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Evaluation of Biosynthesized Zinc Oxide Nanocrystals Comparing to Silver and Gold Nanoparticles against the Susceptibility of Genus *Staphylococcus*

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ABSTRACT

Staphylococci spp are ubiquitous Gram-positive bacteria is often associated with infections, especially nosocomial infections, and antibiotic resistance. Study pathogenic bacteria and its use as a tool in the technology of Nanobiology and molecular genetics research of the latest research trends of modern characterization and definition of different multiresistant of bacteria including *Staphylococci*. The *Staphylococci* are widespread all over the world and particularly in Saudi Arabia. The present work was conducted to evaluate the effect of five different types of nanoparticles (biosynthesized zinc oxide, spherical silver & gold nanoparticles and rod silver & gold nanoparticles) and their antibacterial impact on the *Staphylococcus* species. Ninety-six isolates of *Staphylococcus* species viz; *Staphylococcus aureus. Staphylococcus epidermidis , MRSA* were collected from different sources during March 2011G to June 2011G. All isolates were isolated from in-patients and out-patients department of Royal Commission Hospital in Yanbu Industrial, Saudi Arabia. High percentage isolation from males(55%) than females (45%). *Staphylococcus epidermidis* from males was (47%), (28%) and)(25%). for *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus (MRSA.* Isolates from females were *Staphylococcus* aureus with higher percent of (47%), (30%) and (23%) for MRSA, *Staphylococcus epidermidis*. *Staphylococcus aureus* from wound swab were the highest percent (51.42%) followed by vaginal swab (25.71%). *Staphylococcus epidermidis* were founded with higher percentage in blood (37.14%) and wound swab (34.21%) respectively related to other. The highest percentage of Methicillin-resistant *Staphylococcus aureus (MRSA)*(80.77%) were isolated from wound swab, while those from nostrils were (19.23%) .*Staphylococcus* species were isolates in highest percentage from hospital Emergency department with *Staphylococcus aureus* (59.37%) , Methicillin-resistant *Staphylococcus aureus (MRSA)* (28.13%)and *Staphylococcus epidermidis* (12.5%)respectively. Evaluation of the antibacterial property of Zinc oxide, Silver and Gold nanoparticles as an alternative to conventional antibacterial agents *Staphylococci* isolates from hospital sources we screened them. Gold and Silver rods Nanoparticles to be sensitive to all isolates of *Staphylococcus* species. Zinc oxide Nanoparticles gave sensitivity impact range (52%) and (48%). Gold and Silver spherical nanoparticles did not showed any effect on *Staphylococci* species. Zinc Oxide Nanoparticles gave bactericidal impact (25%) and bacteriostatic impact (75%) for of *Staphylococci* species. Detecting the association of nanoparticles with *Staphylococci* isolates imaging by scanning electron microscope (SEM) of some bacteriostatic isolates for Zinc Oxide nanoparticles on *Staphylococcus aureus*, *Staphylococcus epidermidis* and Methicillin resistant *Staphylococcus aureus(MRSA)*, showed some Overlapping Bacterial cells with lower their number and appearing some appendages with deformities in external shape. Molecular analysis was applied by Multiplex polymerase chain reaction (PCR) used for the identification of genes within *Staphylococcal* pathogens using six primer. The range of Molecular gene typing ranging for *S. aureus* and *MRSA* by *TSST-1*,*mecA*,*femA* ,*icaAB* and *atlE eta* , for *Staphylococcus epidermidis*.

INTRODUCTION

The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology today. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological micro-organisms such as bacteria. Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine **(Park Y., Hong Y.N., Weyers A., Kim Y. S., and Linhardt R.J., 2011).** Biological methods are regarded as safe, cost-effective, sustainable and environment-friendly processes for the synthesis of nanoparticles **(Kalimuthu K., Babu R.S., Venkataraman D., Mohd B., and Gurunathan S,2008).** Gold nanoparticles have been successfully synthesized using various **Bacteria (Nicole Jones1, Binata Ray1, Koodali T. Ranjit2 & Adhar C. Manna 2008)**, Fungi **(Ahmad A., Mukherjee P., Senapati S. Mandal D, Khan M.I., and Kumar R., 2003).**

Over the recent decade, gold nanoparticles (NPs), **(L.A. Dykman, V.A. Bogatyrev, S.Y. Shchyogolev, N.G. Khlebtsov,2008),** have attracted significant interest as a novel platform for various applications such as nanobiotechnology and biomedicine, **(Jigna Parekh, Nehal Karathia, Sumitra Chanda ,2006)** because of convenient surface bioconjugation **(Fatemeh Arabi1, Mojtaba Imandar, Masoud Negahdary, Mohsen Imandar, Mahdi Torkamani Noughabi, Hajar Akbari-dastjerdi1, Mohamad Fazilati , 2012) , (Jigna Parekh, Nehal Karathia, Sumitra Chanda ,2006)** and **(Venkataraman, A. 2009)**, with molecular probes. Recently published examples include applications of NPs to biosensorics **(Bar, H.; Bhui, Kr.D.; Sahoo, P.G.; Sarkar, P.; De, P.S.; Misra, A. ,2009)**, clinical chemistry **(Jones SA, Bowler PG, Walker M, Parsons D. 2004)** , **(M. Hu, J. Chen, Z.-Y.d Li , L. Au, G.V. Hartland, X. Li, M. Marqueze, Y. Xia, Chem. Soc. Rev. 35, 1084 2006)** & **(Anker JN, Hall WP, Lyandres O, Shah NC, Zhao J, Van Duyne RP., 2008)**, immunoassays **(Boisselier, E.; Astruc, D. ,2009),** immune response enhancement **(Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. ,2005)** & **(Jain PK, Huang XH, El-Sayed IH, El-Sayed MA., 2008)**, detection and control of microorganisms **(Stewart ME, Anderton CR, Thompson LB, Maria J, Gray SK, Rogers JA,2008),** and targeted delivery of drugs or genetic and immunological substances **(Dickerson, B.E.; Dreaden, C.E.; Huang, X., El—Sayed, H. I.; Chu, H.;Pushpanketh, S.; McDonald, H.I., El-Sayed, A.M. , 2008)** & **(Payne, C.M, Anderson, L.J.E. ; Hafner, J.H. ,2013),** The development of new resistant

strains of bacteria to current antibiotics **(Ganesh Kumara, K.Govindarajub, G.Singaravelub & D.Adhikesavaluc.2009).** has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides **(RM, Mohamed MB, Ramadan MA, Verwanger T, Krammer B., 2009)**. The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs **(Rai, M. K. S.D. Deshmukh, A.P. Ingle and A.K. Gade, 2012)** & **(N. Marcato, D.P.; De Souza, H.I.; Alves, L.O.; Espsito, E. 2007)** Consequently, there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy Investigations have been carried out on the biological activities of gold nanoparticles; however, the effects of nanoparticles on the activities of antibiotics have not been demonstrated. Nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and health-related applications.

The present study deals with the effect of gold, silver and biologically synthesized ZnO nanoparticles as the antibacterial activity against Staphylococcus sp.

Multiplex polymerase chain reaction (PCR) is defined as the multaneous amplification of multiple regions of DNA templates by adding more than one primer pair to the amplification reaction mixture. Since first being described in 1988 (**Schoske R., Pete M., Christian R. and John B. 2003)**, PCR multiplexing has been applied in many areas of DNA testing including the analysis of deletions, mutations, and Short Tandem Repeats. Furthermore, the wide availability of genetic information due to the publishing of the sequence of the human genome makes the demand for multiplex PCR even greater. For example, more than 1.4million Single Nucleotide Polymorphisms have been identified in the human genome. Multiplex PCR primer sets have been used for linkage studies to track genetic diseases (**Sivadon V., Quincampoix J., Prunier E. and Hoffmeyer P.,2009)**. .

MATERIALS AND METHODS

Bacterial isolation:

Ninety-six isolates of *Staphylococcus* species used in the study were isolated from patients at the clinical microbiology laboratory from Royal Commission Hospital in Yanbu Industrial during March to June 2011. The isolates were collected from twelve different sources (wound swab , Urine, High Vaginal Swab, tissues, blood, Ear, Nostril, Axilla , Groin, Ascitic fluid,

Spinal fluid, Endotracheal tube) of the patients based on their gender from different fourteen Clinical department (Intensive Care Unit, Neonatal Intensive Care Unit, Obstetric Ward, Operating Room, Female Surgical Ward, Female Medical Ward, Female Orthopedic Ward, Male Surgical Ward, Male Medical Ward, Male Orthopedic Ward, Pediatric Ward, Emergency Room, Public Health Department, Dermatology Clinic and Orthopedic)in the royal hospital . All isolates were grown on blood agar and incubated overnight for 20 – 24 hours at $37C^0$. **'Table S1'.**

Bacterial identification:

Bacterial culture of *staphylococcus* is performed on blood agar (BD, Becton, Dickinson and Company Sparks, MD 221734SA38800 Le Pont de Claix/France) add (Sheep Blood, defibrinated), Muller Hinton agar (BD , Becton, Dickinson and Company Sparks, MD 221275 SA 38800 Le Pont de Claix/France), DNase agar (BD , Becton, Dickinson and Company Sparks, MD 221856 SA 38800 Le Pont de Claix/France), Mannitol salt agar (BD , Becton, Dickinson and Company Sparks, MD 221173 SA 38800 Le Pont de Claix/France) .All isolates under study were identified by different biochemical tests.

Relationship between Sex and Age:

The age of all isolates from different clinical departments for male and female.high percentage isolation from males(55%) than females (45%), **'Table S10'.** It was also found that most of isolation were done from wound swab of males, it was noted that males reported higher injury rate (31-60) years than females (16- 30) years, **'FigureS1' & 'FigureS2'**.

- **Relationship between *Staphylococcus* species and Sex**

All species of *Staphylococcus (Staphylococcus aureus*, *Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus aureus (MRSA))* were isolated from different clinical departments for the male and female. High isolation percentage from males(55%) than females (45%). Percentage isolation of *Staphylococcus aureus* from males was (37%), followed by (36%)for *Staphylococcus epidermidis* and Methicillin-resistant *Staphylococcus aureus (MRSA)* (27%) (Table S2), Fig. 3 & 4. Analysis by Chi-square test shows significant results, the calculated value was 6.1638 > 5.991 (critical value at 5% significance level). This showed that the isolate and the gender were dependent, not separable,**'Table S3'.**

Isolates from females were *Staphylococcus aureus* with higher percent of (47%) followed by Methicillin-resistant *Staphylococcus aureus (MRSA) (*30%), then *Staphylococcus epidermidis* (23%) Fig.(S5). Percentage isolation of *Staphylococcus epidermidis* from males was (47%), followed by (28%) for *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus (MRSA)* (25%), '**Figure S6'.**

- **Relationship between of *Staphylococcus* species and Age group**

All species of *Staphylococcus (Staphylococcus* aureus, *Staphylococcus epidermidis,* Methicillin-resistant *Staphylococcus aureus (MRSA)* were isolated from different department for the male and female, Table (S4). Analysis by Chi-square test shows significant results, where the Chi-square calculated value was 21.95 > 15.507 (critical value at 5% significance level). This showed that the isolate and the age group were dependent, not separable, Table (S5). In,'**Figure S5' &** '**Figure S8'**, the number of isolated *Staphylococcus aureus as well as* Methicillin-resistant *Staphylococcus aureus (MRSA)* increased by the age starting from "Born" and reaches the highest number in the age group of 16-30 age and 31-60 respectively. The number starts decreasing as the age increases. Also, the number of *Staphylococcus epidermidis* isolates is initially increasing in" Born" group and reaches the highest by the age 1-15 and increasing as age increases.The data in, '**Figure S6' &** '**Figure S7',** Showed that percentage of *Staphylococcus epidermidis* in new born for females was highest (100%) followed by males (78%) in newborn. *Staphylococcus aureus* in males for the age group (16-30) years was highest (67%) while in females between age group (16-30) years (53%). Methicillin-resistant *Staphylococcus aureus (MRSR)* in females for the age group (31-60) years was highest (43%) while in males between(31-60) years (37.5%), '**Figure S8' &** '**Figure S9'.**

- **Relationship between Sources and species of *Staphylococcus***

All species of *Staphylococcus (Staphylococcus aureus, Staphylococcus epidermidis,* Methicillin-resistant *Staphylococcus aureus (MRSA))* were isolated from different various sources, Table (S6). *Staphylococcus aureus* from wound swab showed highest percent (51.42%) followed by vaginal swab (25.71%). *Staphylococcus epidermidis* showed higher percentage in blood (37.14%) and wound swab (34.21%) respectively related to other isolates. The highest percentage of Methicillin-resistant *Staphylococcus aureus (MRSA)*

(80.77%) were isolated from wound swab, while those from nostrils were (19.23%), '**Figure S10'.**

- **Relationship between *Staphylococcus* species in different Departments**

All species of *Staphylococcus (Staphylococcus aureus, Staphylococcus epidermidis,* Methicillin-resistant *Staphylococcus aureus (MRSA)* were isolated from different major clinical departments in the hospital (TableS7). A highest percentage of *Staphylococcus* species were isolated from hospital emergency department with *Staphylococcus aureus* (59.37%), Methicillin-resistant *Staphylococcus aureus (MRSA)* (28.13%) and *Staphylococcus epidermidis* (12.5%) respectively '**Figure S11'.**

RESULTS

Bacterial identification:

Bacterial isolates were identified by Catalase test, Coagulase test, DNase agar, Mannitol agar, *MRSA* Confirmatory agar and Novobiocin disc antibiotic. (Catalase test the appearance of gas bubbles for all *Staphylococci*. Coagulase test the microorganisms clumping would be in the plasma to clot by converting fibrinogen to fibrin for *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* .) .The color changes in DNase agar (Pink) and Mannitol agar (Yellow) was positive for *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus (MRSA)*. Confirmatory agar was a positive result on the plates for Methicillin-resistant *Staphylococcus aureus (MRSA)*. Novobiocin disc antibiotic distinguish *Staphylococcus epidermidis* from other species of *Staphylococci*, where *Staphylococcus epidermidis* sensitive for Novobiocin antibiotic while be resistant to other species of *Staphylococci. Staphylococcus* is an important pathogen frequently responsible for nosocomial infections, it a Gram-positive, non-motile, encapsulated, facultative anaerobes, aerobes, a cocci shaped, and form in grape-like clusters.

Antimicrobial Resistance Testing:

All isolates were subjected to antibiotic susceptibility testing and studied for extended spectrum β-lactamase production according to the National Committee for Clinical Laboratory Standards. These isolates are collected from different sources, mostly from the Wound swab 53% while the rest of the isolates were small percentages from blood, High

Vaginal Swab (H.V.S), Nostril, Axilla, Groin, Urine, Ascitic fluid, Spinal fluid, Endotracheal tube (ETT) and Tissue.

The present study described the validation of susceptibility to different β-lactamase antibiotics, Ampicillin(AMP), Penicillin(P), Erythromycin(E), Oxacillin(OX), Amoxacillin(AMC), Gentamicin(GM), Cefuroxime(CXM), Teicoplanin(TEC), Tigecyclin(TGC), Rifampicin(RA)and Vancomycin(VA). Ninety six isolates of *Staphylococci* species from different clinical specimens. Ampicillin (Amp) resistance for all *Staphylococci* isolates was the highest (87%), Penicillin (P) (51%), Erythromycin (E) (39%), Oxacillin (OX) (31%) ,Amoxacillin (AMC) and Gentamicin (GM) (24%), Cefuroxime (CXM) (21%) ,Teicoplanin (TEC)(8%),Tigecyclin (TGC)(6%), Rifampicin (RA)(3%) and Vancomycin(VA)(1%). Cefuroxime (CXM) sensitivity for all *Staphylococci* isolates was the highest(69%), Amoxacillin (AMC) (68%), Gentamicin (GM)(67%),Oxacillin (OX)(61%), Erythromycin (E)(49%), Penicillin (P)(41%),Vancomycin (VA)(30%), Rifampicin (RA)(28%),Tigecyclin (TGC)(25%),Teicoplanin (TEC)(23%)and Ampicillin (Amp)(4%) '**Figure S12**'.

Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes. Those that target the bacterial cell wall penicillin(P), Ampicillin (AMP), Oxacillin (OX) and Amoxicillin (AMC), Glycopeptides (Vancomycin (VA) and Teicoplanin (TEC)) and cephalosporins (CXM)) or the cell membrane (polymixins), or interfere with essential bacterial enzymes (Rifamycins (RA), lipiarmycins, quinolones, and sulfonamides) have bactericidal activities. Those that target protein synthesis (aminoglycosides (GM), macrolides (Erythromycin (E), and Tetracyclines (Tigecycline (TGC)) are usually bacteriostatic (**Boisselier, E.,Astruc, D. 2009)**

Antibacterial Activity of Nanoparticles:

Antibacterial activity of Gold, Silver and Zinc Oxide Nanoparticles were applied on the tested bacteria. The zone of inhibition was divided according to Vancomycin (VA) and Teicoplanin (TEC) disks as a standard for gram positive bacteria. Effect of gold, silver and zinc oxide nanoparticles (resistant for antibiotics) which (≤ 10 mm) in diameter. Intermediate effect which ranging from (> 10 to 14 mm) in diameter and High effect for gold , silver and

zinc oxide nanoparticles (sensitive for antibiotics) which (> 14 mm) in diameter for gram positive bacteria (**Zhou Y., Ying K., Subrata K., Jeffrey D & Hong L. , 2012)**.

We observed the Gold rods Nanoparticles and Silver rods Nanoparticles were sensitive to all isolates of *Staphylococcus* species. Zinc oxide Nanoparticles gave sensitivity impact of (52%) and intermediate impact (48%) related to inhibition zone for all of *Staphylococcus* species '**Figure S13**'. The spherical Gold and spherical Silver nanoparticles did not show any effect on *Staphylococci* species**,** '**Figure S14' & 'Figure S15'.** The other three nanoparticles of Gold and Silver Rod and Zinc oxide showed a sensitivity to all bacterial isolates. Gold and Silver Rod nanoparticles appeared more effective than the Zinc oxide**,** '**Figure S16', 'Figure S17' & 'Figure S18**'. Thus, all nanoparticles used were arranged in the following antibacterial effect.

Gold / Silver (Spherical) < Zinc oxide < Gold and Silver Rod nanoparticles.

Evaluation of Nanoparticles Effects:

Bacteriostatic (BS) slow their bacterial growth and bactericidal (BC) kill bacteria, aspect for the effect Gold rod, silver rod and Zinc Oxide Nanoparticles towards bacterial isolates from different sources used in this study have been confirmed by inoculating the bacterial cells from zone of inhibition on (N.A) Nutrient ag. The data were recorded according to growth on Nutrient agar (N.A) as bacteriostatic (BS) or non-growth as bactericidal. Gold rod and Silver Rod Nanoparticles were bactericidal (BC) effect for of *Staphylococci* while Zinc Oxide Nanoparticles gave bactericidal (BC) effect with percentage (25%) and bacteriostatic (BS) impact (75%) for of *Staphylococci* species '**Figure S19**'. The antibacterial effect of nanoparticles under study was evaluated on the three *Staphylococcus* species.

Imaging by Scanning Electron Microscope:

The Scanning Electron Microscope of treated Methicillin-resistant *Staphylococcus aureus (MRSA)* showed spherical cells in irregular clusters '**Figure 20-A' and 'Figure S21-A'.** The antimicrobial activity of Nano-sized Zinc Oxide nanoparticles on Methicillin-resistant *Staphylococcus aureus (MRSA)* cells, showed some appendages on the cells wall, deformities in external shape of cells, Overlapping Bacterial cells and decreasing number of Bacterial cells, **'Figure20-B' & 'Figure S21-B'.** Bacterial Surfaces *Staphylococcus aureus* was revealed by the high-resolution Scanning Electron Microscope showed irregular clusters of spherical cells**,** '**Figure 22-A' & 'Figure 23-A'.** Treating the bacterial cell of

Staphylococcus aureus by Zinc Oxide nanoparticles, showed some appendages on the cells wall, deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells, **Fig(.22-B) and Fig(.23-B).** The Scanning Electron Microscope was to evaluate the surface morphology of treated *Staphylococcus epidermidis* with Zinc Oxide nanoparticles showed spherical cells in irregular clusters**, Fig.(24-A) and Fig.(S25-A).** The treated bacterial cells were significantly changed and showed major damage with appendages, deformities in external shape of cells, Overlapping Bacterial cells and decreasing number of Bacterial cells, **Fig.(24-B) and Fig.(S25-B).** Which was characterized by the formation of "pits" in their cell walls.

Molecular Detection by Multiplex polymerase chain reaction (PCR):

Molecular analysis was applied by Multiplex polymerase chain reaction (PCR) for the detection and identification of genes within *Staphylococcal* pathogens. A multiplex polymerase chain reaction (PCR) method has been developed using six primer pairs to detect different genes using 50 base pairs (bp) and 100bp DNA ladder marker. The range of Molecular gene typing ranging between 93bp to 326bp for *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* by *TSST* (**Ubukata K., Matsuhashi M. and Konno M. , 1989)** , while the bands border were from 546bp to 682bp for *Staphylococcus epidermidis* using *icaAB* and *atlE* genes (**Schoske R., Pete M., Christian R. and John B., 2003)**. Sixteen isolation of *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* were positive for the *femA* gene at 132bp. This allowed the using of this gene as an internal positive control, Fifteen isolates of *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* were positive for *mecA* gene at163bp. This gene was responsible for antibiotic resistant Methicillin, Two isolates of *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* were positive for the *TSST-1* gene at326bp which is responsible for Toxic shock syndrome in some *Staphylococci species*,' **Figure 26'**, None was positive for *eta* gene at 102bp to that was responsible for Exfoliative toxins. Six isolates of *Staphylococcus epidermidis* were positive for *atlE* gene at 682 bp which is responsible for the initial adherence. Three isolates of *Staphylococcus epidermidis* were positive for *icaAB* gene at546bp that is responsible for mediates the formation of the biofilm, **'Figure 27'.**

In conclusion, this study demonstrates the ability of the detection of genes to discriminate between infecting *Staphylococcus* strains and considered biological tests, they may potentiate the clinical criteria used for the diagnosis of septicemia or catheter-related infections.

DISCUSSION

Bacterial identification:

Ninety-six samples of *Staphylococcus* species were recovered from clinical specimens in bacteriology laboratories from Royal Commission Hospital in Yanbu Industrial during March to June 2011. These isolates were collected from different sources. Isolation from males was higher than females, in males (55%) and females (45%). Average all distribution of *Staphylococcus species* isolated from different department for the male and female. Percentage isolation of *S. aureus* was (37%), followed by (36%) for *S. epidermidis* and *(MRSA)* (27%). The data showed that percentage of *S. epidermidis* in newborn for females was highest (100%) followed by males (78%) in newborn. *S. aureus* in males for the age group (16-30) years was highest (67%) while in females between age group (16-30) years (53%). *(MRSR)* in females for the age group (31-60) years was highest (43%) while in males between (31-60) years.

Identification of *Staphylococcus* species was based on the comparison between biochemical tests, which reflect the metabolic activities of the isolate, and data published for known genera and species. These different isolates were identified by Catalase test the emergence of sustained gas bubbles for all *Staphylococcus,* (**Cheesbrough M.,2006)**. Coagulase test microorganisms clumping would be in the plasma to clot by converting fibrinogen to fibrin for *S. aureus* and *(MRSA)*. (**Cheesbrough M.,2006)**. Coloring changes in the DNase agar pink appeared on the plate for *Staphylococcus aureus* and *(MRSA)*. Coloring changes in the Mannitol agar strip were yellow for *S. aureus* and *(MRSA), MRSA* Confirmatory agar was a positive result on the plates for Methicillin-resistant *Staphylococcus aureus (MRSA)* and *Staphylococcus epidermidis* sensitive for Novobiocin antibiotic while be resistant to other species of *Staphylococcus.*

Antimicrobial Resistance of *Staphylococcus*:

Staphylococcus strains caused considerable morbidity and mortality in community environments and as a nosocomial acquired pathogen and has become the head leading cause of nosocomial infection during the last decades, (**Abraham M., Nandita D., Sudi I. and Maori L., 2009)**. showed that among Ninety-six isolates of *Staphylococcus* species. (87%) were resistance to Ampicillin (Amp), this was the highest resistance percentage shown from the *Staphylococcus* species related to other used β-lactamase antibiotics whereas

the isolated have changed in their resistance to others penicillin, Glycopeptides, cephalosporins, aminoglycosides, macrolides & tetracyclines. The resistance percentage for Penicillins (P) was (51%) and (39%) for Erythromycin (E) and(31%) for Oxacillin (OX) and (24%) for Amoxicillin (AMC), Gentamicin (GM) and (21%) for Cefuroxime (CXM) and (8%) for Teicoplanin (TEC) and (6%) for Tigecycline (TGC) and (3%) for Rifampicin (RA) and (1%) for Vancomycin (VA). *Staphylococcus* showed the highest sensitivity were percentage (69%) to Cephalosporins (CXM) antibiotics for all isolates this was the highest resistance percentage shown from the *Staphylococcus species* related to other used β-lactamase antibiotics. The sensitivity percentage for Amoxicillin (AMC) was (68%) and (67%) for Gentamicin (GM) and (61%) for Oxacillin (OX) and (49%) for Erythromycin (E) and (41%) for Penicillin (P) and (30%) for Vancomycin (VA) and (28%) for Rifampicin (RA) and (25%) for Tigecycline (TGC) and (23%) for Teicoplanin (TEC) and (4%) for ampicillin (Amp) and this was the least resistance percentage shown from the *Staphylococcus*.

Although the mechanisms of antibiotics include interference with cell wall synthesis, inhibition of protein synthesis, interference with include acid synthesis, Antibacterial drugs that work by inhibiting bacterial cell wall synthesis include the β -lactams, such as the Penicillins, Cephalosporins, Carbapenems, Glycopeptides, Vancomycin and Teicoplanin. β -Lactams agents inhibit the synthesis of the bacterial cell wall by interfering with the enzymes required for the synthesis of the Peptidoglycan layer. Macrolides, Aminoglycosides, Tetracyclines, Chloramphenicol, Streptogramins, and Oxazolidinones produce their antibacterial effects by inhibiting protein synthesis. Inhibition of a metabolic pathway and disruption of bacterial membrane but bacteria may be showed the resistance to one or more classes of antimicrobial agents, or may acquire resistance by de novo mutation or via the acquisition of resistance genes from other organisms, (**Tenover C., 2006)**. .

Antibacterial of Nanoparticles:

The activity of silver spherical nanoparticles and silver rods nanoparticles on bacterial growth, whereas all isolates of *staphylococcus* species were sensitive to the silver rods nanoparticles and bactericidal to the silver rods nanoparticles in this study while all isolates of *staphylococcus* species do not respond to the silver spherical nanoparticles in this study.

The mechanism of the bactericidal effect of silver that "Nanosilver represents a special physicochemical system which confers their antimicrobial activities via Ag". If this conclusion is verified then most bioaccumulation and toxicity issues relating to silver nanoparticles can be considered from the point of view of the toxic potential of ionic silver, which is documented sufficiently well. As under natural environmental conditions the ionic silver is environmental risks of nano silver toxicity is not as severe as the popular perception may suggest. The bactericidal effect of silver nanoparticles on microorganisms is connected not merely with the release of silver ions in solution. Following their report, silver nanoparticles can also be attached to the surface of the cell membrane and disturb its proper function drastically. They are also able to penetrate into the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds, **(Ras S.,2007)**. The viologen pendant stabilized silver nanoparticles of the present finding exhibited excellent antibacterial activity against the bacterial pathogens *Staphylococcus*, demonstrated that highly reactive metal oxide nanoparticles exhibit excellent biocidal activity against Gram-positive and Gram-negative bacteria. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag treatment. It was also shown that Ag binds to functional groups of proteins, resulting in protein denaturation. Studies have reported that the positive charge of the Ag ions is crucial for its antimicrobial activity through the electrostatic attraction between negatively charged cell membrane of microorganism and positively charged nanoparticles **(Kumar V, 2009)**.

The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed as antibacterial agent. This cloth with nano silver can be used in hospitals to prevent or to minimize bacterial infections and will lead to new generation of development of dressing incorporating antimicrobial agents to prevent pathogens infection **(Karthick R. 2011)**. Also proposed that the antibacterial mechanism of silver nanoparticles which leads to growth inhibition in bacteria, which is subsequently followed by induced membrane cell damage, stating that the damage could be caused by interactions of silver nanoparticles with compounds such as DNA. This interaction may prevent cell division and DNA replication, ultimately leading to cell death **(Rai M ., Deshmukh D., Ingle A. & Gade A.,2012)**. Therefore, the effectiveness of silver nanoparticles may be these have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the

chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. However, Ag ions or salts have only limited usefulness as antimicrobial agents for several reasons, including the interfering effects of salts and antimicrobial mechanism of the continuous release of enough concentration of silver Ag ion from the metal form. In contrast, these kinds of limitations can be overcome by the use of Ag nanoparticles. However, to use Ag in various fields against microorganisms, it is essential to prepare the Ag with cost-effective methods and to know the mechanism of the antimicrobial effect. Generally, silver (Ag) nanoparticles can be prepped cost effectively and that these silver (Ag) nanoparticles are homogeneous and stable **(Kim J., EunyKuk K., Jong K., Sung J. & Hyun K.,2007)**.

The activity of gold spherical nanoparticles and gold rods nanoparticles on bacterial growth, whereas all isolates of *staphylococcus* species were sensitive to the gold rods nanoparticles and bactericidal to the gold rods nanoparticles in this study while all isolates of *staphylococcus species* do not respond to the gold spherical nanoparticles in this study.

Gold rods nanoparticles would be attracted to the cell membrane, whereas Gold spherical nanoparticles would not be attracted as strongly. Once bound to the cell, the amphiphilicity of the mixed monolayer of Gold nanorods could induce a variety of further interactions. In summary, this study has demonstrated that the toxicity of the gold nanoparticles is related to their interactions with the cell membrane (**Guie H. 2012)**. Gold nanoparticles closely bind to the surface of the microorganisms causing visible damage to the cells, this makes the particles easily interact with outer membrane components of the cell and makes significant changes and damage on their surfaces leading to cell death. Gold nanoparticles generate holes in the cell wall, resulting in the leakage of cell contents leads to death, in another way it can binds to the DNA of bacteria and inhibit the DNA transcription. Different antibacterial mechanism for poly-allylamine hydrochloride adding to various colloids of gold nanoparticles is investigated facilitated the delivery of a large number of gold nanoparticles that strongly bound to poly-allylamine hydrochloride on the bacterial cell surface. Bacteria cell wall tends to attract positively charged poly-allylamine hydrochloride due to the total charge of cell wall being negative. As a result, cell walls encounter high stress as they accumulate poly-allylamine hydrochloride and gold nanoparticles. Poly-allylamine

hydrochloride is not responsible for the toxic effects of nanoparticles for bacteria. Once gold nanoparticles penetrate the cell wall and enter the cytoplasm, poly-allylamine hydrochloride is more likely to have direct contact with the cell membrane through damaged cell wall. Then, poly-allylamine hydrochloride could play a role in accelerating cell wall breakdown and cytoplasm release (**Zhou Y., Ying K., Subrata K., Jeffrey D . and Hong L., 2012)**. Generally, gold nanoparticles provide non-toxic routes to drug and gene delivery application. Gold nanoparticles are capable of delivering large biomolecules. The gold nanoparticles were utilized to facilitate the specific interactions between anticancer drugs and DNA. This may created a valuable application of metal nanoparticles in the relative biomedical area, **Sadowski Z. 2009)**.

The activity of Zinc oxide nanoparticles on bacterial growth, whereas the some isolates of *Staphylococcus species* were sensitivity percentage (52%) to the Zinc oxide nanoparticles while some isolates of *Staphylococcus species* intermediate percentage (48%) to the Zinc oxide nanoparticles in this study. Also, some isolates of *Staphylococcu*s species were bactericidal percentage (25%) while some isolates of *Staphylococcus species* bacteriostatic percentage (75%).

