# Effect of Titanium Nanoparticles Biosynthesized by *Lactobacillus crispatus* against Multi Drug Resistance Bacteria isolated from patient with Recurrent UTI

*Jehan Abdul Sattar Salman, **Fattma Abodi Ali, ***Kawther Hkeem Ibrahem

*Department of Biology, Science College, Mustansiriyah University, Baghdad, Iraq

**Department of Microbiology, College of Health Sciences, Hawler Medical University

***Department of Soil and Water Resources, Agriculture College, Kirkuk University

## Abstract:

***Background:*** The synthesis of metal nanoparticles using microorganisms has received great interest due to their optical, chemical, photoelectrical and electronic properties. The microorganisms are used as possible "nanofactories" for development of clean, nontoxic and environmentally friendly methods for producing nanoparticles, the Nanoparticle are synthesized using various biosources such as bacteria, fungi, yeast, plant extract

***Objectives:*** The present study focuses on the biosynthesis of titanium nanoparticles using Iraqi *Lactobacillus crispatus* isolate and their inhibitory effect against multi drug resistance bacteria isolated from patient with Recurrent UTI and against their ESBL and Metallo beta lactamase.

***Materiales and Methods:*** *Lactobacillus crispatus*was isolated from vagina of Iraqi healthy women then identified throughoutcultural, microscopical and biochemical test. Fifty samples of urine were collected from women suffering from recurrent UTI who had certain clinical symptoms; these samples were collected from Rizgary Teaching Hospital and Baghdad Teaching Hospital from August to September, 2013 with ages ranging from 18-60 years. The urine specimens were inoculated on both blood and MacConkeys agar plates. The isolates were identified throughout cultural, microscopical, biochemical test and Vitek 2 system. Isolates were tested against 14 antibiotics by using Kirby-Bauer disc diffusion and detection of Extend spectrum β- lactamases by double disc diffusion synergy method and detection of Metallo-β lactamase by disc potentiating test and Samples of synthesized nanoparticles were characterized after 72 hours of incubation. The formation of metal oxide TiO2 nanoparticles was confirmed by X-ray diffraction (XRD) technique, Atomic Force Microscopy (AFM) and Scanning electron microscopic (SEM) and antibacterial activity of $TiO_2$ nanoparticles synthesized by *L. crispatus* were determined on the basis of minimum inhibitory concentration (MIC) values Effect of $TiO_2$ nanoparticles on metallo-β lactamase and evaluation of combined effect between Antibiotics and synthesized $TiO_2$ nanoparticles.

***Results:*** Fifty samples of urine were collected from patient suffering from recurrent UTI who had certain clinical symptoms. Forty four isolates (88%) were isolated and identified from fifty samples of urine, 13 isolates (29.5%) belonged to *Escherichia coli* which was the most predominant and 6 isolates (13.6%) belonged to *Klebsiella pneumonia*, 5 isolates (11.3%) belonged to *Psudomonasaeruginosa* , 2 isolates (4.5%) belonged to *Acinetobacterbaumannii*, one isolate belonged to *M.morganii*, 11 isolates (25%) belonged to *S.aureus*and 6 isolates (13.6%) belonged to *S.saprophyticus*Moreover , these isolates were tested for susceptibility to (13) antibiotics and for their ability to produce extended β –lactmase and metalo β- lactmaseenzymes. All isolates were resist to Ampicillin, Amoxicillin/ clavulanicacid and Aztreonam. Inhibitory effect of synthesized $TiO_2$ nanoparticles was studied against growth and ESBL and Metallo beta lactamase of recurrent UTI causative bacteria. Results showed the MIC of $TiO_2$ nanoparticles was (32) mg\ml against *E. coli, M.morganii, A.baumanii and S.aureus*, and (64) mg\ml against *K.pneumoniae*, *P.aeruginosa* and *S.saprophyticus* isolates.$TiO_2$ nanoparticles showed inhibitory effect on metalloβ lactmase produced by *E.coli, M.morganii*and *A.baumanii.*, thereduction in inhibition zone diameter of MBL positive isolates of *A.baumanii(1)* and *E.coli*from (2o to 25) mm after treatment with $TiO_2$ nanoparticles compared to control without treatment with $TiO_2$ nanoparticles (25-35,20-30) mm respectively, while inhibition zone diameters of *M.morganii*and *A.*baumanii (2) were(15,20),(10,20) mm respectively after treatment compared to control with inhibition zone diameters (23,30), (30,38) mm. The combined effect between Antibiotics and synthesized $TiO_2$ nanoparticles was also investigated against multi resistant recurrent UTI causative bacteria. The antibacterial activities of some antibiotics such as Ampicillin and Gentamycin have been increased in the presence of $TiO_2$ nanoparticle (subMIC) against all isolates.

