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Influence Semiconductor laser on irradiated *sak* gene from *Staphylococcus aureus*

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Abstract

Objective: The aim of this study in order that study of mutant *sak* gene from mutant *S. aureus* after exposition to Semiconductor laser.

Study design: Cross-sectional study design in descriptive study design.

Background: Staphylococcus is cocci that able to cause a specific defect in any tissue, organ or part resistance to antibiotics, gram positive, anaerobic, yellowish-golden color. PCR technique is depended on the amplification of specific in target DNA. PCR has been participated for decades in detection of pathogens especially in clinical samples but in recent years have appeared importance of PCR as molecular typing tool. Semiconductor laser also called the diode laser depends on Semiconductor material, the advantages of this laser is small in size, consumes little energy used in many applications such as precision devices and laser printers.

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Methodology: This study was managed in the Iraqi laboratories to find out influence of Semiconductor laser beams on irradated *sak* gene from *S. aureus* with a wavelength 532 ±10 nm and 2000 mw on *S. aureus* isolated from skin injuries, burn, nose, blood and tonsils. The bacterial isolates exposed to laser affected and influence on the growth with increase in time for radiation.

Results: The percentage of killing rate increased reach to 88.6%, this increase is due to continuous-pattern laser because the temperature emitted from laser that cause death to bacterial cells. Polymerase chain reaction (PCR) technique was used to screened *sak* gene and analysis PCR product on agarose gel electrophoress, results showed positive isolates possess this gene with 492 bp in size product when compared with DNA ladder after exposition to Semiconductor laser in different time (10, 20, 30) min of irradiated *S. aureus.*

Conclusions: Semiconductor laser influence on *S. aureus* in different time with long period with increase percentage of killing and there are mutation in irradiated *sak* gene.

Key words: *sak* gene, Semiconductor laser , Genotypic deection, Continuous pattern laser

Introduction

Staphylococcus is cocci that capable to cause a specific defect in each tissue, organ or part resistance to antibiotics, gram positive, anaerobic and yellowish-golden color [1], grape-like clusters. It has a capsule composed of polysaccharides [2, 3, 4]. It is an opportunistic pathogen that impact on the human body for instance, skin, tissues, bloodstream as well as the lymph and spread and cause disease [5], for example pneumonia, blood poisoning, bone, joint inflammation, inflammation of the internal portion of the heart and food poisoning [6]. These bacteria have a high capability to invade the host tissue and spread it each quickly and reason defects due to it has virulence factors such as the production of enzymes and toxins that work to induce the injury and destroy living tissues [7, 8, 9].

Semiconductor laser else called the diode laser depends on Semiconductor material, the significant of this laser is small in size, consumes less energy used in several applications for instance precision devices and laser printers. Semiconductor laser is a laser with its efficient

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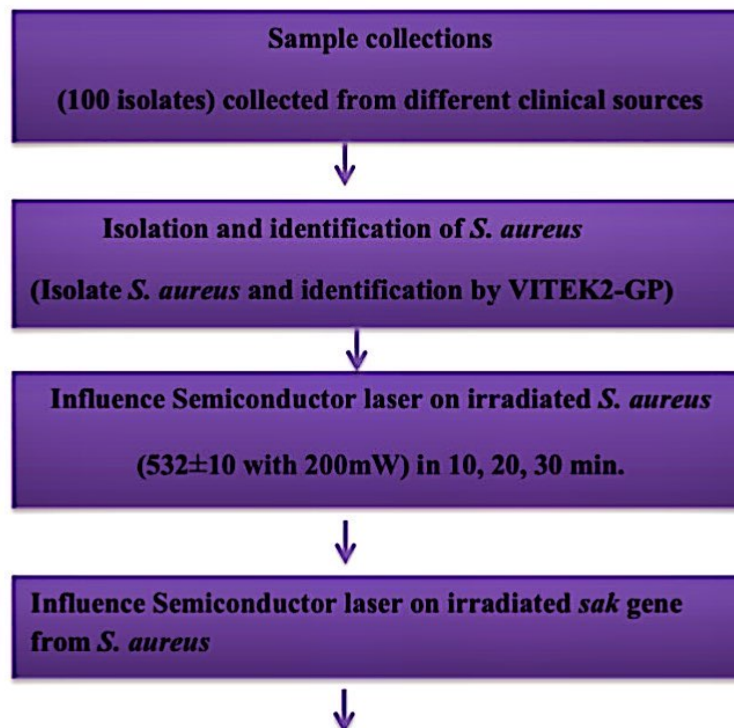
energy to disinfect on the surfaces of the instill have bactericidal effect due to rapid heat generation during irradiation by laser that reason thermal damage to neighboring tissues. Semiconductor laser, it has a small body, light weight, long life and elevation [10].