The mechanism by which the zinc oxide nanoparticles are able to penetrate the bacteria is completely. Zinc oxide nanoparticles changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting in cell death. It is observed that zinc oxide nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compounds such as DNA. it is that DNA may have lost its replication ability and cellular proteins become inactive after treatment with zinc oxide nanoparticles. In addition, zinc oxide nanoparticles prevent of grow and division. Because the zinc oxide heavy metals toxic and react with proteins; therefore they bind protein molecules; as a result cellular metabolism is inhibited causing cell death, also of microorganism that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates “electromagnetic” attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly, while bacterial inhibition depends upon the concentrations of Zinc oxide nanoparticles and the number of bacterial cells. Mechanism of the Antibacterial Activity of Zinc oxide nanoparticles, two mechanisms could be involved in the interaction between Zinc oxide nanoparticles and

bacteria: the effect Zinc oxide nanoparticles, both production of the Reactive Oxygen Species of ZnO Zinc oxide nanoparticles or deposition of Zinc oxide nanoparticles within the cytoplasm on the surface of *Staphylococcus* leads to disruption of cellular function and/or disruption and disorganization of membranes then either inhibition of bacterial growth or killing of *Staphylococcus* cells **(Bennimath V., Chidanand C., Prakash B. & Chandrakanth K. , 2011)** & **(Ubukata K., Matsuhashi M. & Konno M. ,1989)**. This may be due to the destructive effect of Zinc oxide nanoparticles with the cells and increased production of active oxygen such as H_2O_2 leads to the cell death. Zinc oxide nanoparticles, after its adherence to the surface of the cell membrane, results in disturbance in its respiration as it interacts with enzymes of the respiration chains of bacteria. In prokaryotic systems, cell death due to interactions between reactive oxygen species Reactive Oxygen Species and proteins, DNA, or membrane structures can be induced by oxidative stress. The preliminary findings suggest that Zinc oxide nanoparticles can be used externally to control the spreading of bacterial infections. It would be interesting to determine if any derivatives of Zinc oxide nanoparticles with various chemical groups or bioagents are more effective at eliminating various microorganisms. In the prevention and control of bacterial spreading and infections, the main target is the cell wall structure. The cell wall of most pathogenic bacteria is composed of surface proteins for adhesion and colonization, and components such as polysaccharides and teichoic acid that protect against host defenses and environmental conditions. These components are charged macromolecules; therefore, specific interactions to disrupt their main function and location may be triggered by introducing specific groups on the surface of the Zinc oxide nanoparticles. It has been reported that certain long-chain polycations coated onto surfaces can efficiently kill on Gram-positive bacteria **(Jones N., Binata R., Koodali T . & A, dhar C.2008)**.

Bacterial Surfaces as Revealed by Scanning Electron Microscope (SEM):

Scanning electron microscopy (SEM) is one of the best suited out of a variety of procedures to visualize the external appearance of bacteria. Bacteria live in various environments and their preparation for SEM thus takes their nature into consideration. Antimicrobial activity of different nanoparticles against *Staphylococcus* was investigated as a model for Gram-positive bacteria.

Studying the external shape of some isolates of *Staphylococcus* species after exposure to Zinc Oxide nanoparticles for 24 hours using the Scanning electron microscope showed cease on

some cells while the isolates showed some deformities in the external shape of cell and exit of the components of some cells in this study. Using the other nanoparticles as silver rod and gold rod nanoparticles caused lysis of all *Staphylococcus species* cells which made difficult to investigated by (SEM).

These particles were shown to be an effective bacteriostatic. Scanning electron microscopy (SEM) was used to study the nanoparticles action of this Nanoscale material. The results confirmed that the treated *Staphylococcus* cells were damaged, showing formation of "pits" in the cell wall of the bacteria while the Zinc Oxide nanoparticles were found to accumulate in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in the death of the cell.

Morphological changes on bacterial cells were observed by Scanning electron microscope (SEM) on some cells surface of *Staphylococcus* species, *(MRSA), S. aureus* and *S. epidermidis*, showed many irregular fragments on the cell surface, damage to cell surfaces. This increased the permeability of the cell membrane or leakage of cell contents could be caused Reactive Oxygen Species. Cell fragments could be the products derived from leakage of cytoplasmic contents in damaged cells. A Previous study was signed to active formation of bactericidal Reactive Oxygen Species by nanoparticles. This explained by increasing protein leakage with nanoparticles treatment that decreases the growth and reproduction of bacterial cells. The present study confirmed the antibacterial effect of nanoparticles against various microorganisms which can endanger human beings (**Lee H., Ryu D., Sojae C. & Dong L., 2011**). The lysis of some isolated bacteria after treated with nanoparticles refers to unbalance between two groups of enzymes and hydrolase syntheses of which performs cells lysis **(Holtje J. 1998)**. It has been demonstrated that the 'field-emission source' scanning electron microscope with high resolution. It is possible to examine the three-dimensional fine structure of bacterial surfaces not otherwise revealed by the conventional scanning electron microscope. In this study, we describe the surface structures of some Gram-negative and Gram-positive bacteria revealed using this high resolution scanning electron microscopic technique. Nanosized particles, of either simple or composite nature, display unique physical and chemical properties and represent an increasingly important material in the development of novel Nanodevices which can be used in numerous physical, biological, biomedical, and pharmaceutical applications (**Sondi I & Salopek B.,2004)**. A number of recent achievements offer the possibility of generating new types of nanostructured materials with designed

surface and structural properties **(Ghosh K. & Tarasankar P., 2007)**. Resistance of bacteria to bactericides and antibiotics has increased in recent years due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials.

Multiplex Polymerase Chain Reaction (MPCR):

A multiplex PCR assay for detection of toxin genotypes genes of *Staphylococci* enterotoxins A to E. Six primers used (*eta, icaAB, mecA, FemA, tst* and *atlE*) for DNA PCR amplification have been identified *eta, icaAB, mecA, FemA, tst* and *atlE*. In this study, and they are easily distinguished by gel electrophoresis. This PCR-based assay differentiated multiple genes within one reaction. Molecular test is very important it emphasizes and supports phenotypic test. The results differed in sixteen isolations where genes appeared in some isolates *S. aureus* and *(MRSA)* did not appear in the other isolates, uses four primers *eta, mecA, FemA* and *tst* among the 16 strains tested strains were collected from wound swab (pus) and high vaginal swab (HVS) in this study. Sixteen strains from *(MRSA),* were fifteen isolation of *S. aureus* and *Methicillin-resistant Staphylococcus aureus* were positive for *mecA* gene this showed the band at 163bp this the gene responsible for antibiotic resistant Methicillin, two isolates of *S. aureus* and *Methicillin-resistant Staphylococcus aureus* were positive for the *TSST-1* gene this showed the band at 326bp this the gene responsible for toxic shock syndrome in some *Staphylococcus species*, None were positive for *eta* gene this showed the band at 102bp 326bp this the gene responsible for exfoliative toxins in some *Staphylococcus species*, all of the sixteen samples tested contained the *femA* gene this gene used as an internal positive control .

Description multiplex PCR-based diagnostic protocol to detect the genes for enterotoxins ETA, *ETB* and *TSST-1* and the *mecA* gene in DNA extracted from human isolates of *S. aureus* and *(MRSA)*. This procedure is an improvement over our previously described PCR protocols, where individual primers were used to identify the *Staphylococcal* toxin genes. *femA* was found to be present in all of the strains studied. The gene product of *femA* has been suggested to have a role in cell wall metabolism and is reported to be present in all *S. aureus* and *(MRSA)* species during the active growth phase. The use of multiplex PCR to characterize *Staphylococcal* strains and their resistance to methicillin. The study showed the presence of the gene responsible toxic shock syndrome toxin 1 *(TSST)* and the gene responsible for methicillin resistance *(mecA)* and the gene responsible for exfoliative toxins

A *(eta A)*, **(Mehrotra M., Wang G. & Johnson W., 2000)**. The polymerase chain reaction (PCR) was used to amplify both the *(MRSA)* specific sequence gene and mecA gene with the amplify. All isolates showed the presence of a gene mecA, suggesting a link gene mecA to isolates resistant to the antibiotic Methicillin (**Nicole Jones1, Binata Ray1, Koodali T. Ranjit2 & Adhar C. Manna,2008)**. By multiplex PCR the tst gene encoding TSST-1 was detected of *S. aureus* and *(MRSA)*. TSST-1 the type of toxicities and symbolizes the genetic component in *Staphylococcus aureus* known *sapI*. Several studies have shown that the TSST-1 gene is more prevalent in Methicillin-resistant *Staphylococcus aureus (MRSA)* than in methicillin-susceptible *Staphylococcus aureus*. Methicillin resistance is either due to expression of *mecA* gene or the synthesis of methicillinase or due to both. All the isolates were subjected to PCR amplification of *mecA* gene. PCR detection isolates were carrying mecA gene. The repression of *mecA* gene and the resulting absence of *(MRSA)* in some of the isolates could be due to several factors. Both genetic and environmental factors play a significant role in the expression of *(MRSA)*. The genetic factor could be repression of *mecA*, which are its co-repressors. The induction of *mecA* gene occurs through a signaling pathway initiated by the interaction of β-lactams, a trans-membrane protein. Therefore, selective pressure generated by indiscriminate use of antibiotic therapy is an important environmental factor in the induction of *mecA* gene. The present study demonstrated the production of methicillinase in some of *(MRSA) MRSA* strains isolated. It also suggests that *S. aureus*, suspected to be carriers of mecA, should be the subject for both phenotypic and genotypic analysis to confirm their *(MRSA)* status (**Khan, A., Sultan A., Tyagi A., Zahoor S., Akram M. & Chetana V. 2007)**. Designed primers for amplification of *mecA* gene which is present in the plasmid DNA of *S. aureus* strain from PCR. The amplified part has sequenced, have been found mecA gene of *S. aureus* strain M600. The *mecA* gene plays an important role in β-lactamase antigen resistance including methicillin (**Bennimath V., Chidanand C., Prakash B. & Chandrakanth K., 2011)**. The *mecA* gene is a gene found in bacterial cells. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin, erythromycin, tetracycline and other penicillin-like antibiotics. The most commonly known carrier of the *mecA* gene is the bacterium known as *(MRSA)*. It was also found in *Staphylococcus aureus* strain and *Streptococcus pneumoniae* strains resistant to penicillin-like antibiotics. In *Staphylococcus* species, *mecA* is spread on the *SCCmec* genetic element. The *mecA* gene does not allow the ringlike structure of penicillin-like antibiotics to attack the enzymes that help form the cell wall of the bacterium, and hence, the bacteria is allowed to replicate as normal. The gene encodes the protein PBP2A (Penicillin-binding

protein 2A). PBP2A has a low affinity for beta-lactam antibiotics such as methicillin and penicillin. This enables transpeptidase activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis (**Ubukata K., Matsuhashi M. and Konno M.,1989)**.

The results differed in eight isolates of *S. epidermidis* where the genes appeared in some isolates did not appear in the other isolates, two primers *icaAB* and *atlE* by PCR Multiplex as shown in, among the 8 strains tested strains were collected from blood in this study. Six isolates of *Staphylococcus epidermidis* were positive for *atlE* gene this showed the band at 682 bp for the gene that responsible for its involved in initial adherence, three isolates of *Staphylococcus epidermidis* were positive for *icaAB* gene this showed the band at 546bp this the gene responsible for mediates the formation of the biofilm.

The PCR detection of putative virulence genes was for contaminating strains, sepsis-related strains, catheter strains. Multiplex PCR was used to explore the *atlE* gene, which is involved in initial adherence and adherence are cell-surface components or appendages of bacteria that facilitate bacterial adhesion or adherence to other cells or to inanimate surfaces. Adhesins are a type of virulence factor. Adherence is an essential step in bacterial pathogenesis or infection, required for colonizing a new host, the intercellular adhesion gene cluster (*icaAB*), which mediates the formation of the biofilm. Whereas the *atlE* gene was almost ubiquitously amplified, the *icaAB* gene was detected significantly more in infecting strains than in contaminating strains and thus appeared to be related to the potential virulence of *Staphylococcus epidermidis*. In other respects, investigation of the second stage of biofilm formation demonstrated that cell aggregation and biofilm accumulation were mediated by the products of the chromosomal ica gene locus, which comprises three intercellular adhesion genes (*icaA, icaB,* and *icaC*) organized in an operon structure and which leads to the biosynthesis of polysaccharide intercellular adhesin. The *atlE* gene, which encodes a vitronectin-binding cell surface protein involved in primary attachment. Vitronectin is a protein encoded by the genes. Vitronectin is an abundant glycoprotein found in serum and the extracellular matrix and promotes cell adhesion, was ubiquitously amplified in *Staphylococcus epidermidis* strains and virulence. Also, study was to whether of ica and that of the *atlE* gene might discriminate between virulent *Staphylococcus epidermidis* strains that cause real sepsis and nonvirulent *Staphylococcus epidermidis* strains that contaminate blood cultures[23]. The detection by PCR for the genes *atlE*, *icaA*, and *icaD* by amplification method

was performed in *Staphylococcus epidermidis* of isolates from prosthetic joint infections and isolates from skin as controls. The *atlE* was significantly more in prosthetic joint infections frequent strains about other strains were controls. Most prosthetic joint infections strains were positive for icaA and *icaD,* **(Sivadon V., Quincampoix J., Prunier E. & Hoffmeyer P.,2009)**. *Staphylococcus epidermidis* has become the leading cause of foreign-body infections due to its biofilm formation on all kinds of medical device surfaces. The biofilm development of *Staphylococcus epidermidis* includes two steps: the initial attachment phase and the accumulative phase. In the accumulative phase, the polysaccharide intercellular adhesin, encoded by the *icaADB* genes, is the major component mediating intercellular adhesion. Showing a tendency towards an increasing proportion of this subpopulation in *staphylococci*-associated infections. Biofilm production by *Staphylococcus* is an important virulence determinant mediated by the *icaADBC* gene -encoded polysaccharide intercellular adhesin or by surface and extracellular proteins. This study reveals that in the *Staphylococcus epidermidis ica* gene loci and controls the biofilm phenotype, primarily by regulating *icaADBC* gene transcription and polysaccharide intercellular adhesin production (**Schoske R., Pete M., Christian R. & John B., 2003)**. Validation of the multiplex PCR primers was performed and interpreted using 24 isolates of *Staphylococcus* isolates which were characterized by their toxin gene profiles by using individual primers. We conclude that the multiplex primer sets described here are reliable and specific in detecting the toxin genes of *Staphylococci.* Considering the low cost and much shorter time required to detect the six genes of *Staphylococci* species by multiplex PCR, we believe this to be a powerful tool for studying the genotypes of *staphylococcal* isolates. This procedure was specially developed to fit into the daily work pattern of a routine clinical laboratory, since genotypic detection of drug resistance and the presence of toxin genes are becoming an important component of the diagnostic inventory of such laboratories.

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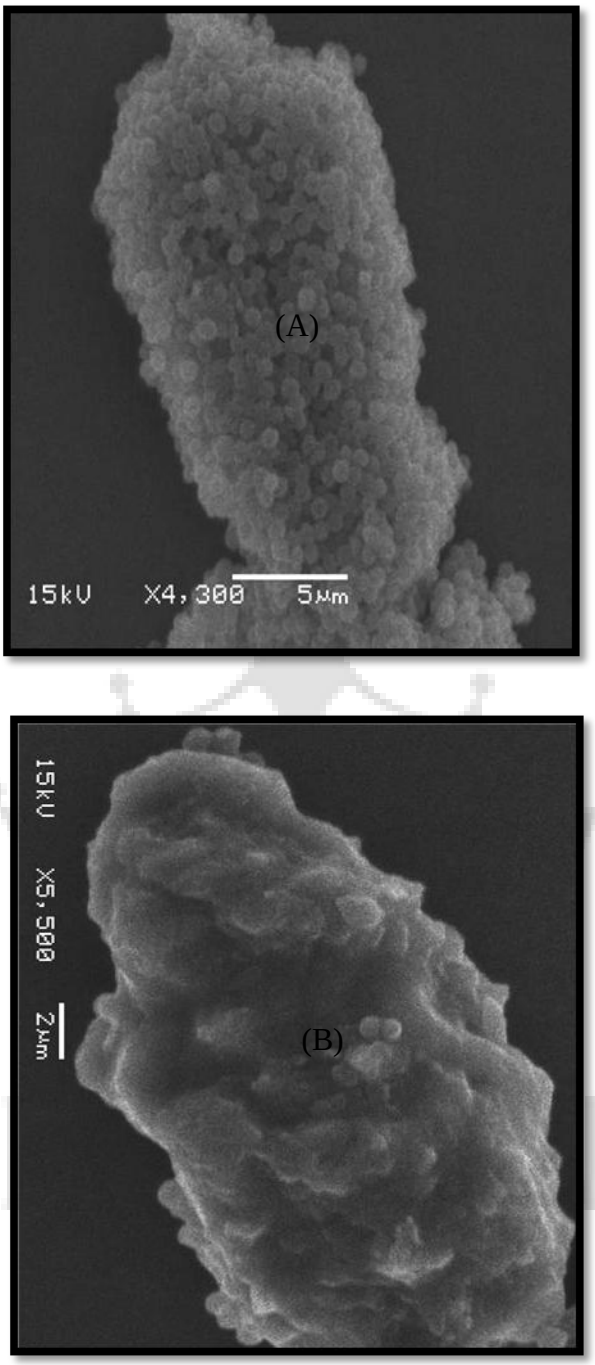
Figure captions list:

- **'Figure20':** Scanning Electron Microscope of Methicillin-resistant *Staphylococcus aureus (MRSA)* (SEM), (A) Non-treated Bacterial cells showed spherical cells in irregular clusters,X4,300; (B) After treated with Zinc Oxide nanoparticles, showed some appendages on Bacterial cells wall, some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X5,500).
- **'Figure22'**: Scanning Electron Microscope of *Staphylococcus aureus* (SEM). (A) Non treated Bacterial cells showed spherical cells in irregular clusters,(X5,000). (B) After treated with Zinc Oxide nanoparticles, showed some appendages on Bacterial cells wall, some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X4,500).

