one or several types of bacteria. They consist of one layer continuous or several layers and have a three-dimensional structure or may take other forms ⁽⁵⁾. The bacterial biofilm had given protection against severe conditions and is responsible for 65% of pathological infections, including urinary tract infection, middle ear, gum, tooth decay as well as injuries related to medical instruments ⁽⁶⁾. The formation of biofilms is one of the most virulent factors that facilitate staphylococcal involvement and tissue stabilization, it contributes to the elimination of immune host defenses, resulting in continuous and recurrent infections due to the difficulty of eliminating pathogens ⁽⁷⁾. Coagulase-negative staphylococci, which is a constituent of biofilms

* The research is a part of M.Sc. thesis in the case of the second researcher.

increases antibiotic resistance about 100 to 1000 times more than an biofilm formation ⁽⁸⁾. This membrane is formed by the adhesion of intercellular polysaccharides and is encoded by *icaADBC* operon ⁽⁹⁾, and a major clinical problem mainly due to the high levels of antimicrobial therapy as well as the immune resistance of the host ⁽¹⁰⁾.

The human body is affected when exposed to radiation; radiation causes significant damage to the DNA level, thus causing changes in genes, which are reflected in birth defects, cancer tumors and death ^(11, 12). γ -ray and x-rays come on the list of the most dangerous radiation on living cells because they have the ability to penetrate and transmit energy by electromagnetic waves that velocity of light converges at its speed, as well as moving in the vacuum ⁽¹³⁾. The effect of the magnetic field on the synthesis of DNA and on ionic transcription of the membrane ⁽¹⁴⁾. Novak et al. ⁽¹⁵⁾ shows that the great influence of the magnetic field on the bacterial cell structure is more pronounced on the properties and the cell membrane's viability. While Kamel et al. ⁽¹⁶⁾ were found that *Staphylococcus aureus* is exposed to a stable magnetic field at different stresses (400, 800, 1200 and 1600) Gauss, which inhibits growth, and this effect varies depending on the type of microorganism and magnetic field strength.

The Aim of the Study

Due to the effect of radiographs by MRI, CAT and x-ray on the molecular level of *Staphylococcus aureus*, and the resulting of potential for increased pathogenic factors and the difficulty of controlling them with antibacterial agents, the study showed a genotypic characterization of *Staphylococcus* isolated of workers bodies in units: MRI, CAT, x-ray and restaurants as well as tested their ability to biofilms formation.

Materials and methods

a- Collection of samples: The samples were collected from hospitals in Diwaniya city: Diwaniya Teaching Hospital, Maternity and Children Teaching Hospital in Al-Diwaniya, Al-Hussein hospital for children, General Hospitals Al Hamzah, General Hospitals Afek and General Hospitals Al-Shamiya from different areas of workers body in MRI, CAT, x-ray and restaurants, for a period of (1/11/2016) till (1/4/2017), with a total of (200) samples, distributed as follows:

- 50 samples of people working in the MRI unit.
- 50 samples of people working in the CAT unit.
- 50 samples of people working in the x-ray unit.
- 50 samples of people working in restaurants.

Wipes were taken from different areas including: nose, ear, skin, wounds and urine with different age of both sexes. transport media swabs (Amies) were used in the isolation process to ensure their vitality.

b- Culture of samples: The swabs were transferred directly to the laboratory within 20-30 minutes. The samples were cultured on media of blood and MacConkey agar, then incubated at 37°C for 24 hours, as well as incubation of media that did not show growth within 24 hours for another 24 hours before being negative.

c- Diagnosis of bacterial isolate by API-20 Staph. system: Use the API-20 Staph. system which is manufactured by BioMerieux company/ France to diagnose isolates under study. This system is used to diagnose all species of *Staphylococcus* to obtain reliable results and to distinguish the bacterial species produced coagulase by *Staph. aureus* is the other species were produced this enzyme ⁽¹⁷⁾.

d- Biofilm formation test: by transferring the growing bacterial colonies to a glass test tube containing Tryptic soy broth with 1% glucose, incubated at 37° C for 24 hours, and then pouring the samples and washing the tubes with a sterile saline phosphate solution, drying and dyeing them with Crystalline violet dyes and safranin for three minutes, after which the excess dye is washed with distilled water, then placed upside down to dry, and the observed biofilms formation in the bottom of the pipes and inner walls in a violet layer ⁽¹⁸⁾.

e- Polymerase chain reaction test: the test was conducted to investigate some of the causes of virulence and antibiotic resistance in *Staphylococcus* (*Staph. epidermidis*, *Staph. haemolyticus* and *Staph. aureus*), as follows:

1. Bacterial genomic DNA extraction according to the genomic DNA Extraction Kit (Presto™ Mini gDNA Bacteria Kit) and instructions supplied by the American Generaid Company.
2. DNA Examination by the Nano drop device for the detection and measurement of nucleic acid concentrations. It is detected by determining the concentration of the DNA (ng/μl) and measuring its purity by reading the absorbance at a wavelength of 260-280 nm.
3. Preparation of PCR Master Mix by using the AccuPower® PCR PreMix kit and its processed instructions from Bioneer (South Korea).
4. Thermocycler Program to DNA amplified was programmed device according to the Townsend et al.⁽¹⁹⁾ method for each primer. The thermocyclers for each primer were repeated to 30 cycles.
5. Preparation of agarose gel according to method of Sambrook et al.⁽²⁰⁾.
6. Agarose gel electrophoresis (1.5%) under 100 volts and 80 mA at 60 minute for detection of extracted DNA bands and amplified DNA representing amplified sizes or PCR products, according to method of Sambrouk et al.⁽²⁰⁾.

g- Statistical analysis: all data of the results were arrangement and classified by Microsoft office excel-2013 program, and the results data were analyzed by using the tool of Data Analysis in the Analysis ToolPak of add-on scheduling software⁽²¹⁾. Completely randomized design (CRD) was depended as an experimental design of the results data, as well as a comparison of the means using the least significant difference (LSD) test to determine the significant differences at the probability level ($P \leq 0.05$)⁽²²⁾.

Results

1- Isolation and Diagnosis

The current study aimed to isolation and diagnosis of *Staphylococcus* bacteria from different areas of workers bodies in the units: MRI, CAT and x-ray as well as the restaurants with a total of 200 samples were distributed equally among these four groups, covering different areas of the body such as: nose, ear, skin, wounds and urine with different ages of both sexes (males and females). The results of the statistical analysis of primary isolation (Fig. 1) at ($P < 0.05$) showed the highest male isolation percentage compared to females for bacterial isolates in units of MRI, CAT and x-ray. While the percentage of females was not in restaurants compared with high percentage of male, which achieved the highest percentage of the other groups under study, and that the percentage of males to females somewhat converged in MRI unit and the same applies to the units of CAT and x-ray of where the percentage of females in both groups as well as males with the difference between them.

The results of the distribution of bacterial isolates according to the sex of workers from the sources of isolation can be explained by the nature of workers in these units and the consequent frequent cleaning or sterilization operations, as well as the nature of work and its specific type of male sex compared with females as in restaurants. Among other, the process of direct contact between patients and staff in the units under study may be the cause of this isolation result due to its role in transmitting pathogens within MRI, CAT and x-ray which are characterized by a high level of cleanliness and sterilization unlike other low-level restaurants in that area. On the other hand, bacterial isolates of *Staphylococcus* species were diagnosed by using API-20 Staph. system due to it was characterized by speed and accuracy in the detection of bacterial species as well as the reduction of the process of cultural pollution (Photo 1). The interactions of bacterial isolates in conformity with the tests of this system, and then translated to the corresponding numbers of the result of interactions that were interpreted by the analytical analysis index with API-20 Staph. system.

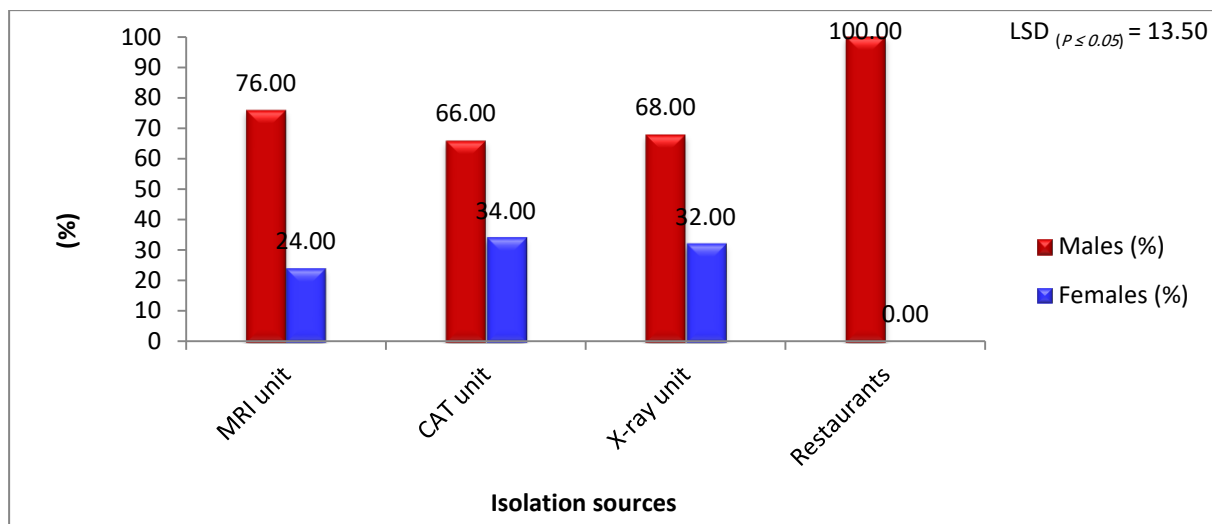


Fig. 1: Distribution of bacterial isolates by sex of workers from isolation sources (n = 200)