PCR technique is rely on the amplification of certain in target DNA [11]. PCR has been participated for decades in disclosure of pathogens especially in clinical samples but in recent years have appeared significant of PCR as molecular typing tool [12]. PCR-based methods have been utilized routinely in reference laboratories beside, standard method to detection and typing MRSA via SCC*mec* complies that contains *mec*A gene and *ccr* gene [13].

Methodology

Clinical examination inclusive collection of sample of different sources, identification via VITEK2-GP, Influence efficiency of laser radiation on *S. aureus* and influence Semiconductor laser, PCR revelation on *sak* gene in this tratise as shown in figure (1).



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PCR detection of irradiated *sak* gene from *S. aureus*

(492 bp size of product of *sak* gene)

Figure (1): Scheme for Study design of this research.

Sample collections

- A total of 100 *S. aureus* isolates were composed of Baghdad hospitals in 2021.These isolates were identified. The isolates were injected on Nutrient agar, the results were stay for 24 h of incubation at 37°C according to the criteria established by [14].

Isolation and identification of *S. aureus*

Morphological Examination

Fundamental diagnostic of bacterial isolates established on morphological characteristics of colonies in culture media inclusive colony shape, colony composition, colony color and edges. It were deliberated on MacConky agar, Blood agar and eventually on Mannitol salt agar [15].

Microscopic Examination

Gram stain reaction was accomplished picked one colony and fixed on microscopic slide to appear the cell shape and arrangement [15].

Identification of *S.aureus* by Vitek2 GP

Identification of the bacterial isolates was managed utilizing Vitek2 GP system for *S.aureus* according to the procedure proposed via the manufacturing corporation. There are 64 biochemical tests measuration carbon resources utilization, enzymatic activities and antibiotics resistance. This system is designed for the achievment of 64 standard biochemical

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tests from a single colony of purified isolate. The bacterial suspension was ready for Vitek2 GP suspension solution and the turbidity modified to 0.5 McFarland (1.5×10^8CFU/ml). The Gram positive (GP) card is utilized for the automated identification of utmost important Gram-positive bacteria. The GP identification card is based on accomplished biochemical methods, Results from the connotation of the card to provide anaerobic status and other microbes with less need for air, depending on the suitability of each test and as instructed by the company [16, 17]. French and newly developed substrates with a sterile Pasteur pipette, were inoculated according to the manufactures directive. After incubating at 37°C for 24 hrs., the identification of the isolate utilizing the automatic analytical, rapid identification at species plane had been done, The suspension tubes and GP card were placed in the cassette with one negative control well. The device works during a period of nurture on the analysis and storage of biochemical paradigm are subjective and after a whilst cuddling analyzed the apparatus software these patterns printed diagnostic report for each card inside Reader / Incubator, depending on instructions Biomerieux corporation [18, 19, 20, 21].

Influence Semiconductor laser on irradiated *S. aureus*

S. aureus cultivation was done depending on [22] with several modifications as succeed: *S. aureus* were cultivated in Nutrient broth at 37° C for 24 hr. to arrive the stationary-phase culture, subsequently, culture was centrifuged (5000 rpm for 10 minutes). The pellet was suspended in 150 ml of sterile normal saline, thereafter 1 ml of this solution was displayed to Semiconductor laser in various time (10, 20, 30) min. in rapprochement with control (without display to laser).

Influence Semiconductor laser on irradiated *sak* gene from *S. aureus*

PCR detection of irradiated *sak* gene of *S. aureus*

A bacterial colony was placed into a small tube including distilled water at 5ml exposed to Semiconductor laser in 10, 20, 30 min. rapprochement with control (before exposure to the

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laser), then cultured of the headmost dilution that cultivated on the growth medium (Nutrient agar) and calculate the numeral of colonies as well as the percentage of killing, brood in the incubator for 24hr. at temperature of 37°C, subsequently, extraction DNA by DNA extraction kits of ZYMO corporation.