***Conclusions:*** Titanium nanoparticles has more efficient antibacterial agent and can be useful in the treatment of infectious diseases caused bybacteria, the combination of antibiotics and metal nanoparticles could increase the antibiotics' efficacy against resistant pathogens. In addition, nanoparticle–antibiotic conjugates lower the amount of both agents in the dosage, which reduces harmfulness and increases antimicrobial properties.

***Keywords:*** *Titanium nanoparticles, UTI, Esbl, Metallo enzyme.*

## Introduction:

In past two decades the synthesis of metal nanoparticles using microorganisms has received great interest due to their optical, chemical, photoelectrical and electronic properties. The microorganisms are used as possible "nanofactories" for development of clean, nontoxic and environmentally friendly methods for producing nanoparticles [(1)]. The Nanoparticle are synthesized using various biosources such as bacteria, fungi, yeast, plant extract; Synthesis using bio-organisms is compatible with the green chemistry principles. The bio-organism is eco-friendly as are the reducing agent employed and the capping agent of the reaction. Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the elemental metal through enzymes generated by the cell activities [(2)]. The TiO2 nanoparticles are synthesized using various methods such as sol gel, hydrothermal, flame combustion, solvothermal, fungal mediated biosynthesis etc. The microorganisms such as *Lactobacillus sp.* and *Saccharomyces cerevisae*are used for the synthesis of Titanium dioxide nanoparticles [(3)]. Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. It is estimated that there are about 150 million cases in the world per year [(4)]. Although antimicrobial therapy is generally effective in eradicating urogintalinfections, there is still a high incidence of recurrence [(5)]. The ESBL producing *E. coli* are difficult to treat due to their resistance to wide spectrum of antibiotics including the third generation cephalosporine. Factors often responsible for *Escherichia coli* resistance include R-factor on plasmids, resistance genes on the chromosomes, production of β- lactamase and extended spectrum β-lactamase enzymes [(6)]. Recurrent complicated urinary tract infections represent a risk for ascending infection or urosepsis, *Escherichia coli* is the most common organism in all patient groups, but *Klebsiella, Pseudomonas, Proteus,* and other organisms are more common in patients with certain risk factors for complicated urinary tract infections [(7)]. The worldwide spread of acquired Metallo beta lactamase in clinically important pathogens such as *Pseudomonas* spp., *Acinetobacter*spp. And members of Enterobacteriaceae has become a great concern and detection of MBL producing organisms in the clinical microbiology laboratory is a matter of major importance for the choice of appropriate therapeutic schemes and the implementation of infection control measures [(8)]. Nano-Materials are called a wonder of modern medicine. It's stated that antibiotics kill perhaps a half dozen different diseases-causing organisms but Nano-materials can kill some 650 cell [(9)]. The antibacterial efficacy of metal nanoparticles has been suggested to be due to their high surface-to-volume ratio rather than to the sole effect of metal-ion release. A high surface-to-volume ratio is generally accompanied by increased production of reactive oxygen species, including free radicals. These characteristics allow nanoparticles to interact closely with microbial membranes, damage their structure, and inactivate bacteria [(10)]. In this paper, we report the evaluation of antibacterial efficacy of $TiO_2$ nanoparticlesbiosynthesized by *Lactobacillus crispatus* against multi

drug resistant UTI pathogens and evaluation of combined effect between Antibiotics and synthesized $TiO_2$ nanoparticles.

## Materials and Methods:

### *Lactobacillus crispatus*

*Lactobacillus crispatus*was isolated from vagina of Iraqi healthy women, then identified throughout cultural, microscopical and biochemical test according to Carr *et al*., [11].

### Recurrent UTI Causative Bacteria:

Fifty samples of urine were collected from women suffering from recurrent UTI who had certain clinical symptoms; these samples were collected from Rizgary Teaching Hospital and Baghdad Teaching Hospital from August to September, 2013 with ages ranging from 18-60 years.

The urine specimens were inoculated on both blood and MacConkeys agar plates by direct streaking method using a loop to deliver a loopful of the urine specimens after incubation overnight at 37C°. The isolates were identifiedthroughout cultural, microscopical, biochemical test according to the criteria established by Forbes *et al*., [12] and Vitek 2 system.