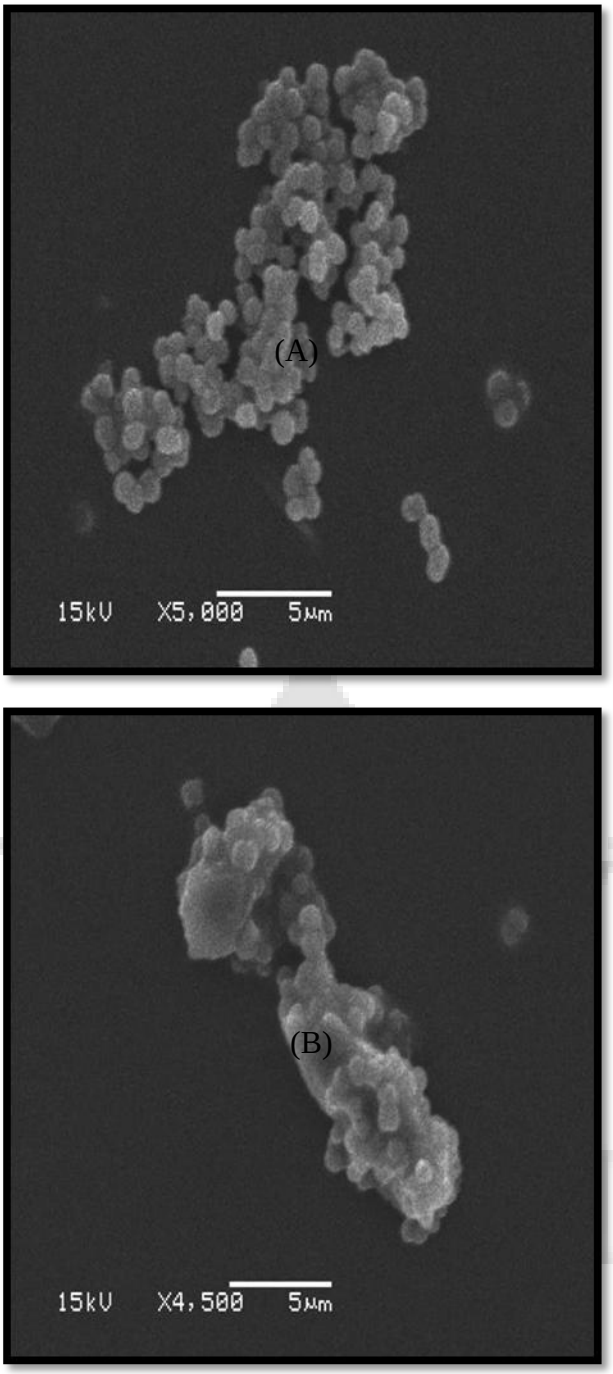
- **'Figure23':**Scanning Electron Microscope of *Staphylococcus aureus* (SEM), (A) Non treated Bacterial cells showed spherical cells in irregular clusters,(X5,000); (B) After treated with Zinc Oxide nanoparticles, showed some appendages on Bacterial cells wall ,some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X5,000).
- **'Figure24':**Scanning Electron Microscope of *Staphylococcus epidermidis* (SEM), (A) Non-treated Bacterial cells showed spherical cells in irregular clusters (X4,300); (B) After treated with Zinc Oxide nanoparticles, showed some appendages on Bacterial cells wall ,some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X5,000).
- **'Figure26':**Multiplex PCR amplification of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus (MRSA)* with four different primer were identified (*eta, mecA, FemA* and *tst*), 50bp and 100-bp DNA ladder maker; lanes 1 to 8, PCR amplicons from *Staphylococcus* aureus; lanes 9 to 16, PCR amplicons from *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus (MRSA)*: 1, *mecA* plus *femA*;2 *mecA* plus *femA*;3, *mecA* plus *femA*;4, *mecA* plus *femA*;5, *tst* plus *femA*;6, *mecA* plus *femA*; 7, *mecA* plus *femA* ; 8, *mecA* plus *femA*;9, *mecA* plus *femA* and *tst* ; 10, , *mecA* plus *femA*; 11, *mecA* plus *femA*; 12, *mecA* plus *femA*; 13 , *mecA* plus *femA*; 14 , *mecA* plus *femA*; 15 , *mecA* plus *femA*; 16 , *mecA* plus femA; C , negative control; *femA* ranging 132bp, *mecA* ranging 163bp and *tst* ranging 326bp.

'Figure27': Multiplex PCR amplification of *staphylococcus epidermidis* with two different primer were identified (*icaAB* and *atlE*), 100-bp DNA ladder maker; lanes 1 to 8, PCR amplicons *staphylococcus epidermidis*: 1, negative; 2 negative; 3, *atlE* genes; 4, *atlE* plus *icaAB* genes; 5, *atlE* plus *icaAB* genes; 6, *atlE* genes; 7, *atlE* plus *icaAB* genes; 8, *atlE* genes; C negative control; *atlE* ranging 682bp and *icaAB* ranging 546 bp.

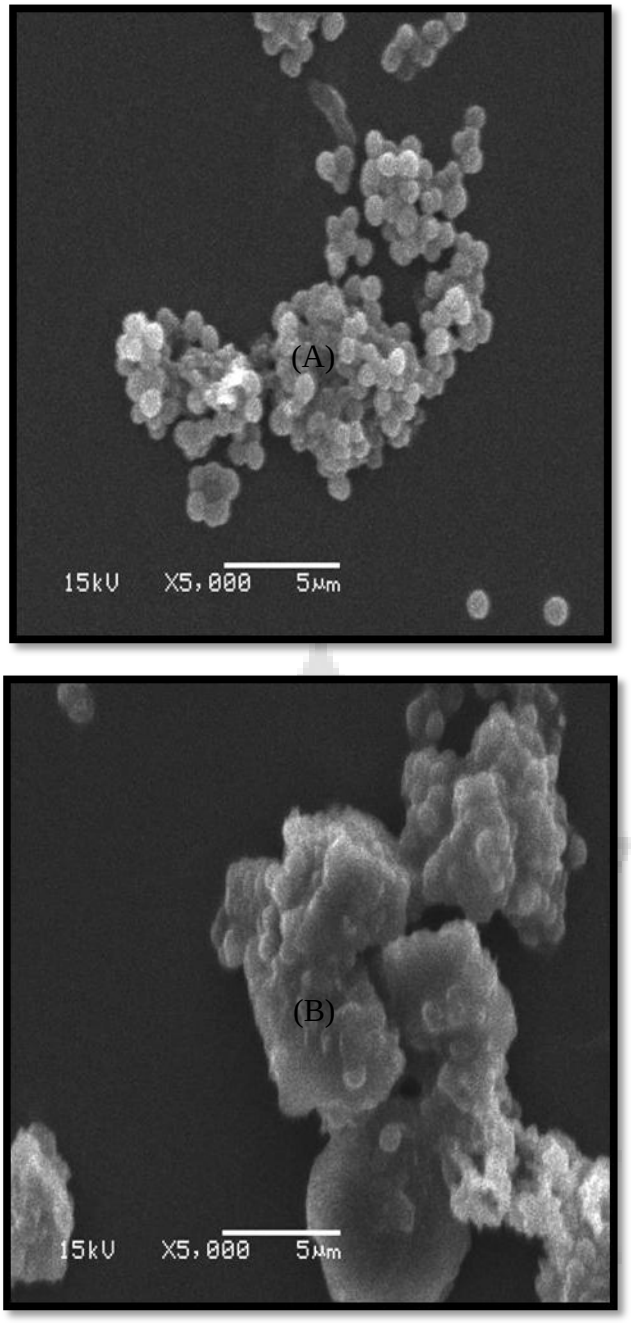
Tables & Figures



(Figure 20)



(Figure 22)



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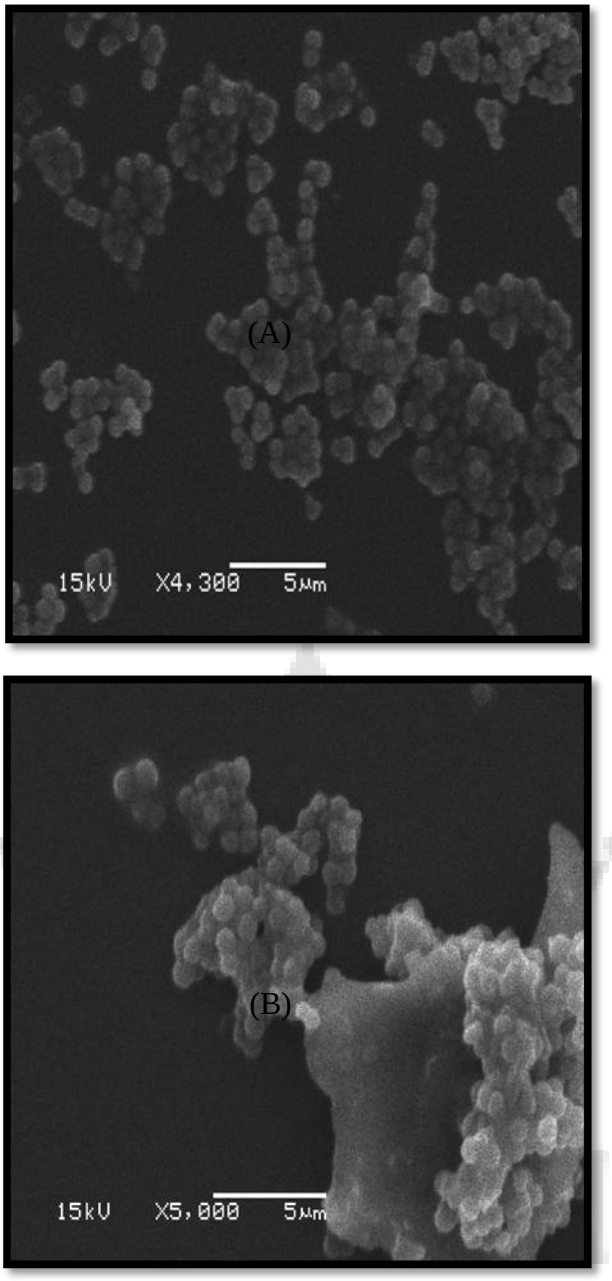

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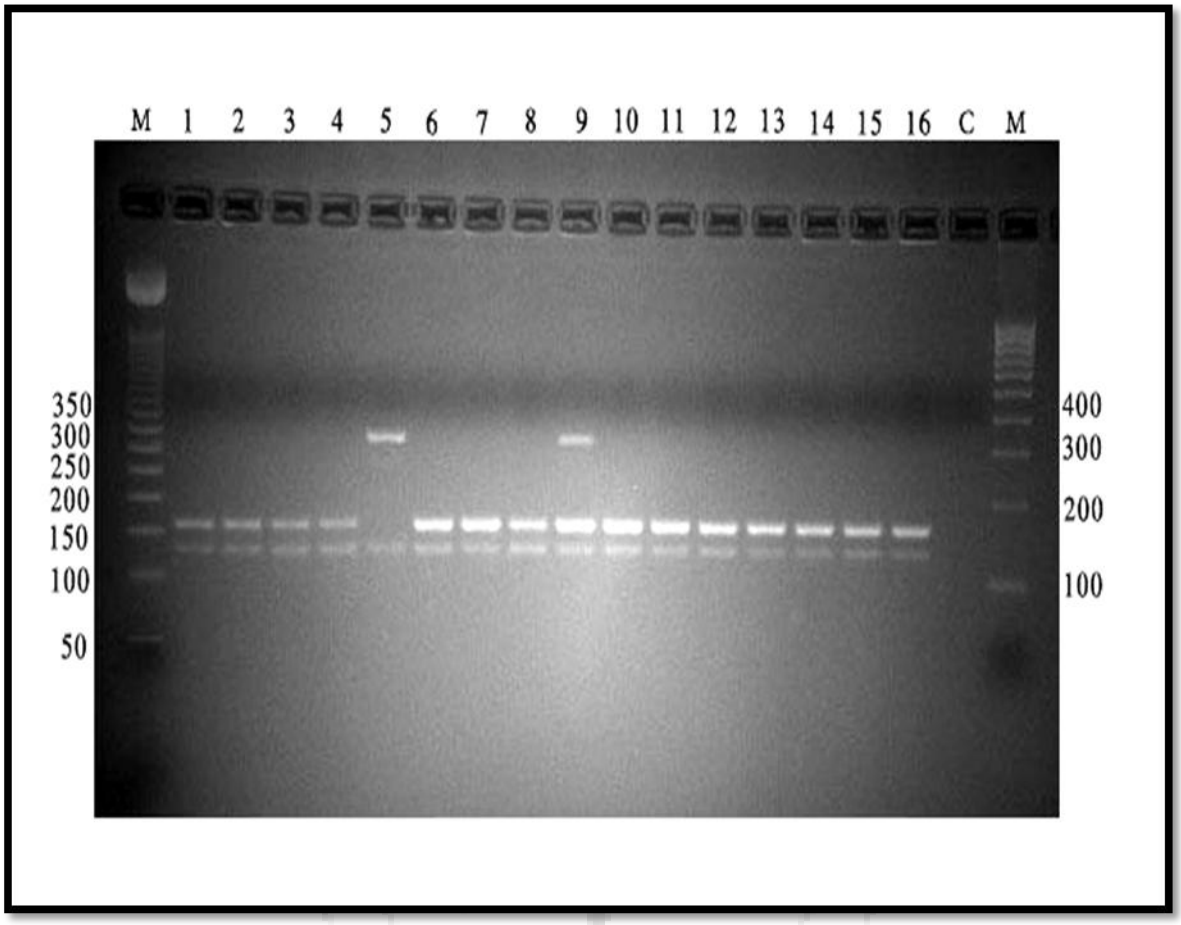
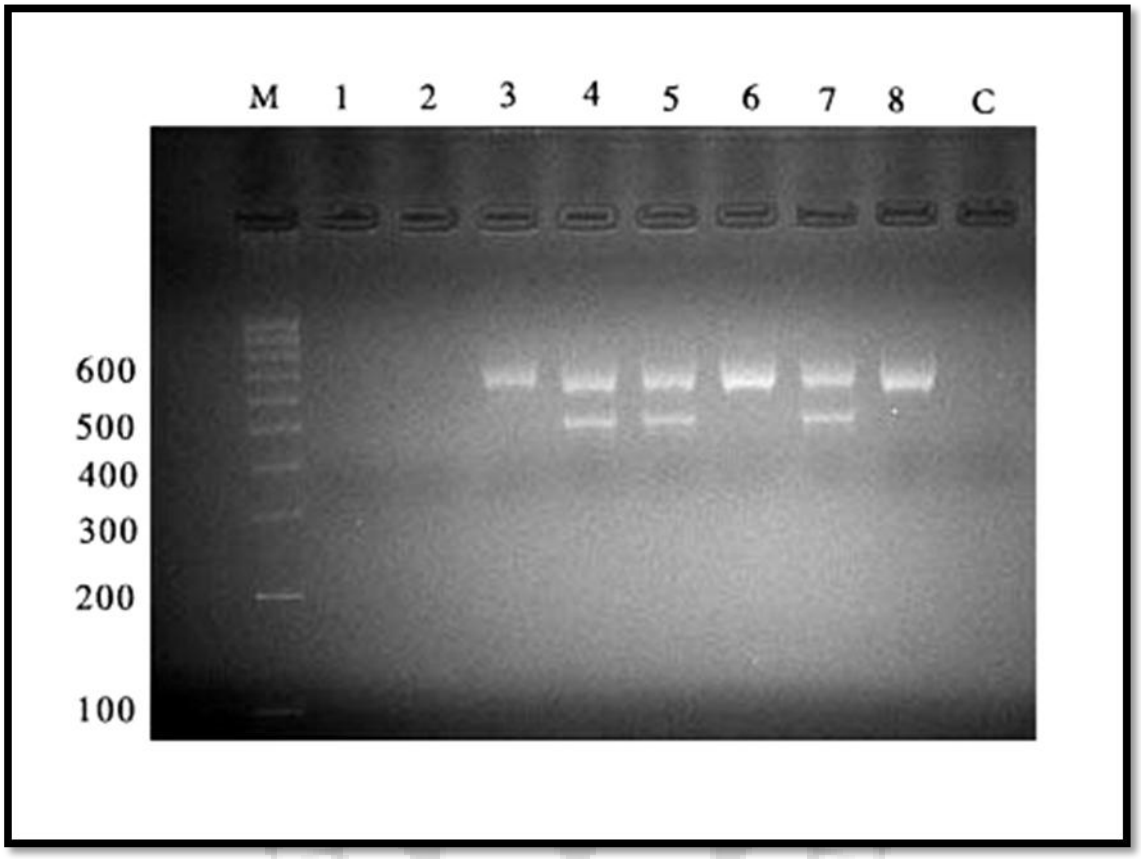