Molecular study of *sak* gene

Extraction of genomic DNA

Genomic DNA of *S. aureus* was extracted via Kit of DNA extraction depending on the manufacturer (Zymo kits) that utilized for extracting DNA as succeed:

1- 50-100 mg of bacterial cells were resuspended in above to 200µl of water to a ZR bashing bead lysis tube (0.1mm and 0.5mm), subsequently 750 µl bashing bead buffer was appended to the tube.

2- Guaranted in a bead beater fitted with a 3ml tube holder gathering and remedy at maximum speed for >5 minutes.

3- The ZR bashing bead lysis tube (0.1mm and 0.5mm) was centrifuged in a microcentrifuge at 10,000 xg for 1 minute.

4- A number 400 µl was transferred to supernatant to a zymo-spinIII-F filter in aggregate tube and centrifuge at 8,000 xg for 1 minute.

5- Addendum of 1,200 of genomic lysis buffer to the filtrate in the aggregate tube from step 4.

6- 800 µl of the mixture from step 5 was transmitted to a zymo-spinIIIC column in aggregate tube and centrifuged at 10,000xg for 1 minute.

7- The aggregate tube was discarded and reiterate step 6.

8- DNA pre-wash buffer 200 µl was appended to the zymo-spinIIIC column in a modern collection tube and centrifuge at 10,000xg for 1 minute.

9- A number of 500 µl g-DNA wash buffer was appended to the zymo-spin IIC column and centrifuged at 10,000xg for 1 minute.

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10- The zymo-spin IIC column was transmitted to a clean 1.5ml microcentrifuge tube and appended 100 µl DNA elution buffer immediately to the column marix, centrifuged at 10,000 xg for 30 seconds to separate the DNA.

11- Ultra-pure DNA was prepared for utilize.

Genotypic detection of *Staphylococcus aureus* via polymerase chain reaction (PCR)

Amplification of *sak gene* of *S.aureus* via PCR was done depending on [23] as exhibition in table 1. The amplification of *sak* gene from the irradiated *S. aureus* was intended as listed in table 2.

Table 1: Components of reaction admixture for amplification of *sak* gene of irradiated *S. aureus*.

No.	Component	Volume (µl)
1	Green master mix	12.5
2	Forward Primer (10 pmol)	1.5
3	Reverse Primer (10 pmol)	1.5
4	DNA template	5
5	Nuclease free water	4.5
Total volume		**25**

The optimal status of polymerase chain reactions (PCR) of *sak* gene into the *S. aureus* was limitted as shown in table 2.

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Table 2: PCR Amplification plan of mutant *sak* gene and *16srRNA* from mutated *Staphylococcus aureus*

Initial denaturation	Denaturation	Annealing	Extension	Final extension
95 °C for 5 min.	94 °C for 1min	52 °C for 1min.	72 °C for 1min.	72°C for 10 min.
	35 cycle			

PCR amplification plan of irradiated *sak* gene depending on [24].

Agarose gel electrophoresis

The amplified PCR output was analyzed via agarose gel electrophoresis depending on [25] as follows:

1- Agarose1% was prepared via dissolving 1g in 100 ml of 1X TBE, then melted via heating with motivation. The agarose was left to cool to 60°C, subsequently ethidium bromide was added (final concentration 0.5 µg/mL).

2- The agarose was teeming into the tapped tray and a comb was put near one edge of the gel.

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3- The gel was let go to harden into it turn into opaque, then the comb and the tape were politely removed.

4- TBE (1X) buffer was teeming into gel tank and the tray was put horizontally in electrophoresis tank.

5- The PCR master mix ready containing loading buffer so the amplified PCR output were loaded immediately in the wells.

6- Aliqout of 5 µl of the DNA ladder (100bp) was laden in single lane that served as marker during the electrophoresis process.

7- The power equipping was set at 7V/cm for 90 min of PCR outputs.

8- The gel of electrophoresis was revealed to UV via ustilizing UV transilliuminator and subsequently photographed utilizing digital camera.

Results and discussion

Study design

- Study design is the formularization of trials and experiments, beide observational studies in medical, clinical or another types of research for example epidemiological including human beings. In this descriptive study design of (100) *S.aureus* via cross-sectional study design collected from various clinical resources.

Sample collections

S.aureus 100 isolate from various clinical resources inclusive skin, burn, nose, blood and tonsils from Baghdad hospitals during 2021. Identification via utilizing vitek2-GP system.