Photo 1: Results of diagnosis by API-20 Staph. system for *Staphylococcus* species

When comparing the isolation percentage between species of *Staphylococcus* genus (Fig. 2), the significant superiority ($P < 0.05$) was evident among them, with the first three species of high percentages: *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. aureus* (33.33, 30.43 and 18.84)% respectively, with a significant difference between them, as well as the lower percentages of other species (8.70, 7.25 and 1.45)%, as shown in the same figure

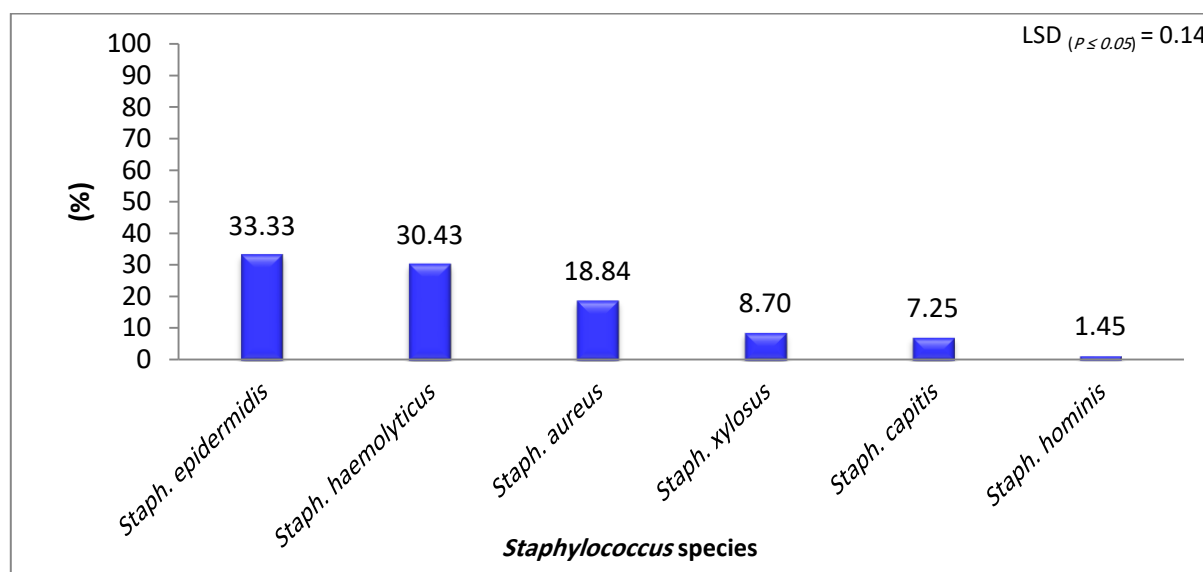


Fig. 2: Distribution of *Staphylococcus* isolates by species (n = 69)

The reason for recording the highest isolation percent of the *Staphylococcus* species under study is due to its presence as a normal flora in the body and a broad-spectrum pathogen that spreads widely throughout the world. In addition, with the increasing colonization of species, opportunistic strains are increasingly present and present, Immunohistochemistry of the host and the acquisition of pathogens are a combination of virulence factors with direct pathogenicity as well as a number of major causes of acquired infections in society and hospitals^(23, 24). When compared with the results of previous studies, the results are consistent with Jabbar⁽²⁵⁾, Jawad⁽²⁶⁾, Aziz⁽²⁷⁾, Ocoru et al.⁽²⁸⁾ and Hassan⁽²⁹⁾ in terms of recording the highest percentage of isolates of the *Staphylococcus* from the specific areas of the body under study (nose, ear, skin, urine and wounds). The reason for this to *Staphylococcus* species have many factors that increase their virulence and make them able to exist in different environments; enzymes are one of the virulence factors produced by bacteria (catalase and urease) and break down complex organic compounds and analyze them into their primary components⁽³⁰⁾. In addition, adapting them to low temperatures (10° C) and dry or wet living conditions as well as high salinity on the one hand, and having the cell wall as osmotic protection and the formation of some strains to the capsule from the other hand is one of the most important means of defense and protection against chemical and mechanical damage^(31, 32).