(Figure 26)



(Figure 27)

Figure captions list :

- **'Figure S 1':** Distribution of the number of isolates from males and females based on the age.
- **'Figure S 2'**: Number of isolates of males and females according to age categories.
- **'Figure S 3'**: Distribution of organisms with respect to gender
- **'Figure S 4'**: Percentage of *Staphylococcus* species for Males and Females.
- **'Figure S 5'**: Percentage of *Staphylococcus*species in Females
- **'Figure S 6'**: Percentage of *Staphylococcus*species in Males.
- **'Figure S 7'**: Distribution of in each isolates with age group.
- **'Figure S 8'**: Distribution of Males in eachisolateswith age group.
- **'Figure S 9'**: Distribution of Females in each isolates with age group.
- **'Figure S 10'**: Distribution of isolates with respect to various sources
- **'Figure S 11'**: Distribution of bacterial isolates with respect to in major departments.
- **'Figure S 12'**: Effect of antibiotics on bacterial sensitivity for all 96 *staphylococcus* isolates.
- **'Figure S 13'**: Antimicrobial Sensitivity of Nanoparticles (Gold, Silver and Zinc oxide) on all of *Staphylococcus* isolates.
- **'Figure S 14'**: The effect of Silver (Spherical) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).
- **'Figure S 15'**: The effect of Silver (Rods) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).
- **'Figure S 16'**: The effect of Gold (Spherical) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).
- **'Figure S 17'**: The effect of Gold (Rods) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).
- **'Figure S 18'**: The effect of Zinc Oxide nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).
- **'Figure S 19'**: Bacteriostatic and Bactericidal effect of Nanoparticles (Gold, Silver and Zinc oxide) on all of *Staphylococcus* isolates.
- **'Figure S 21'**: Scanning Electron Microscope of Methicillin resistant *Staphylococcus aureus (MRSA)* (SEM), (A) Non treated Bacterial cells showed spherical cells in irregular clusters,X5,000; (B) After treated with Zinc Oxide nanoparticles, showed some appendages

on Bacterial cells wall ,some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X3,700).

- **'Figure S 25'**: Scanning Electron Microscope of *Staphylococcus epidermidis* (SEM), (A) Non treated Bacterial cells showed spherical cells in irregular clusters,(X5,000); (B) After treated with Zinc Oxide nanoparticles, showed some appendages on Bacterial cells wall ,some deformities in external shape of cells, some Overlapping Bacterial cells and decreasing number of Bacterial cells,(X3,700).

Tables captions list :

- **'Table S1'**: Distribution of number of isolates in males and females with respect to different age group.
- **'Table S2'**: Showed the Comparison of distribution percentagefor all 96 isolates.
- **'Table S3'**:: Chi-square test analysis for 96 isolated.
- **'Table S4'**: Number and percentage of isolates from males and females with respect to different age group for all 96 samples.
- **'Table S5'**: Chi-square testanalysis for 96 isoletd.
- **'Table S6'**: Distribution of isolates with respects to various sources for male and female.
- **'Table S7'**: Distribution of bacterial isolates with respect to major clinical departments, Intensive Care Unit(ICU), Intensive Care UnitNeonatal(ICUN), Obstetric Ward(OBW), Operating Room(OR), Female Surgical Ward(FSW), Female Medical Ward(FMW), Female Orthopedic Ward(FOW), Male Surgical Ward(MSW), Male Medical Ward(MMW), Male Orthopedic Ward(MOW), Pediatric Ward(PW), Emergency Room(ER), Public Health Department(PHD), Dermatology Clinic (Clinic c) and Orthopedic Clinic (OW).
- **'Table S8'**: Microbiological tests used to distinguish between bacterial species, Positive (+), Negative (-), Resistant (R) and Sensitive (S).

Table (S1): Distribution of number of isolates in males and females with respect to different age group.

Age Categories	# of Males	# of Females
Born 0-1	10	2
Pediatric 1-15	2	4
Teenagers 16-30	11	17
Adults31-60	16	14
Elderly 61-100	14	6
Total	53	43
Average	55%	45%

Table (S2): Showed the Comparison of distribution percentage for all 96 isolates.

Species	Number in Males	percents of isolates %	Number in Females	percents of isolates %	percents of all isolates %
Staphylococcus aureus	15	28%	20	47%	37%
Staphylococcus epidermidis	25	47%	10	30%	36%
Methicillin –resistant *Staphylococcus aureus*	13	25%	13	23%	27%
Average /Total	53	100%	43	100%	100%

Table (S3): Chi-square test analysis for 96 isolated

Species	Male		Female		Male	Female	Male	Female	Male	
	o.f	e.f	o.f	e.f	(o.f-e.f)		$(o.f-e.f)^2$		$(o.f-e.f)^2/e.f$	
Staphylococcus aureus	15	19.32	20	15.68	-4.32	4.32	18.6624	18.6624	0.9659	1.1902
Staphylococcus epidermidis	25	19.32	10	15.68	5.68	-5.68	32.2624	32.2624	1.6668	2.0575
Methicillin resistant *Staphylococcus aureus*	13	14.35	13	11.65	-1.35	1.35	1.8225	1.8225	0.127	0.1564
Column Total	53		43				SUM		**6.1638**	

Table (S4): Number and percentage of isolates from males and females with respect to different age groups for all 96 samples.

Age Group	# / % Males /isolates			# / % Femles /isolates		
	S.aureus	*S.epidermidis*	*MRSA*	*S.aureus*	*S.epidermidis*	*MRSA*
Born	1	7	1	0	2	0
1-15	1	1	0	2	1	1
16-30	8	3	1	9	3	5
31-60	3	7	6	7	1	6
61-100	2	7	5	2	3	1
Average/Total	**15 -16%**	**25 - 26%**	**13-13.5%**	**20-21%**	**10-10.41%**	**13-13.5%**

Table (S5): Chi-square test analysis for 96 isolates

Age group / Organisms		o.f	e.f	(o.f-e.f)	(o.f-e.f)²	(o.f-e.f)²/e.f
Born	*Staphylococcus aureus*	1	4.01	-3.01	9.06	2.26
	Staphylococcus epidermidis	9	4.01	4.99	24.90	6.21
	Methicillin –resistant *Staphylococcus aureus*	1	2.98	-1.98	3.92	1.32
1-15	*Staphylococcus aureus*	3	2.19	0.81	0.66	0.30
	Staphylococcus epidermidis	2	2.19	-0.19	0.04	0.02
	Methicillin –resistant *Staphylococcus aureus*	1	1.63	-0.63	0.40	0.24
16-30	*Staphylococcus aureus*	17	10.57	6.43	41.34	3.91
	Staphylococcus epidermidis	6	10.57	-4.57	20.88	1.98
	Methicillin –resistant *Staphylococcus aureus*	6	7.85	-1.85	3.42	0.44
31-60	*Staphylococcus aureus*	10	10.94	-0.94	0.88	0.08
	Staphylococcus epidermidis	8	10.94	-2.94	8.64	0.79
	Methicillin –resistant *Staphylococcus aureus*	12	8.12	3.88	15.05	1.85

	Staphylococcus aureus	4	7.29	-3.29	10.82	1.48
	Staphylococcus epidermidis	10	7.29	2.71	7.34	1.01
61-100	**Methicillin –resistant *Staphylococcus aureus***	6	5.42	0.58	0.34	0.06
	Column Total				SUM	**21.95**

Table (S6): Distribution of isolates with respects to various sources for male and female.

Sl. No	**Source**	***S. aureus***	***S. epidermidis***	***MRSA***
1	Nostrils Swabs	2	1	5
2	Wound Swabs	18	12	21
3	Urine	3	0	0
4	Blood	1	13	0
5	High Vaginal Swabs	9	2	0
6	Ear Swabs	1	0	0
7	Axilla Swabs	0	1	0
8	Groin Swabs	0	2	0
9	Endo Tracheal Tube	1	1	0
10	Tissue	0	1	0
11	Ascitic Fluid	0	1	0
12	Spinal Fluid	0	1	0
	Total	**35**	**35**	**26**
	Average %	**37%**	**36%**	**27%**

Table(S 7): Distribution of bacterial isolates with respect to major clinical departments, Intensive Care Unit(ICU), Intensive Care UnitNeonatal(ICUN), Obstetric Ward(OBW), Operating Room(OR), Female Surgical Ward(FSW), Female Medical Ward(FMW), Female Orthopedic Ward(FOW), Male Surgical Ward(MSW), Male Medical Ward(MMW), Male Orthopedic Ward(MOW), Pediatric Ward(PW), Emergency Room(ER), Public Health Department(PHD), Dermatology Clinic (Clinic c) and Orthopedic Clinic (OW).

Departments	**Organisms**			**Total**	**Average**
	S. aureus	***S. epidermidis***	***MRSA***		
OBW	1	1	3	5	5.20%
PW	1	3	3	7	7.29%
FSW	4	3	1	8	8.33%
MSW	4	1	2	7	7.29%
OR	0	1	0	1	1%
NICU	0	6	0	6	6.25%
ICU	0	2	7	9	9.37%
MOW	0	2	4	6	6.25%
FOW	1	1	0	2	2%
FMW	1	1	1	3	3%
MMW	1	0	1	2	2%
ER	19	4	9	32	33.33%
PHD	1	0	0	1	1%
Clinic C	1	3	1	5	5.20%
OW	0	0	2	2	2%
				96	100%

Bacterial identification:

Table (S 8): Microbiological tests used to distinguish between bacterial species, Positive (+), Negative (-), Resistant (R) and Sensitive (S).

Species	Catalase	Coagulase	Mannitol	DNAase	MRSA Confirmatory	Novobiocin 5 µg
MRSA	(+)	(+)	(+)	(+)	(+)	(R)
Staphylococcus aureus	(+)	(+)	(+)	(+)	(-)	(R)
Staphylococcus epidermidis	(+)	(-)	(-)	(-)	(-)	(S) > 16 mm

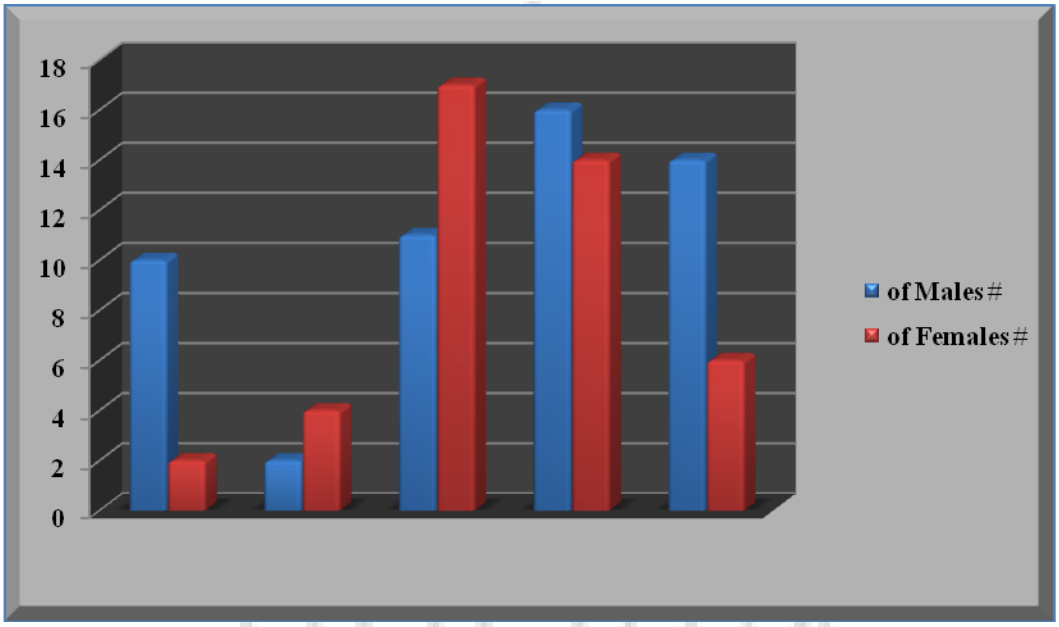


Figure (S1): Distribution of the number of isolates from males and females based on the age.

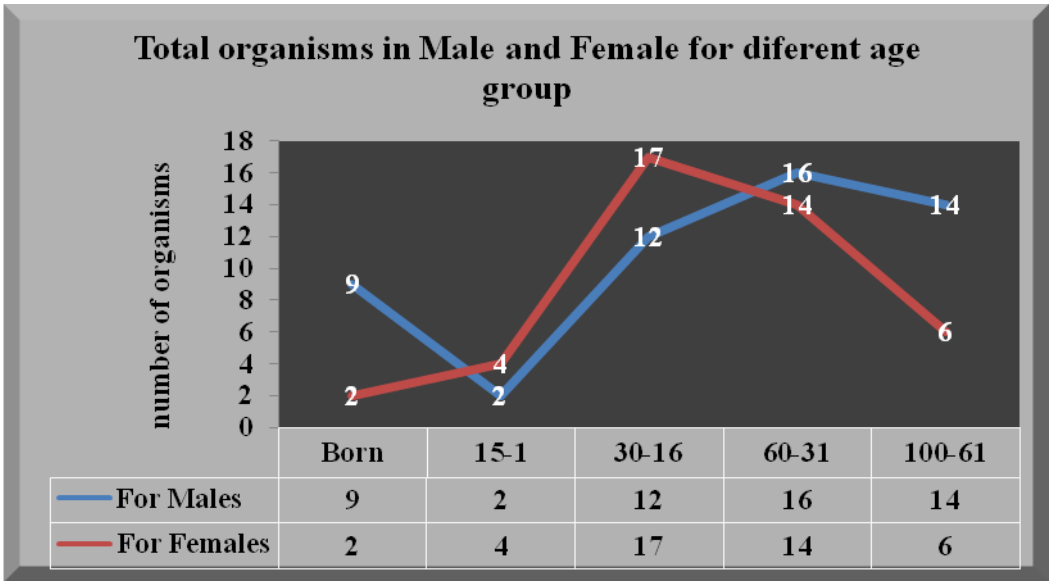


Figure (S2): Number of isolates of males and females according to age categories.