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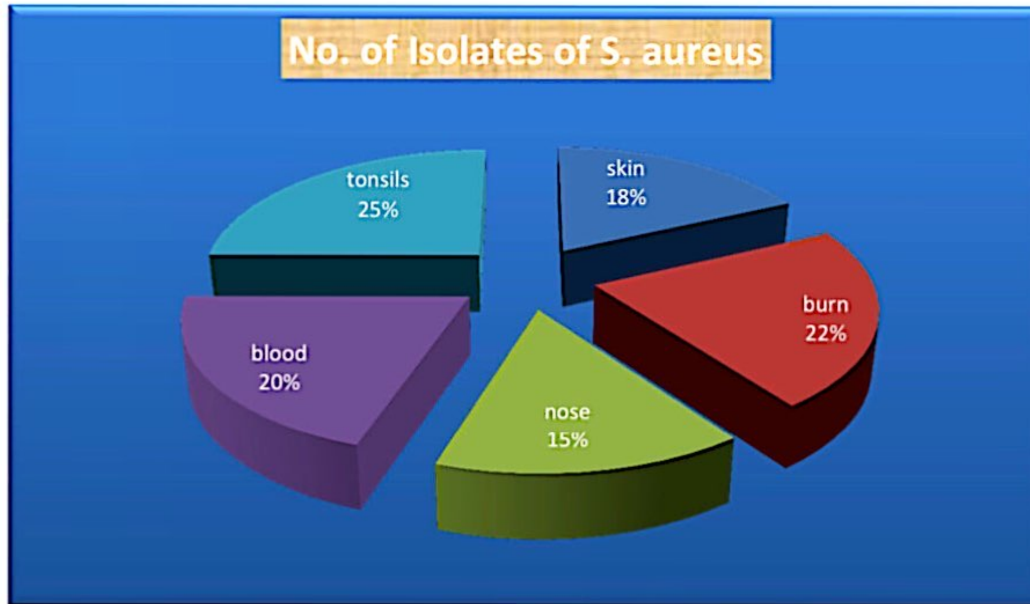

Figure (2): Prevalence of sample collections from Iraqi different clinical sources including skin, burn, nose, blood and tonsils.

Excommunication and identification of *S. aureus*

Morphological and Microscopic examination of *S. aureus*

The collected isolates were at first diagnosed in hospitals accordingly *Staphylococcus*. To confirm this advanced diagnostic, all isolates were civilized on Nutrien agar and Mannitol salt agar due to this media identification for gram positive bacteria [26].

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Figure (3): Excommunication and identification *S. aureus* before insinuation to laser radiation on Nutrient agar medium.

Results in figure (3) perform growth *S. aureus* yellowish-golden color on Nutrient agar and Mannitol Salt agar for excommunication and identification of S. aureus, all isolates positive for VITEK2 GP confirmed *S. aureus*.

Influence Semiconductor laser on irradiated *S. aureus*

The laser light amplification via stimulate emission of radiation and the basis of the laser's work rely on converting electrical energy till light energy that sheds on the portion to be treated via the laser and photons fall on that portion, the energy of those photons is transmitted either randomly. Laser is an significant and fundamental thing and kill many types of bacteria [27].

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Figure (4): Influence Semiconductor laser on irradiated *S. aureus* in wavelength 532±10 with 2000 mW.

Results in figure (4) display growth of irradiated *S. ureus* on nutrient agar after exposed to Semiconductor laser in various time (10, 20, 30) min with wavelength 532+-10 and 2000 Mw.

Laser irradiation reason a defect in the physiological action of a living cell, degree of destroy tissue rely on the dose and stress which leading to decrease in cell growth via loss of effectiveness cell metabolism and internal degeneration of its components [28].

Table (3): Influence Semiconductor laser on irradiated *S. aureus* with percentage of killing in different time.