2- Biofilm formation

The ability of bacterial isolates from is different sources of isolation on the biofilm formation is illustrated by its statistical significance in table (1). The total number of biofilm formation isolates is 40 isolates (70.18%) out of 57 isolates, in addition, 14 isolates of 23 isolates affiliated to *Staph. epidermidis* formed a biofilm (60.87%) of the total number while there were 5 isolates (23.80%) only of 21 isolates for *Staph. haemolyticus* is don't made up of the biofilm, along with the composition of the majority of *Staph. aureus* isolates of the biofilm 10 isolates (76.92%) except 3 isolates (23.07%) were only unable to form the biofilm. When comparing the results of the present study with the results of other studies, they are consistent with the results of Silva et al.⁽³³⁾ regarding isolates of *Staph. haemolyticus*, which a biofilm formation with 60% compared to 57.14% for current study isolates, and

is consistent with the results of Al-Omari et al. ⁽³⁴⁾ regarding isolates of *Staph. aureus* which a biofilm formation with 87.50% versus 76.92% for under study isolates. *Staph. epidermidis* isolates which a biofilm formation with 43.47% it came a little higher than the results of Al-Hilu ⁽³⁵⁾ with 26% and the results of Hassan ⁽²⁹⁾ with 25% of the above-mentioned bacteria, and less than the results of Gad et al. ⁽³⁶⁾ which reached 88.60%. The reason for the formation of biofilm by the bacterial isolates under study is considered one of the most aggressive virulence factors because it indicates the presence of bacteria in polluted and pathogenic environments ⁽³⁷⁾, and it work to protect it from the immune inhibitors of the host by concentrates nutrients, protecting bacterial cells from antibiotics and pharyngeal cells ⁽³⁸⁾. On the other hand, Staphylococcus has the ability to settle inside the host and adhere to live surfaces by forming of biofilm that protect them from host immunological defenses and antimicrobial therapy ⁽³⁹⁾.

Genetic investigation of the diagnostic gene for each species of *Staphylococcus* under study as well as the gene responsible for the formation of biofilms were consistent with the results of phenotypic investigation. Moreover, the earlier explanations related to the results of phenotypic investigation were fully consistent with the results of genetic screening; photo (2) showed that the agarose gel electrophoresis containing the results of the polymerase chain reaction assay proved that all isolates were present had the 16SrRNA, which in turn represented the house-keeping for each bacterium and bore weight of 475 base pairs of *Staph. epidermidis*, 407 base pairs of *Staph. haemolyticus* and 556 base pairs of *Staph. aureus*, respectively ⁽⁴⁰⁾, which proves the identity of each of the three bacterial species.

Some isolates under study have the *icaA* gene (photos 3,4 and 5), which is encoded for the formation of biofilm, are attributed to the same reasons mentioned in the results of phenotypic investigation as well as the formation of biofilm, which was obtained by Prasad et al. ⁽⁴¹⁾ on *Staph. epidermidis* and Pinheiro et al. ⁽⁴²⁾ on *Staph. epidermidis* and *Staph. haemolyticus*, Duran et al. ⁽⁴³⁾ and Santos et al. ⁽⁴⁴⁾ on *Staph. aureus*. The difference in results from previous studies results may be due to the effect of radiation at the genetic level in the creation of a mutation leading to the loss of a particular gene or acquisition of a new gene with characteristics different from that in their natural strains

Table 1: The ability of bacterial isolates according to the sources of their isolation on phenotypic biofilm formation

Bacterial isolates according to isolation sources		Total number	Isolates of biofilm formation	
			n	%
<i>Staph. epidermidis</i>	MRI unit	23	5	21.74
	CAT unit		5	21.74
	X-ray unit		3	13.04
	Restaurants		1	4.35
<i>Staph. haemolyticus</i>	MRI unit	21	3	14.29
	CAT unit		4	19.05
	X-ray unit		4	19.05
	Restaurants		5	23.81
<i>Staph. aureus</i>	MRI unit	13	2	15.38
	CAT unit		1	7.69
	X-ray unit		3	23.08
	Restaurants		4	30.77
Total		57	40	70.18