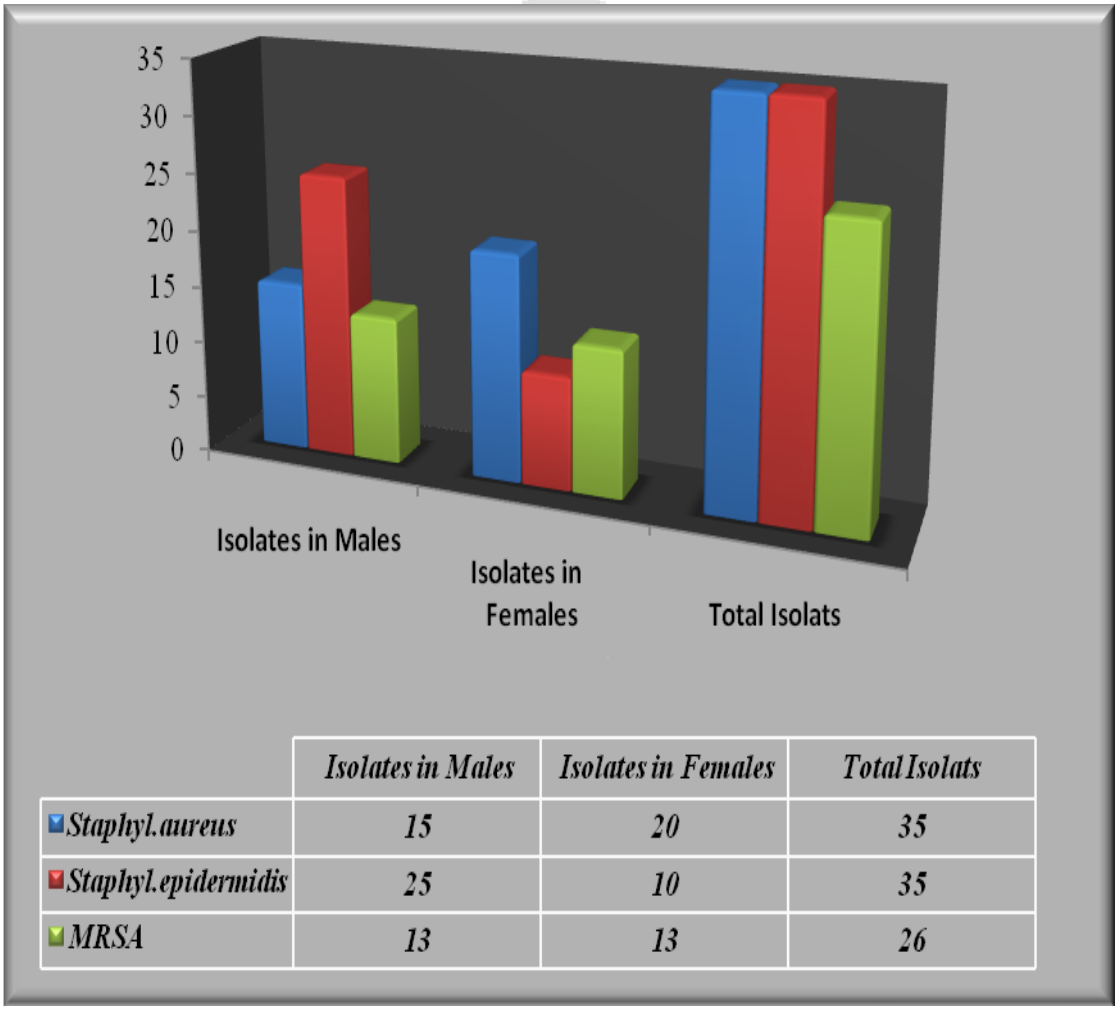


Figure (S3): Distribution of organisms with respect to gender

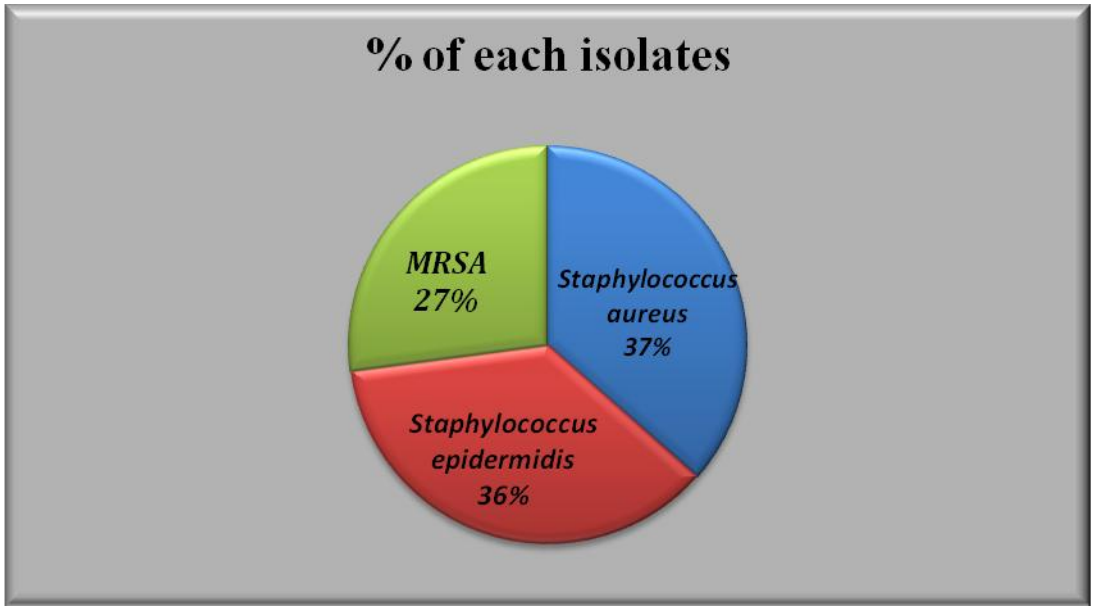


Figure (S4): Percentage of *Staphylococcus* species for Males and Females.

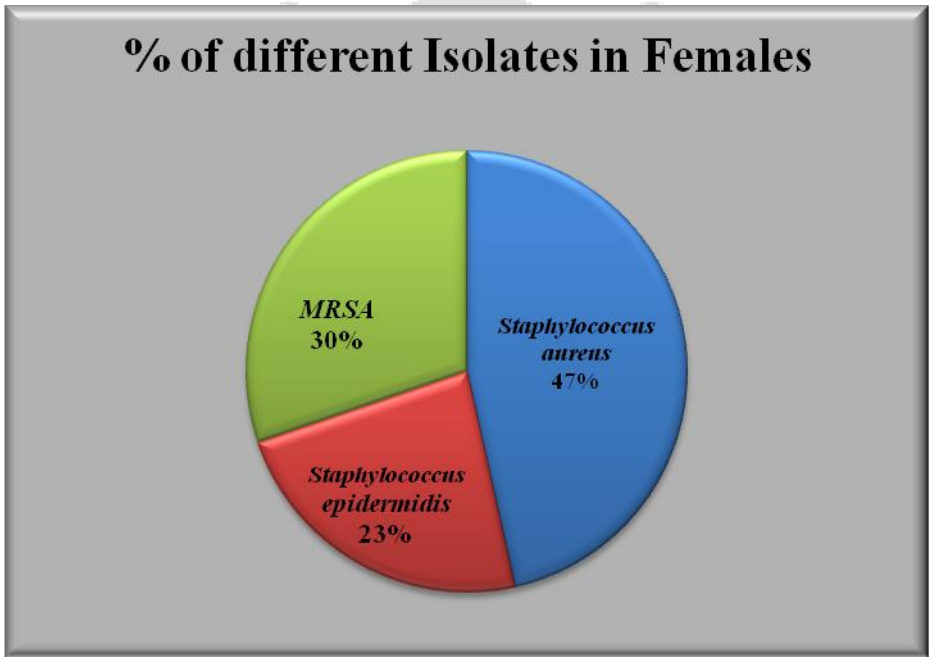


Figure (S5): Percentage of *Staphylococcus* species in Females

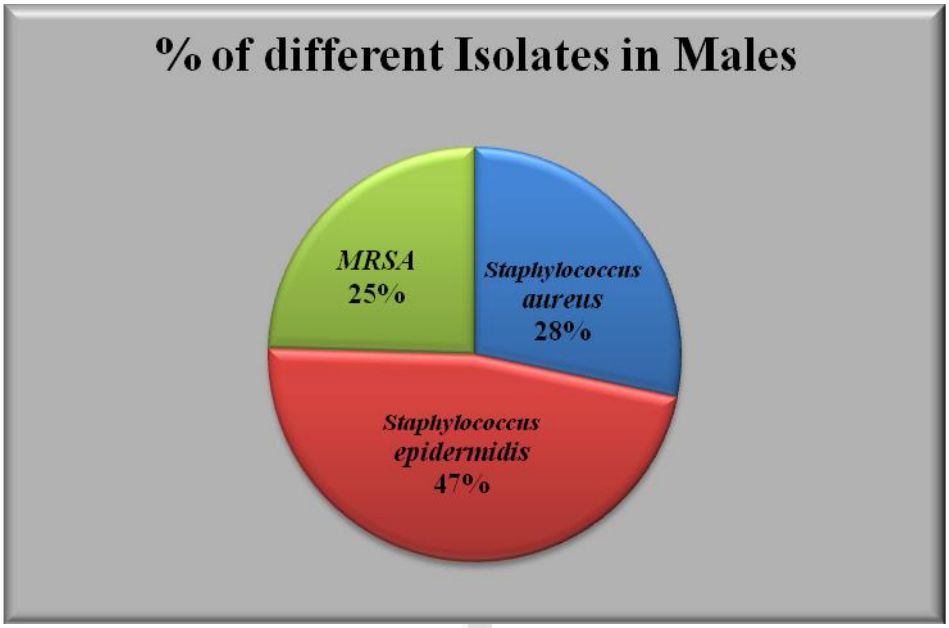
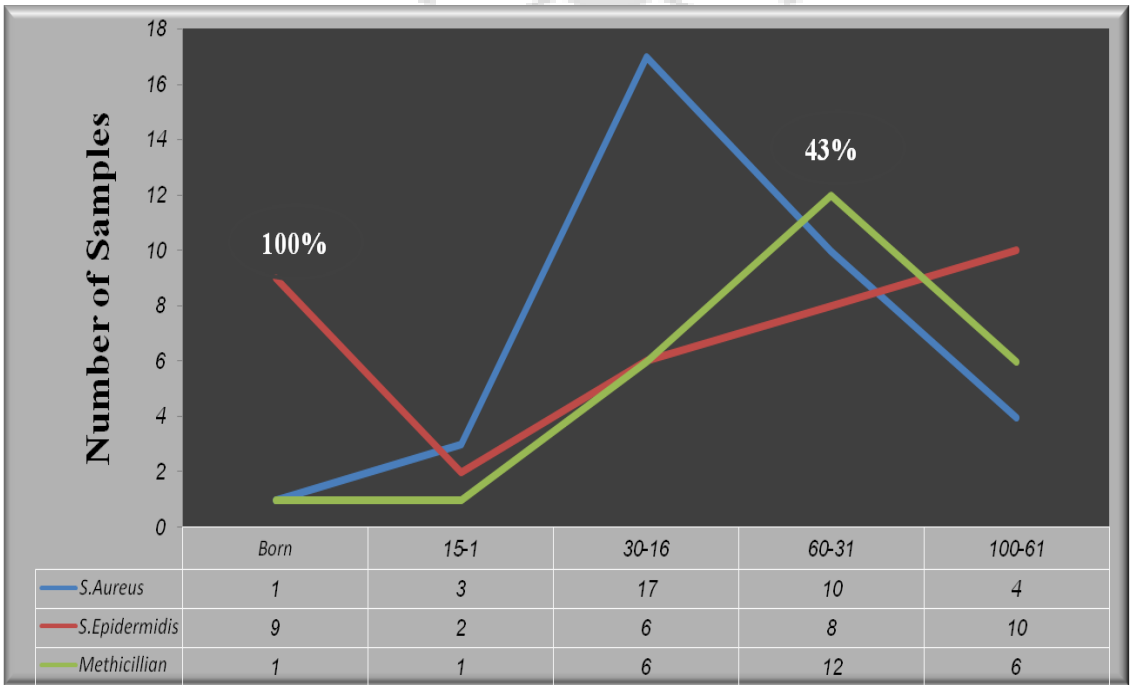


Figure (S6): Percentage of *Staphylococcus* species in Males



	Born	15-1	30-16	60-31	100-61
S.Aureus	1	3	17	10	4
S.Epidermidis	9	2	6	8	10
Methicillian	1	1	6	12	6

Figure (S7): Distribution of in each isolates with age group.