Time(min)	Percentage of killing (%)
10	**58.800[a] ± 3.458**

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20	$73.333^{b} \pm 2.512$
30	$88.633^{c} \pm 3.433$

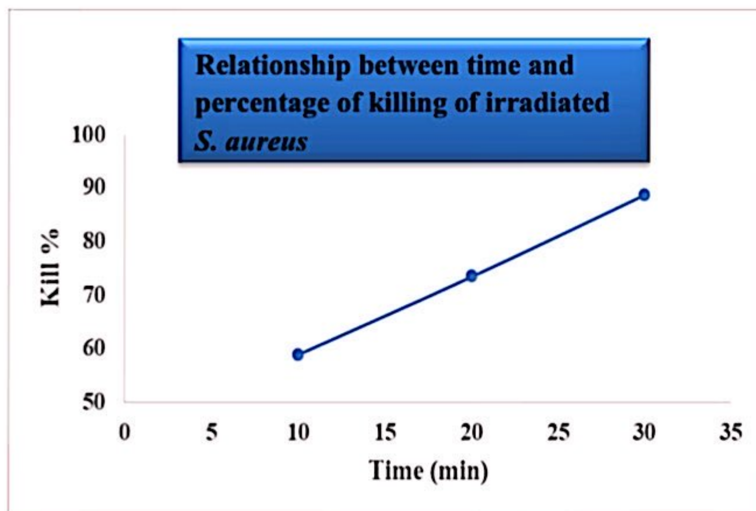

Figure (5): Relationship between time and percentage of killing rates on irradiated *S. aureus* colonies in different time 10, 20 and 30 min.

Results in figure (5) display increase percentage of killing irradated *S. aureus* when revealed to long time that perform the relationship between the time of insinuation and percentage of killing rates of rradiated *S. aureus* in diverse time 10, 20 and 30 min. The killing rate raise with increase time peroid reach to 88.6 % because insinuation to continuous mode laser causes destroy to living cell subsequently dead [29, 30].

Results in table (3) mainfest influence of Semiconductor laser radiation on *S. aureus* with viable cell (95 cells in 10 min; 12 cells in 20 min and 48 cell in 30 min) rapprochement control 400 viable cell, the percentage of killing are extremely and the viable cells are less than control.

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Influence Semiconductor laser on irradiated *sak* gene from *S. aureus*

PCR revelation of irradiated *sak* gene of *S. aureus*

PCR is the technique which offers a rapid tool with elevate sensitivity and specificity for the revelation of bacteria as rapprochement to conventional methods [31].

Table (3) : Oligonucleotide primers utilized for the amplification of *sak* gene of irradiated *S. aureus*.

Primers	Sequences of primers	No. base pair	Size of product	Refrences
Forward primer	5'AGAGATTGATTGTGAAAGAAGTGTT 3'	25	492	[32, 24]
Reverse primer	3'CGAAGTACCTGCCTAAAAAAGGAT 5'	24		

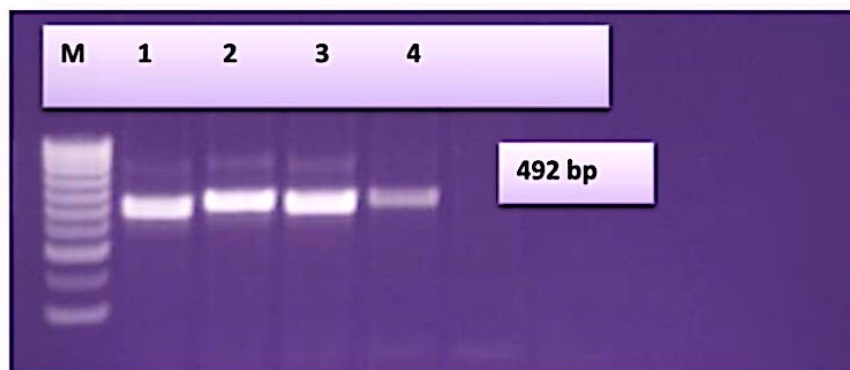

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Figure (6): Agarose gel electrophoresis (1%agarose, 7 V/cm^{2}for 60 min) of *sak* gene (492bp amplicon). Lane M 1000bp DNA ladder, lanes 1-4 perform bands size of out put of irradiated *S.aureus*.

Positive results in figure (6) displayed possess *sak* gene with 492 bp *sak* gene with 492 bp in size product from irradiated *S. aureus* when rapprochement with DNA ladder after exhibition to semiconductor laser in diverse time. Polymerase chain reaction (PCR) technique utilized to screen *sak* gene and analysis PCR out put on agarose gel electrophoresis.

Many types of PCR displayed sensitivity, especially with small numeral of bacteria, nested PCR is one of revelation with a less bacteria in clinical sample [33, 34].

Statistical analysis :

Results of Statistical Analysis System- SAS program confirmed significant.