LSD ($P \leq 0.05$) = 0.004

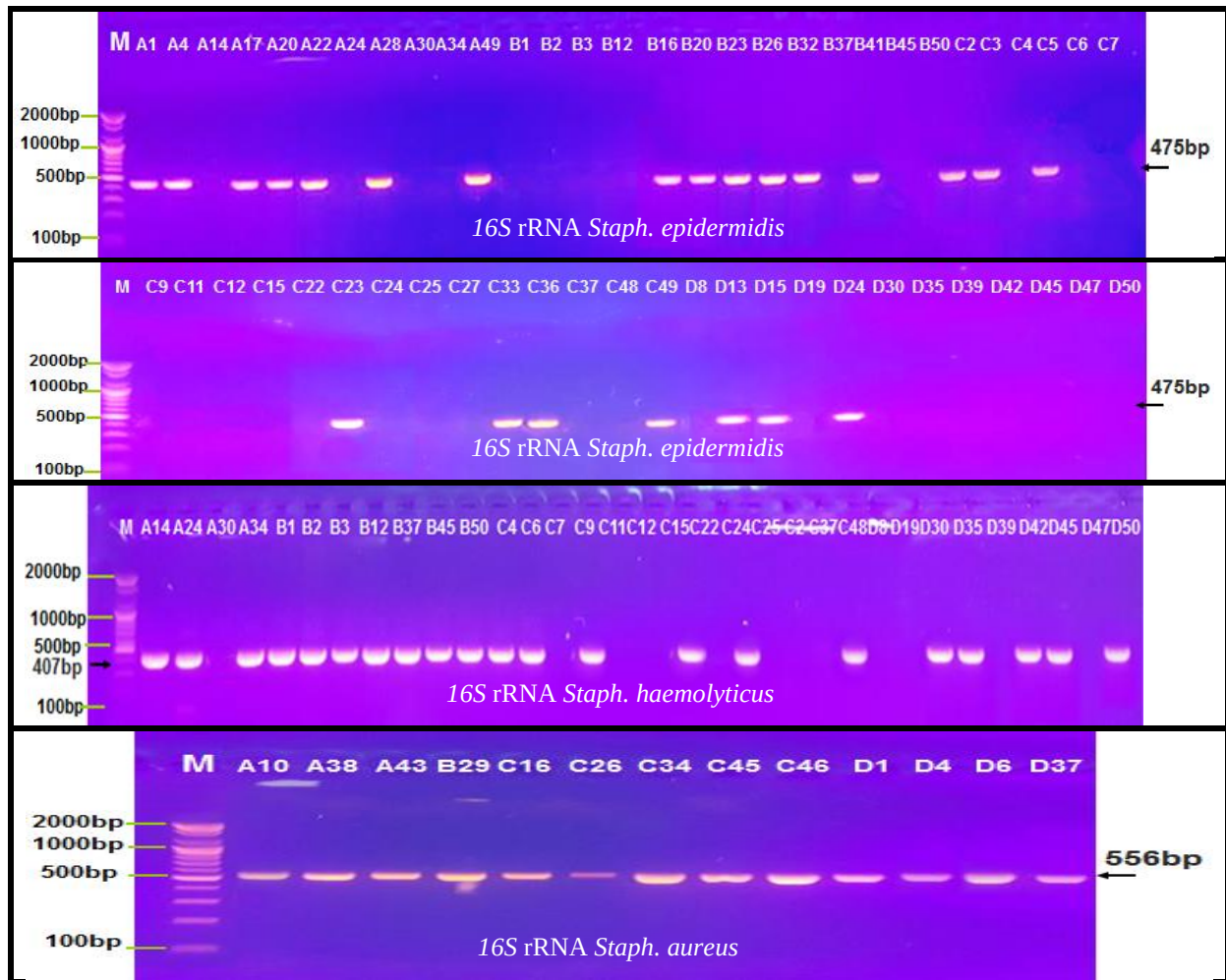


Photo 2: Agarose gel electrophoresis containing the results of PCR to investigation of 16SrRNA for *Staphylococcus* species, M (Marker) from 100-2000 base pair (bp), while symbols refer to the numbering of isolates

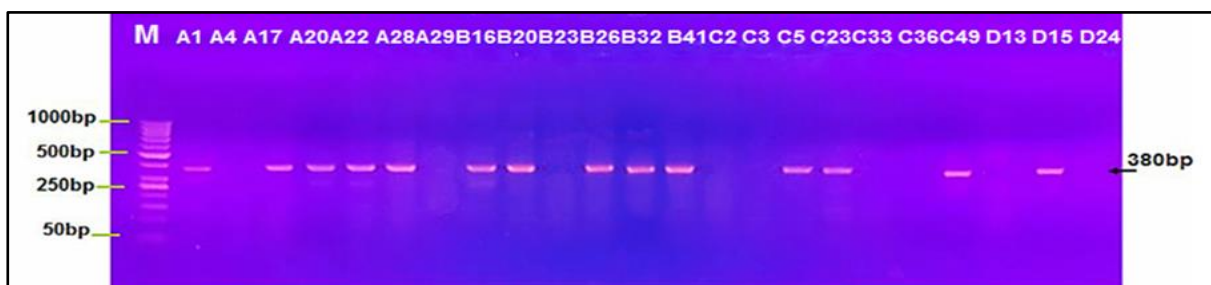


Photo 3: Agarose gel electrophoresis containing the results of PCR to investigation of *icaA* in *Staph. epidermidis*, M (Marker) from 50-1000 base pair (bp), while symbols refer to the numbering of isolates