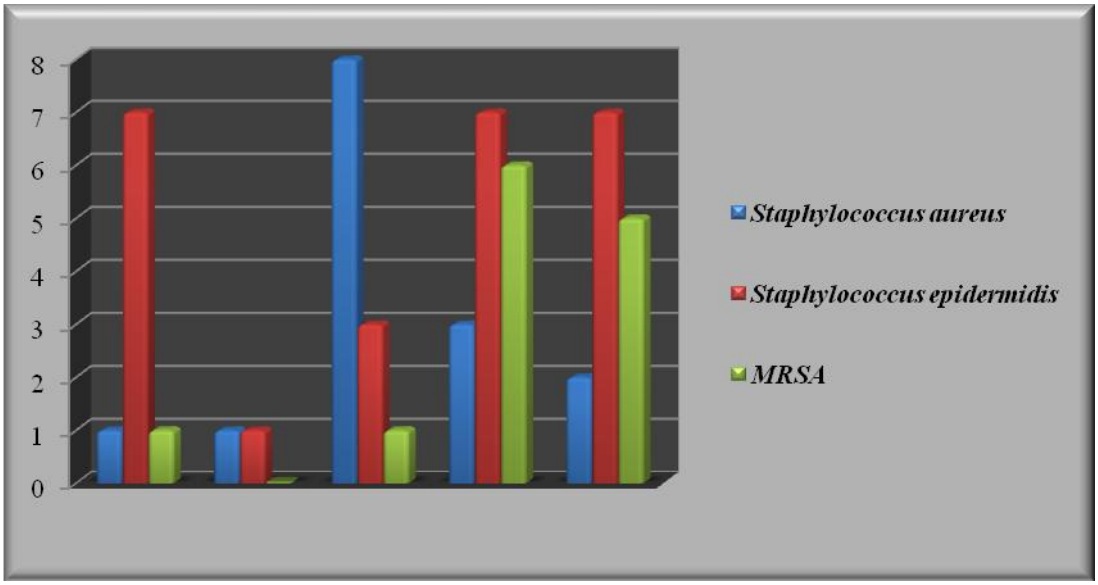


Figure (S8): Distribution of Males in each isolates with age group

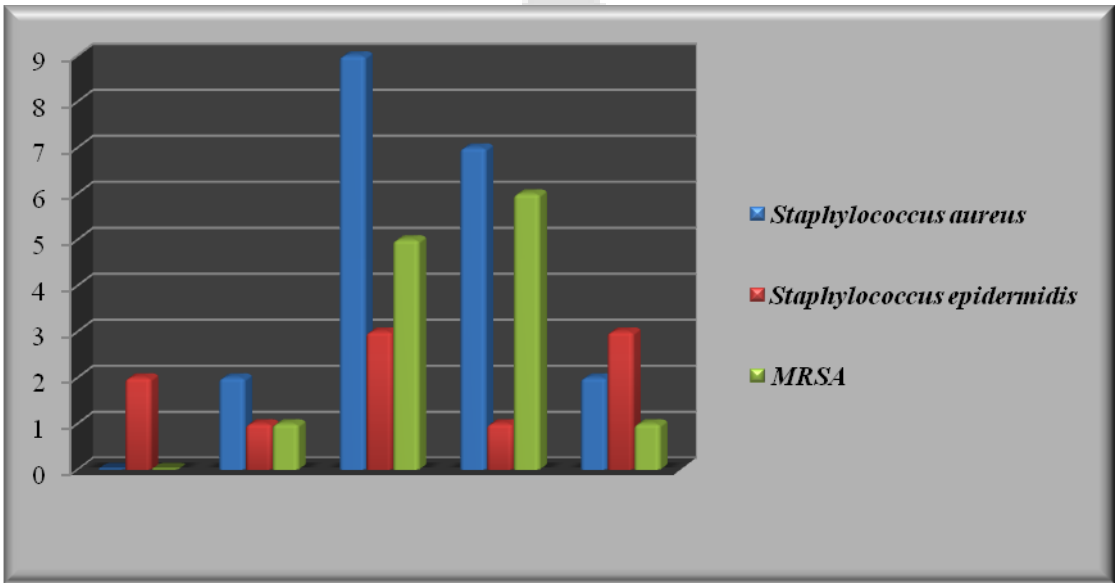


Figure (S9): Distribution of Females in each isolates with age group

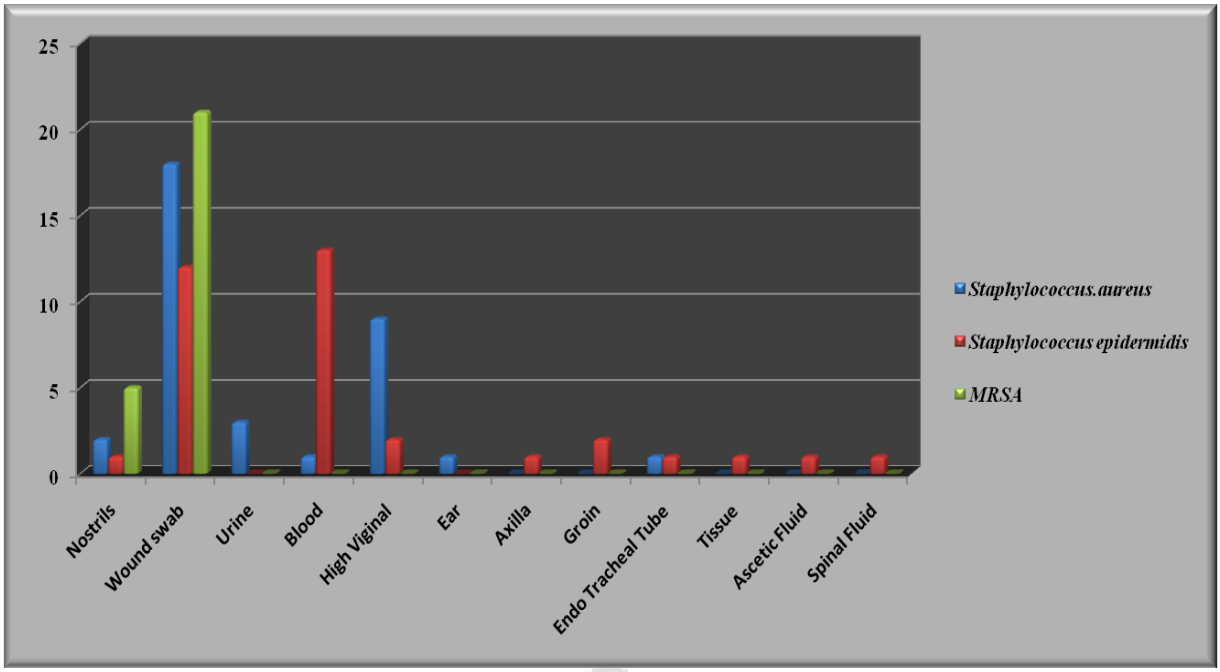


Figure (S10): Distribution of isolates with respect to various sources

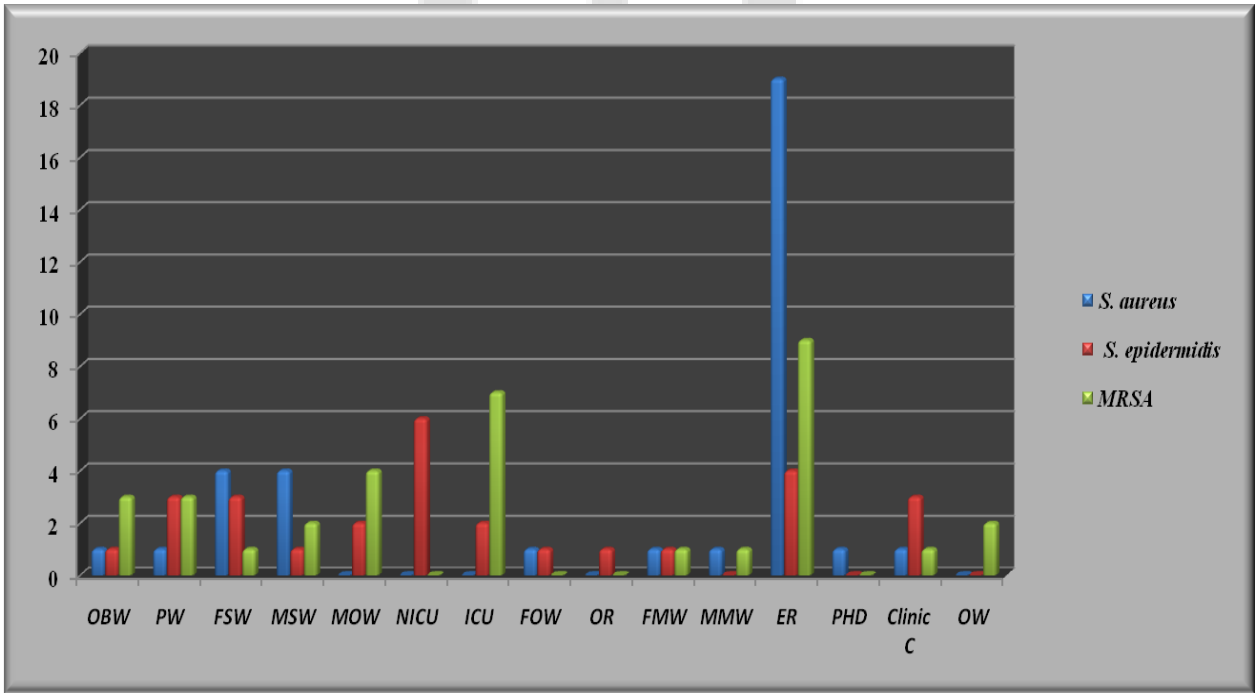


Figure (S11): Distribution of bacterial isolates with respect to in major departments

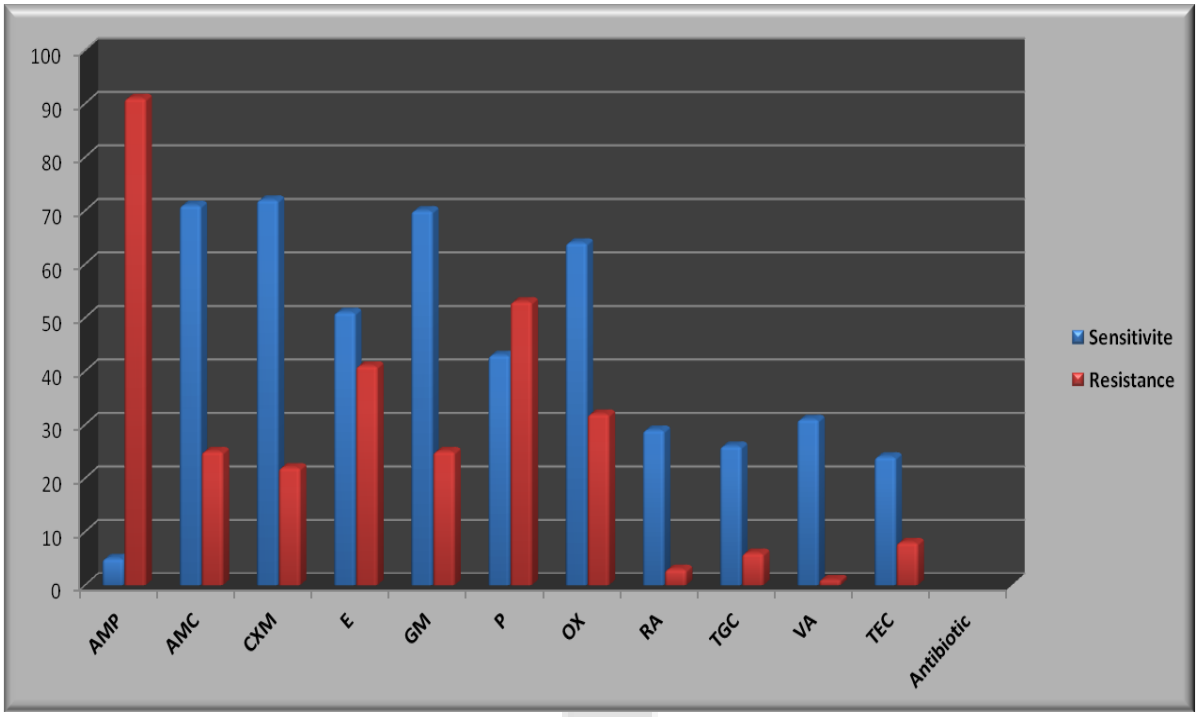


Figure (S12): Effect of Antibiotics on bacterial sensitivity for all 96 *staphylococcus* isolates

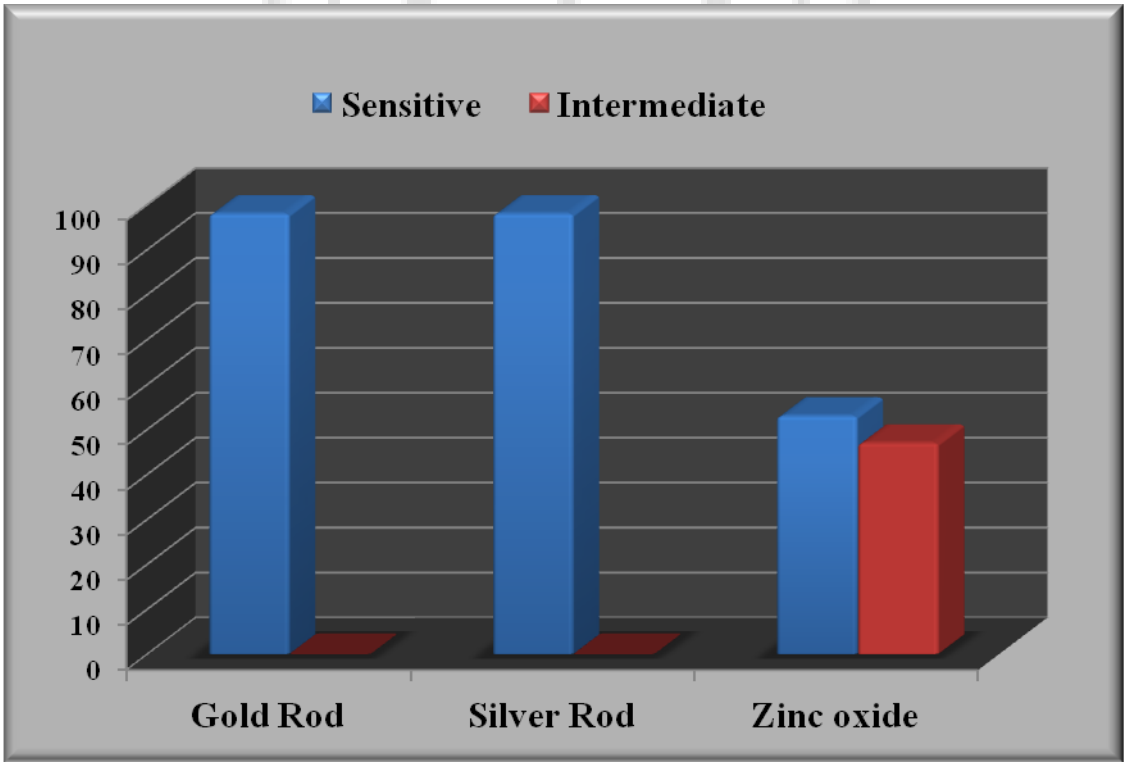


Figure (S13): Antimicrobial Sensitivity of Nanoparticles (Gold, Silver and Zinc oxide) on all of *Staphylococcus* isolates

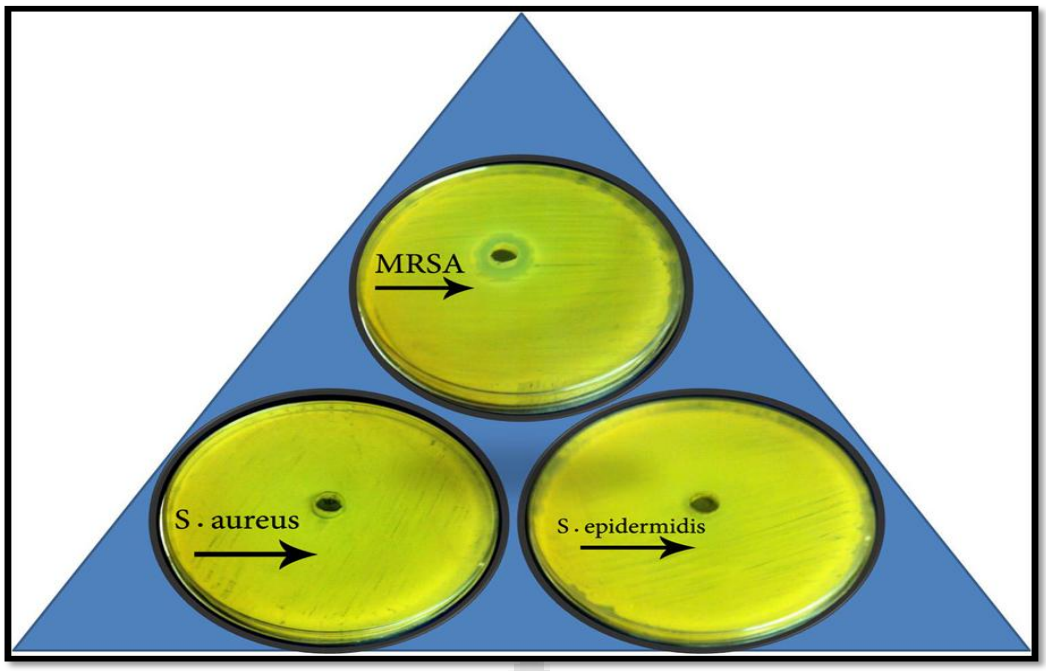


Figure (S14): The effect of Silver (Spherical) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).

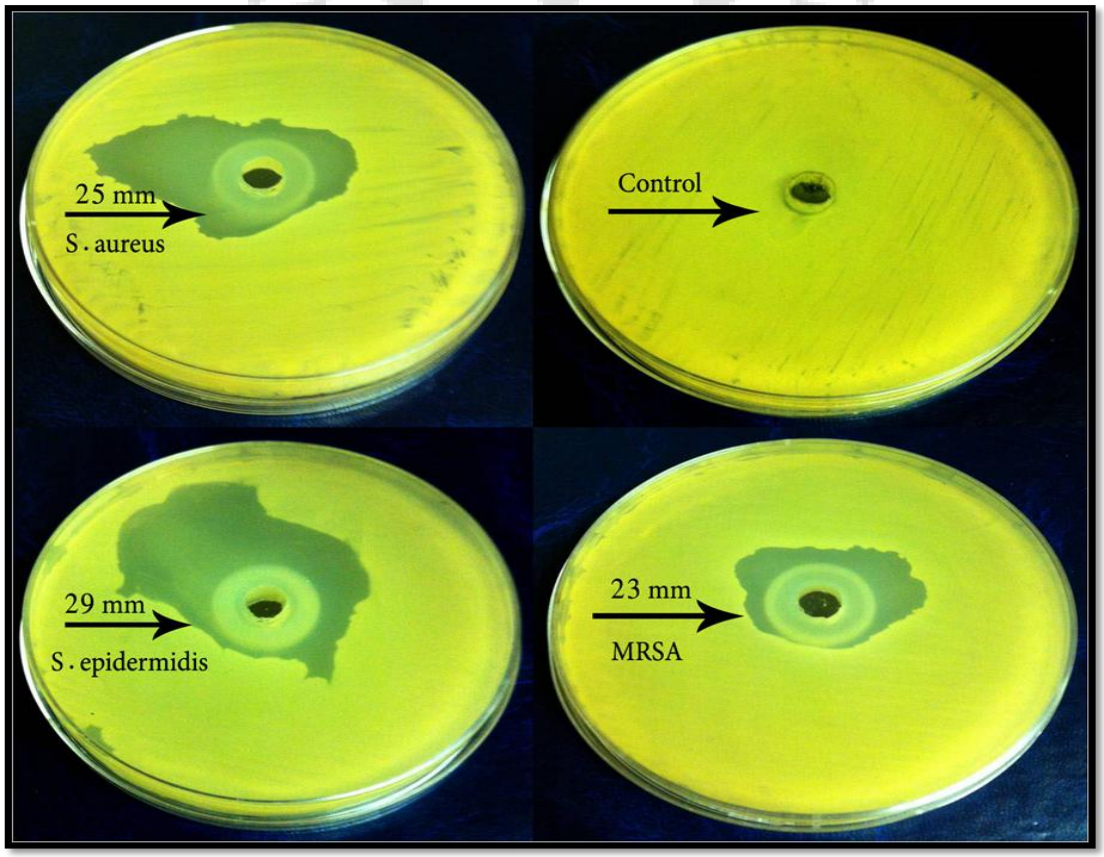


Figure (S15): The effect of Silver (Rods) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).