Conclusions

1- Semiconductor laser influence on *S. aureus* in different time with long period with increase percentage of killing.
2- There are mutation in irradiated *sak* gene.

Acknowledgment about author

Researcher Dr. Nebras Rada Mohammed Ph.D. in Biotechnology with a micro specialization, Genetic Engineering, Molecular Genetics and Protein Engineering, a researcher, creator, inventor and author, a lecturer at the University College of Al-Turath University college, a Bachelor's degree in Microbiology and a Master's degree in Molecular Biology in Microbiology from Al-Mustansiriya University, an arbitrator, international resident and consultant In medical laboratories, an expert in medical laboratories and a holder of the title of a scientist project, an arbitrator, a distinguished publisher, a silver supporter of scientific platforms, a chairman of a committee in a scientific society, receiving accolades from international intellectual property, the Best Arab Woman Award 2020, also

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References

1-**Lowy**, F. D. (1998). *Staphylococcus aureus* infections. New England journal of medicine 339.8 : 520-532.

2-**Alalem**, A. M. (2008). Antibiotic resistant *Staphylococcus aureus* infection studies in hospitals.

3- **Rasigade**, J. P. and Vandenesch, F. (2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. Infect. Genet. Evol. 21: 510-4.

4-**Waldvogel**, F.A. (1990). staphylococcus aurues (including toxic shock syndronal) in Mandell GL ,Douglas R6 ,bennett JE (ends) principles and practice of infections disease 3nd. .churchill living ston,London. .pp 1489-1510.

5-**Aston**, J. L. (2010). Risk factors for treatment failure in patients receiving vancomycin for hospital-acquired methicillin-resistant *Staphylococcus aureus* pneumonia. Surgical infections 11.1 : 21-28.

6-**Omoe**, K. (2002). Detection of seg, seh, and sei genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring seg, seh, or sei genes. Journal of clinical microbiology 40.3 : 857-862.

7-**Quinn**, P. J. (2006). Veterinary Microbiology and Microbial diseases printed and bound in Great Britain by International Ltd-Padstow Corn wall.

8-**Ray**, C. G. and Kenneth J. R. (2010). Sherris medical microbiology. Vol. 6. McGraw-Hill.

9-**Fritz**, S. A. (2011).The natural history of contemporary *Staphylococcus aureus* nasal colonization in community children. The Pediatric infectious disease journal 30.4 : 349.

10-**Khodaverdian**, V. (2013). Discovery of antivirulence agents against methicillin-resistant *Staphylococcus aureus*. Antimicrobial agents and chemotherapy. 57.8 : 3645-3652.

Scanned with CamScanner

11-Brown, D.F; David,E.; Peter, H. M; Donald M. D; Geoffrey, L. R.; Towner, K. J. and Michael, W. D. W. (2005) on behalf of the Joint Working Party of the British Society for Antimicrobial Chemotherapy, Hospital Infection Society and Infection Control Nurses Association Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA) Journal of Antimicrobial Chemotherapy .56: 1000–1018.

12-Van Leeuwen, W. (2003). Molecular Approaches for Epidemiological Characterization of *Staphylococcus aureus*. *In*, MRSA Current Perspectives (Ad C. Fluit and Franz-Josef Schmitz, Eds). Caister Academic Press, Norfolk, England: 95-136.

13-Mehndiratta, P.L.; Bhalla P, Ahmed, A. and Sharma, Y.D. (2009). Molecular typing of methicillin-resistant *staphylococcus aureus* strains by PCR-RFLP Of SPA Gene: A Reference laboratory perspective. Indian J Med Microbiol .27:116-22.

14- Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. (2007). Baily and Scott's Diagnostic Microbiology. 11th edition. Mosby, Inc. Baltimore, USA., 384-398.

15-Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley, J. T. and William, S. T. (1994). Berge's Manual of Determinative Bacteriology. 9th. ed. Williams and Wilkins, USA . P.532-551.

16- Harly, J.P. and Prescott, L.M (2002). Laboratory Exercises Microbiology, 5th ed. Mc Graw-Hill Companies, Inc.,New York .8 (13): 456-459.

17-Biomeruex corporation. (2010) . VITEK . USA .

18- Collins , M.D. and Lawson, P.A. (2000). The genus A biotrophia (Kawamura *et al.*) is not monophiletic: proposal of Granulicatella gen. nov., Granulicatella adiacens comb.nov., Granulicatella elegans comb. nov.and Granulicatella balaenopterae comb. nov. Int. J. Syst. Evol. Microbiol. ;50:365-369.