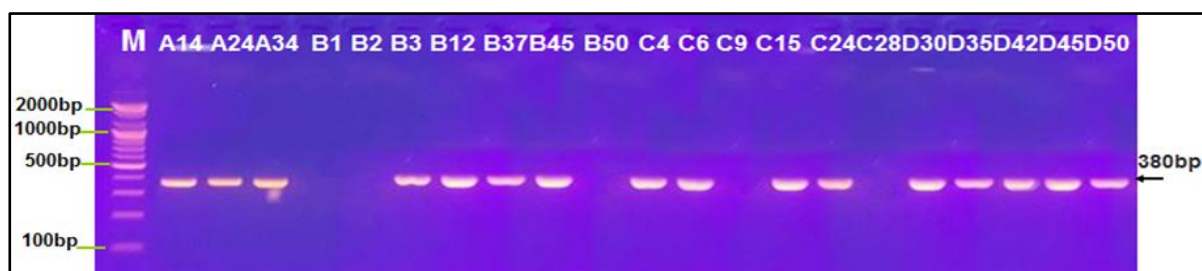


Photo 4: Agarose gel electrophoresis containing the results of PCR to investigation of *icaA* in *Staph. haemolyticus*, M (Marker) from 100-2000 base pair (bp), while symbols refer to the numbering of isolates



Photo 5: Agarose gel electrophoresis containing the results of PCR to investigation of *icaA* in *Staph. aureus*, M (Marker) from 100-2000 base pair (bp), while symbols refer to the numbering of isolates

Conclusion

The bacterial isolation percent of males was higher than that of females in the units of MRI, CAT and x-ray, as well as restaurants where there was no percent of female. *Staphylococcus* species (*Staph. epidermidis*, *Staph. haemolyticus* and *Staph. aureus*) had the highest isolation percent recorded in the current study. The results of the genetic investigation were consistent with the results of the phenotypic investigation in terms of the isolates had the encoded gene to biofilm formation (*icaA*), as well as the diagnostic gene (*16SrRNA*) for each species of *Staphylococcus*.

References:

1. El-Jakee, J.; Ata, S.; Nagwa, M.; Bakry, S.A.; Zouelfakar, E.; Elgabry, V. and Gad El-Said, W.A. (2008). Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. American-Eurasian J. Agric. Environ. Sci., 4(2): 221-229.
2. Anderson, M.; Lefebvre, S. and Weese, J. (2008). Evaluation of prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel attending an international equine veterinary conference. Vet. Microbiol., 129(3-4): 410-417.
3. Plata, K.; Rosato, A.E. and Wegrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. Acta Biochimica Polonica, 56(4): 597-612.
4. Jarraud, S.; Mougé, C.; Thioulouse, J.; Lina, G.; Meugnier, H.; Forey, F.; Nesme, X.; Etienne, J. and Vandenesch, F. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect. Immun., 70: 631-641.
5. Bagge, D.; Hjelm, M.; Johansen, C.; Huber, I. and Gram, L. (2001). *Shewanella putrefaciens* adhesion and biofilm formation on food processing surfaces. Appl. Environ. Microbiol., 67(5): 2319-2325.
6. Ansari, S.A.; Baqai, R.; Memon, M.R.; Aziz, M. and Khan, M.K. (2011). Biofilm formation and isolation of *Staphylococcus aureus* from blood culture of patient undergoing oral surgical procedures. J. Pak. Dent. Assoc., 20(3): 181-184.