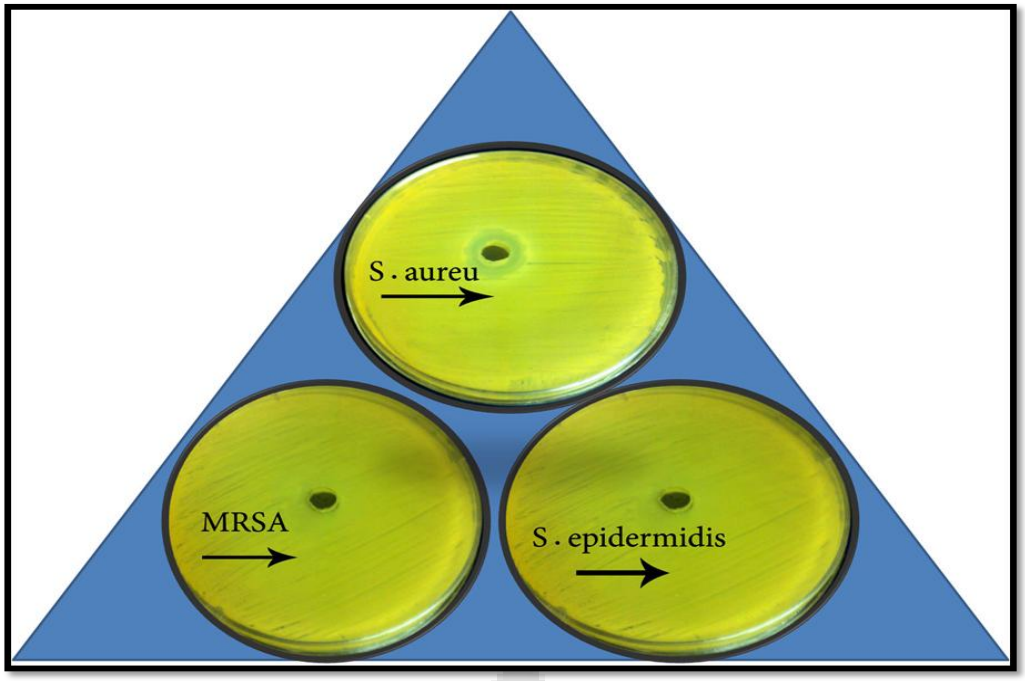


Figure (S16): The effect of Gold (Spherical) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).

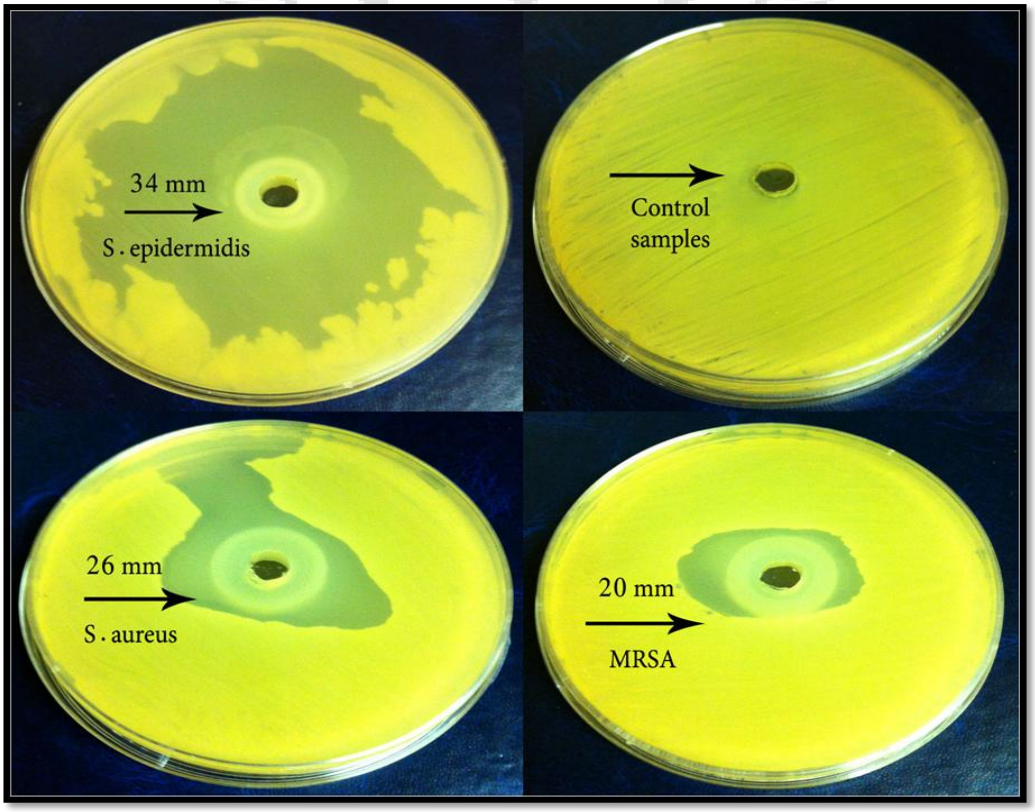


Figure (S17): The effect of Gold (Rods) nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).

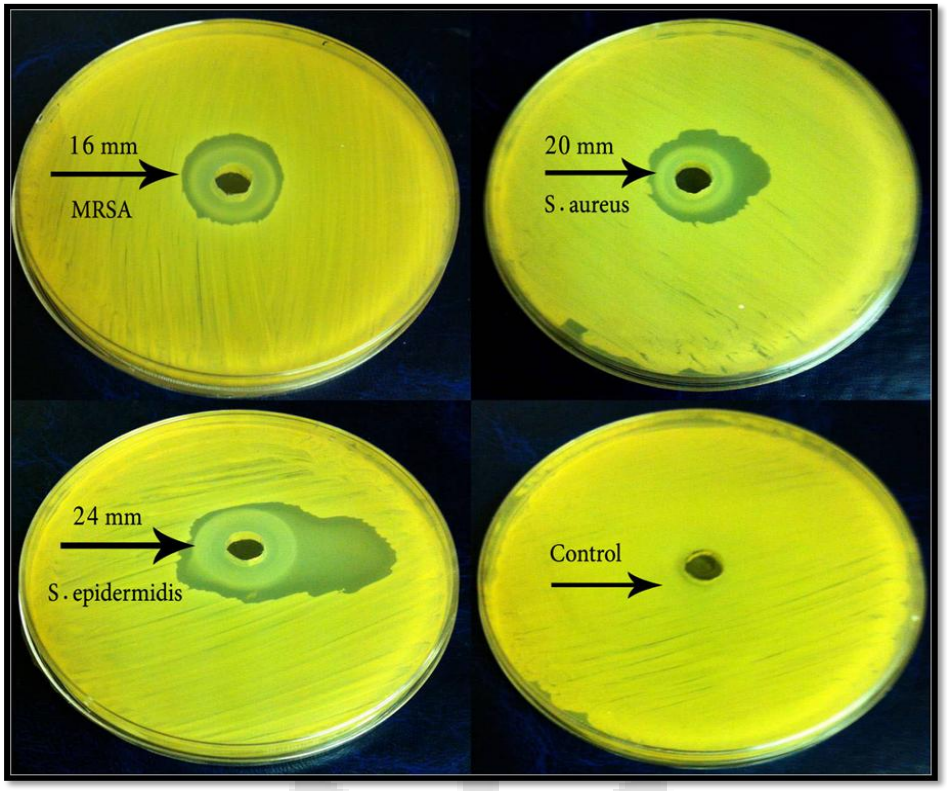


Figure (S18): The effect of Zinc oxide nanoparticles on *Staphylococcus* species showed by the inhibition zone (mm).

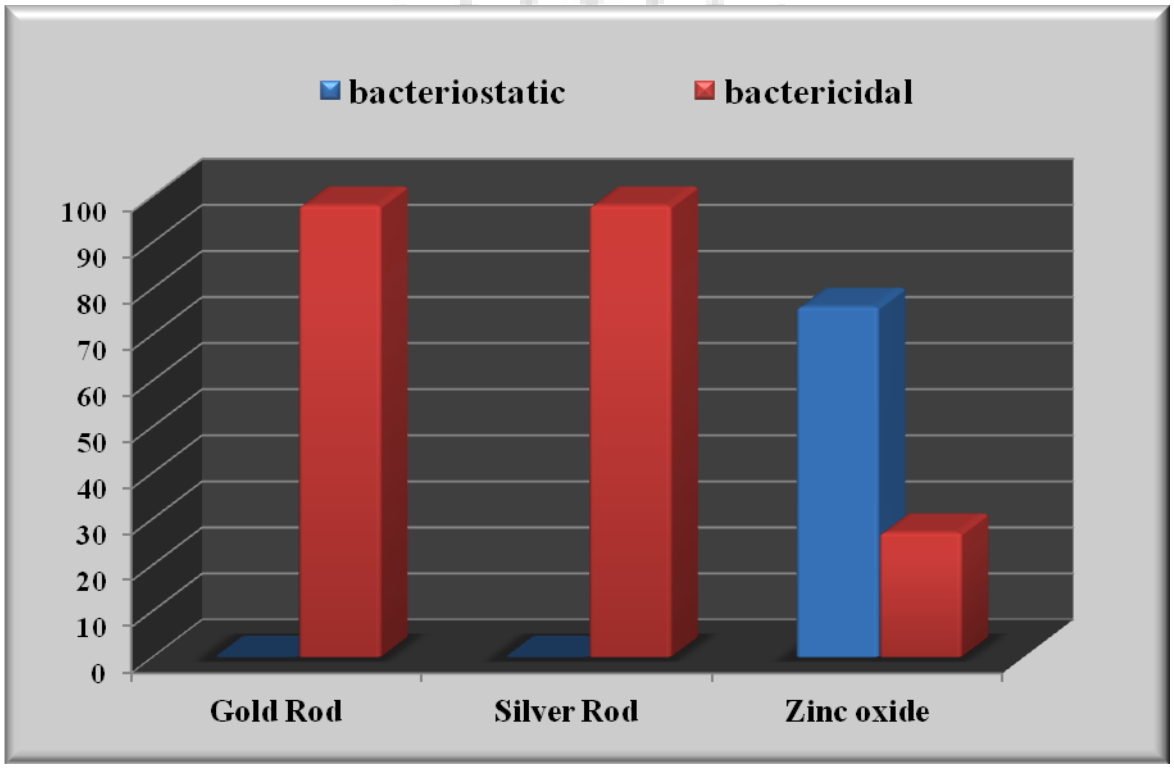


Figure (S19): Bacteriostatic and Bactericidal effect of Nanoparticles (Gold, Silver and Zinc oxide) on all of *Staphylococcus* isolates.

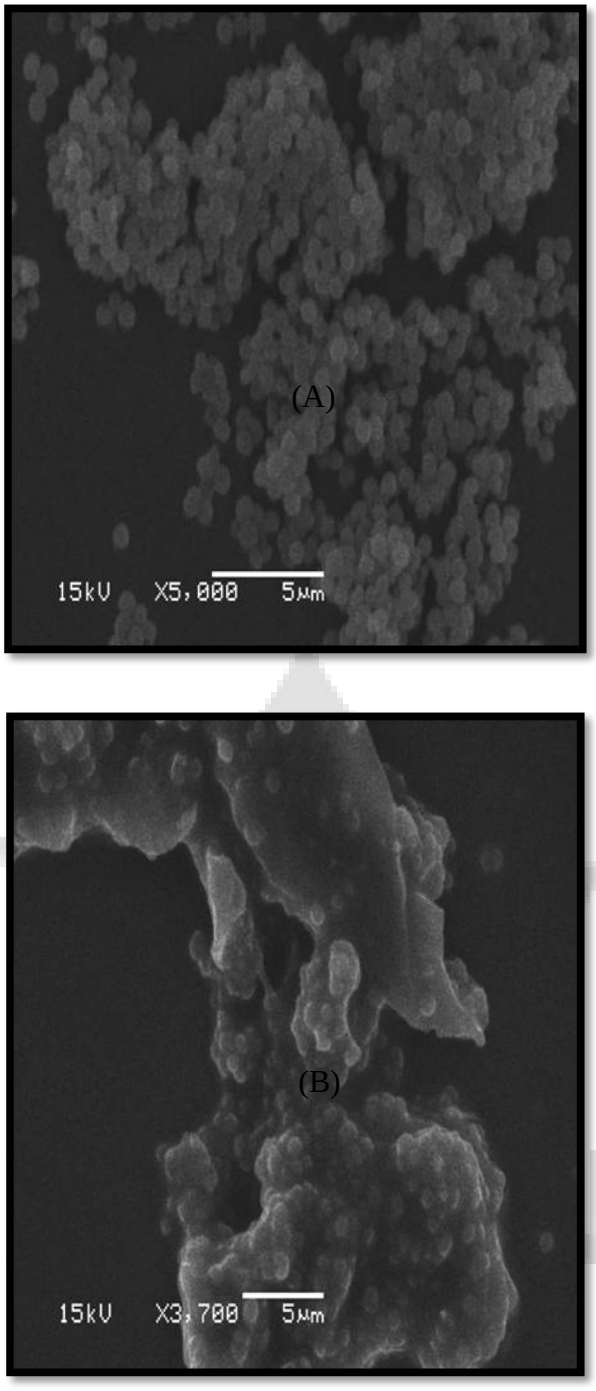


Figure (S21)

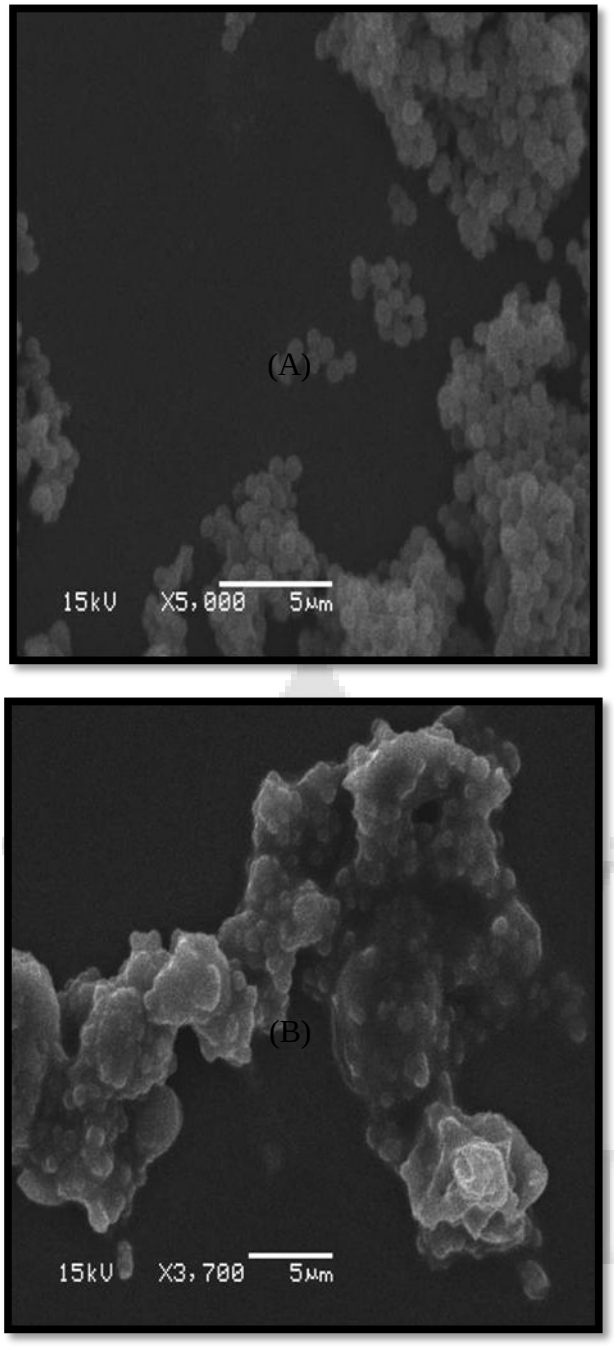


Figure (S25)