19- Carlton, R. M.; Noordman, W. H.; Biswas, B.; de Meester, E. D. and Loessner, M. J. (2005). Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul Toxicol Pharmacol. 43(3):301-12.

Scanned with CamScanner

20-Barros, R. R; Carvalho, G. S; Peralta, J. M.; Facklam, R. R. and Teixeira, L. M. (2001). Phenotypic and Genotypic Characterization of Pediococcus Strains Isolated from Human Clinical Sources. J. Clin. Microbiol. 39:1241-1246.

21-Chang, Y. H.; Han, J.; Chun ,J.; Lee, K. C.; Rhee, M. S.; Kim, Y. B. and Bae, K. S. (2002). *Comamonas koreensis* sp.nov., a non-motile species from wetland in Woopo, Korea. Int. J. Syst. Evol. Microbiol., 52:377-318.

22-Trampuz A.; Piper, K. E.; Steckelberg, J. M. and Patel R. (2006*).* Effect of gamma irradiation on viability and DNA of Staphylococcus epidermidis and *Escherichia coli*. Journal of Medical Microbiology. 55:1271–1275.

23- Kai, Y. M.; Nakagawa,S.; Kryukov,K.; Matsukawa,S.; Tanaka,T.; Iwai,T.; Imanishi,T. and Hirota,K. (2018). Direct PCR amplification of *16S rRNA* genes offers accelerated bacterial identification using the MinIONTM nanopore sequencer Shinichi. bioRxiv preprint first .Japan.

24-Yerasi, G. P. R.; Rajendran, P.; Singaram, A. K. (2014). Isolation, cloning and expression of recombinant staphylokinase gene against thrombosis. Int. J .Pharm. Sci. 6:266-270, Issue 4.

25- Sambrook, J. and Russell, D. (2001). Molecular Cloning: Laboratory Manual. 3rd Ed. Cold Spring Harbor, New York. USA.

26- Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and William, S.T. (1994). Berge's Manual of Determinative Bacteriology. 9th. ed. Williams&Wilkins, USA . P.532-551.

27- Trampuz A.; Piper, K.E.; Steckelberg, J.M. and Patel R. (2006*).* Effect of gamma irradiation on viability and DNA of Staphylococcus epidermidis and *Escherichia coli*. Journal of Medical Microbiology, 55:1271–1275.

28-Meral, G.,(2003). Factors affecting the antibacterial effects of Nd: YAG laser *in vivo*. Lasers in surgery and medicine 32.3 : 197-202.

29-Yuan, X. (2018). Effect of laser irradiation on cell function and its implications in Raman spectroscopy. Applied and Environmental Microbiology 84.8.

30-Ward, G. D. (2000). Bactericidal action of high-power Nd: YAG laser light on *Escherichia coli* in saline suspension. Journal of applied microbiology. 89.3 : 517-525.

Scanned with CamScanner

31-Caltoir, V.; Gilibert, A .; Glaunec , J. M .; Lannay, N.; Bait, L .; and Legr, D. (2010) .Rapid detection of *Pseudomonas aeruginosa* from positive blood culturesby quantitative PCR. Annals.clinical. microbial. Antimicrob. 9 (21):1186-1476.

32-Lu, Y. S.; Ringler, D. H. and Park, J. S. (2000). Characterization of *Pasteurella multocida* isolates from the nares of healthy rabbits with pneumonia. Laboratory Animal Science 28, 691– 697.

33- Madico, G.; Quinn, T. C.; Boman, J. and Gaydos, C. A. (2000). Touchdown enzyme time release-PCR for detection and identification of *Chlamydia trachomatis*, *C. pneumonia* and *C. psittaci* using the 16S and 16S-23S spacer rRNA genes. J. Clin. Microbiol. 38:1085-1093.

34- Apfalter, P., F.; Blasi, J. ;Boman, C. A. ;Gaydos, M. ;Kundi, M.; Maass, A.; Makristathis, A.; Meijer, R.; Nadrchal, K.; Persson, M. L.; Rotter, C. Y.; Tong, G.; Stanek, and Hirschl, A. M. (2001). Multicenter comparison trial of DNA extraction methods and PCR assays for detection of *Chlamydia pneumoniae* in endarterectomy specimens. J. Clin. Microbiol. 39: 519-524.

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