7. Darwish, S.F. and Asfour, H.A. (2013). Investigation of biofilm forming ability in *Staphylococci* causing bovin mastitis using phenotyping and genotypic assay. *Sci. World J.*, 2013: 1-9.
8. Forrest, G.N. and Tamura, K. (2010). Rifampin combination therapy for non-mycobacterial infection. *Clin. Microbiol. Rev.*, 23: 14-34.
9. O'Gara, J.P.; Humphreys, H. and Kapila, K. (2001). *Staphylococcus epidermidis* biofilms: importance and implications. *J. Med. Microbiol.*, 50: 582-587.
10. Jefferson, K.K. (2004). What drives bacteria to produce a biofilm. *FEMS. Microbiol. Lett.*, 236(2): 163-173.
11. Enka (1982). Introduction to Nuclear Physics. Translated by Assem Azzoz, Dar Al-Kuttab Foundation Press, University of Mosul, Iraq. (in Arabic).
12. Al-Dhahir, A. (1983). Risks of Atomic Radiation. *J. Arab Culture*, 1(2): 93 - 98. (in Arabic).
13. Andrei, T.; Kenyl, E.P. and James, M.S. (2006). Effect of gamma irradiation viability and DNA of *Staphylococcus epidermidis* and *Escherichia coli*. *J. Med. Microbiol.*, 55: 1271-1275.
14. Liburdy, R.P.; Callahan, D.E.; Harland, J.; Dunham, E.; Sloma, T.R. and Yaswen, P. (1993). Experimental evidence for 60 Hz magnetic fields operating through the signal transduction cascade: effects on calcium influx and c-MYC mRNA induction. *FEBS. Lett.*, 334: 301-308.
15. Novak, J.; Strasak, L.; Fojt, L.; Slaninova, I. and Vetterl, V. (2007). Effects of low-frequency magnetic fields on the viability of yeast *Saccharomyces cerevisiae*. *Bioelectrochem.*, 70(1): 115-121.
16. Kamel, F.H.; Saeed, G.H. and Qader, S.S. (2014). Magnetic field effect on growth and antibiotic susceptibility of *Staphylococcus aureus*. *J. Al-Nahrain Univ.*, 17(3): 138-143.
17. Greenwood, D. (2012). Medical Microbiology, With Studentconsult Online Access, 18: Medical Microbiology. Elsevier Health Sciences.
18. Hassan, A.; Usman, J.; Kaleem, F.; Omair, M.; Khalid, A. and Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *Braz. J. Infect. Dis.*, 15(4): 305-311.
19. Townsend, K.M.; Boyce, J.D.; Chung, J.Y.; Frost, A.J. and Adler, B. (2001). Genetic organization of *Pasteurella multocida* cap Loci and development of a multiplex capsular PCR typing system. *J. Clin. Microbiol.*, 39: 924-929.
20. Sambrook, J.; Maccallum, P. and Russel, D. (2001). Molecular Cloning: a Laboratory Manual, 3rd ed, Cold Springs Harbour Press, New York, p: 2344.
21. Al-Jassar, A.J. (2016). Principles of Statistics with Practical Applications by Using EXCEL 2013. Al-Jasoor for Training and Statistical Consulting Co. Ltd., First Edition, Iraq, 80 pages. (in Arabic).
22. Steel, R.G.D.; Torrie, J.H. and Dickey, D.A. (1997). Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw-Hill Companeis, Inc., New York, USA.
23. Stryjewski, M.E. and Corey, G.R. (2014). Methicillin-resistant *Staphylococcus aureus*. anevolving pathogen. *Clin. Infect. Dis.*, 58(1): 9-10.
24. Zasshi, K. (2004). Epidemiological investigation of beta-lactam antibiotic induced vancomycin-resistant MRSA from clinical isolated MRSA-comparition of detection rate of BIVR with or without CZX. *Int. J. Antimicrob. Agents*, 78(8): 717-721.
25. Jabbar, A.A. (2013). Bacteriological and genetic study on *Staphylococcus aureus* isolated from patients with diabetes in AL-Diwaniya city. M.Sc. Thesis, College of Education, University of Al-Qadisiyah, Iraq. (in Arabic).
26. Jawad, M.R. (2013). Detection of some genes responsible for virulence and antibiotic resistance in *Staphylococcus aureus* isolated from different clinical cases in the city of AL-Diwaniya. M.Sc. Thesis, Faculty of Science, University of Al-Qadisiyah, Iraq. (in Arabic).
27. Aziz, S.A. (2013). Bacterial urinary tract infection in diabetic patients in Al-Najaf city. M.Sc. Thesis, Collage of Medicine, University of Kufa, Iraq.
28. Ocokoru, C.; Onzima, R.; Govule, P. and Katongole, S. (2015). Prevalence and drug susceptibility of isolates of urinary tract infections among febrile under-fives in Nsambya Hospital, Uganda. *J. Open Sci. Clin. Med.*, 3(6): 199-204.

29. Hassan, R.W. (2017). Molecular study of genes responsible for the production of hemolysin in bacteria causing urinary tract infections and resistance to control factors. M.Sc. Thesis, College of Education, University of Al-Qadisiyah, Iraq. (in Arabic).
30. Baron, E.J. and Tenover, S.M. (1995). Diagnostic Microbiology. 1st ed., Bailey and Scott's, Mosbey Company, Baltimore, Philadelphia, Toronto, USA.
31. O'Riordan, K. and Lee, J.C. (2004). *Staphylococcus aureus* capsular polysaccharides. J. Clin. Microbiol. Infect. Dis., 17(1): 218-234.
32. MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd ed., The Williams and Wilkins, London, UK.
33. Pinheiro, L.; Brito, C.I.; de Oliveira, A.; Martins, P.Y.F.; Pereira, V.C. and da Cunha, M.D.L.R.D.S. (2015). *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*: molecular detection of cytotoxin and enterotoxin genes. Toxins, 7(9): 3688-3699.
34. Silva, P.V.; Cruz, R.S.; Keim, L.S.; De Paula, G.R.; Carvalho, B.T.F.; Coelho, L.R.; Carvalho, M.C.D.; Rosa, J.M.C.; Figueredo, A.M. and Teixeira, L.A. (2013). The antimicrobial susceptibility, biofilm formation and genotypic profiles of *Staphylococcus haemolyticus* from bloodstream infections. Mem. Inst. Oswaldo Cruz. Rio de Janeiro., 108(6): 812-816.
35. Al-Omari, A.W.; Mohamad, A.M. and Raoof, W.M. (2013). Detection of biofilm formation in some pathogenic bacteria using tube and Congo red agar methods. J. Rafidain Sci., 24(6): 55-65.
36. Al-Hilu, S.A. (2014). Molecular study of virulence factors of some coagulase negative *Staphylococci* isolated from different infections. Ph.D. Thesis, Faculty of Science, University of Kufa, Iraq.
37. Gad, M.G.F.; El-Feky, M.A.; El-Rehewy, M.S.; Hassan, M.A.; Abolella, H. and Abd El-Baky, R.M. (2009). Detection of *icaA*, *icaD* genes and biofilm production by *Staphylococcus epidermidis* isolated from urinary tract catheterized patient. J. Infect. Dev. Countries, 3(5): 342-351.
38. Hola, V.; Ruzicka, F. and Votava, M. (2006). The dynamics of *Staphylococcus epidermis* biofilm formation in relation to nutrition, temperature and time. Scripta Medica, 79(3): 169-174.
39. Garofalo, C.K.; Hooton, T.M.; Martin, S.M.; Stamm, W.E.; Palermo, J.J.; Gordon, J.I. and Hultgren, S.J. (2007). *Escherichia coli* from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation. Infect. Immun., 75(1): 52-60.
40. Ermolaeva, S.A.; Varfolomeev, A.F.; Chernukha, M.Y.; Yurov, D.S.; Vasiliev, M.M.; Kaminskaya, A.A. and Shimizu, T. (2011). Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. J. Med. Microbiol., 60(1): 75-83.
41. Prasad, S.; Nayak, N.; Satpathy, G.; Nag, H.L.; Venkatesh, P.; Ramakrishnan, S.; Ghose, S. and Nag, T.C. (2012). Molecular and phenotypic characterization of *Staphylococcus epidermidis* in implant related infection. Ind. J. Med. Res., 136(3): 483-490.
42. Ghebremedhin, B.; Layer, F.; Konig, W. and Konig, B. (2008). Genetic classification and distinguishing of *Staphylococcus* species based on different partial *gap*, *16S* rRNA, *hsp60*, *rpoB*, *sodA*, and *tuf* gene sequences. J. Clin. Microbiol., 46(3): 1019-1025.
43. Duran, N.; Ozer, B.; Duran, G.G.; Onlen, Y. and Demir, C. (2012). Antibiotic resistance genes & susceptibility patterns in staphylococci. Ind. J. Med. Res., 135: 389-396.
44. Santos, V.M.; Martins, H.B.; Rezende, I.S.; Barbosa, M.S.; Andrade, E.F.; Souza, S.G.; Campos, G.B.; Oliveira, P.S.; Sousa, D.S.; Da Silva, D.C.C.; Amorim, A.T.; Timenetsky, J.; Cruz, M.P.; Yatsuda, R. and Marques, L.M. (2014). Virulence factor profile of *Staphylococcus aureus* isolated from Bovine milk from Brazil. Food and Nutr. Sci., 5: 1496-1505.



Research Journal of Pharmacy and Technology

Scopus coverage years: 1997, 2005, from 2011 to Present

Publisher: A and V Publication

ISSN: 0974-3618 E-ISSN: 0974-360X

<http://www.scimagojr.com/journalsearch.php?q=21100197160&tip=sid&clean=0>

Acceptance Letter

27 December 2017

Dear Author(s)

Dr./Mr(s). Ali Abd Raheem Al-Nashe / University of Al-Qadisiyah, IRAQ

Dr./Mr(s). Saif Lateef Shakir/ University of Al-Qadisiyah, IRAQ

Greetings, with reference (JPT 59) to your article entitled:

Genotypic Characterization of *Staphylococcus* spp. Isolated from the Bodies of Workers in Units of MRI, CAT, X-Ray, Restaurants, and Testing Their Ability to Biofilms Formation

We wish to bring to your kind notice the following

- ✓ We acknowledge the receipt of the above mentioned article.
- ✓ The above mentioned article(s) has been sent to the reviewer of expert comments
- ✓ The above mentioned article(s) have been accepted for publication in the

Vol:11 (No.:4-5 to get release in April-May 2018):(Research Journal of

Pharmacy and Technology ISSN: 0974360X).