### Antibiotic Susceptibility test

The Antimicrobial susceptibility of pathogenic isolates was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar using overnight culture at a 0.5 McFarland standard followed by incubation at 35C$^o$ for 16 to 18 h. following Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. With commercially available antimicrobial discs. Isolates were tested against the following antimicrobial agents: Amikacin, Amoxicillin/ clavunic acid, Aztreonam, Ceftazidime, Ceftriaxone, Ceftriaxone, Ciprofloxacin, Trimethoprim/ Sulphamethoxazol, Gentamycin, Imipenem, Nalidixic acid, Nitrofurantoin, Tetracycline and Azithromycin.

### Detection of Extend spectrum β-lactamases by double disc diffusion synergy method

To detect ESBL production, the double disc diffusion synergy test (DDST) was used as described by Jarlier*et al*., [14]. Mueller Hinton agar was inoculated with standardized inoculums (corresponding to 0.5 McFarland tube) using sterile cotton swab. An Augmentin (20 µg Amoxicillin and 10 µg of Clavulanic acid-AMC) disk was placed in the center of the plate and test disks of 3$^{rd}$ generation Cephalosporins (Ceftazidime- CAZ 30 µg, Ceftriaxone CRO 30 µg, Cefotaxime-CTX 30 µg) and Aztreonam (ATM 30 µg) disks were placed at 20 mm distance (center to center) from the Amoxicillin-Clavulanic acid disk prior to incubation. The plate was incubated overnight at 37C$^o$. Enhancement of the zone of inhibition of any one of the four drug disks toward Amoxicillin-Clavulanic acid suggested the presence of extended-spectrum beta-lactamases.

### Detection of metallo-β lactamase

Detection of Metallo-β lactamase by disc potentiating test MBLs production was determined by disc potentiating test as described byBashi*et al*., [15]. Two imipenem discs were placed on the Mueller Hinton agar; 5 µl of (5M) EDTA solution was added to one of the discs. The inhibition zones of the imipenemandimipenem-EDTA discs were compared after 16-18 hours of incubation at 35°C$^o$. An increase in the zone size of at least 7 mm around the

imipenem -EDTA disc was recorded as an MBL-positive isolate.

**Synthesis of Tetanium Nanoparticles**

Three flasks were used; each flask was filled with 40 ml of MRS broth. Then 20 ml of $TiO_2$ (0.025m) were added to the first and second flask respectively and both were stirred for half hour on a magnetic stirrer while the third flask contains MRS broth only. Final concentration ultimately would be equivalent. *Lactobacillus* isolate was cultured in first and third flask incubated an aerobically at 37C$^o$ for (24, 48, 72) hours. Second flask was used as blank for first one, the change in color from light brown to dark brown observed and production of sediment will observed as primary detection of produced $TiO_2$ nanoparticles Azhar *et al* [16].

**Characterization of TiO2nanoparticles**

Samples of synthesized nanoparticles were characterized after 72 hours of incubation.The formation of metal oxide TiO2 nanoparticles was confirmed by X-ray diffraction (XRD) technique, Atomic Force Microscopy(AFM) and Scanning electron microscopic(SEM) according to *Salman* et al.,[17].

**Antibacterial activity of $TiO_2$ nanoparticles synthesized by *L. crispatus***

Antibacterial activity of $TiO_2$ nanoparticles were determined on the basis of minimum inhibitory concentration (MIC) values, defined as the lowest concentration of $TiO_2$ nanoparticles at which no visible growth could be observed after incubation for the required time. MIC was determined for all bacterial isolates causing recurrent UTI by broth dilution method as described by Morello*et al*., [18]. Briefly, a stock solution of $TiO_2$ nanoparticles from *L.crispatus* in sterilized distilled water were diluted to concentrations ranging (4, 8, 16, 32, 64, 128) mg/ml.

**Effect of $TiO_2$nanoparticles onmetallo-β lactamase**

Metallo-β lactamase production by UTI causative bacterial isolates were studied by Muller-Hinton method according to Bashir *et al* [15]. With some modification. Briefly. (1 ml) of subMIC $TiO_2$ nanoparticles poured on Mueller-Hinton agar medium,left in room temperature to dry completely, then Plates were inoculated according to Kirby -Bauer method onto plates of Mueller-Hinton agar media. Then two discs of Imipenem antibiotic were placed on the plate; 5 µl of EDTA solution (final concentration is 0.5M) was added to one of them.The inhibition zones of the imipenem and imipenem -EDTA discs were compared after 16-18 hour. of incubation at 35C$^\circ$.inhibition zone was measured (mm) and compared with standared measurement.

**Evaluation of combined effect between Antibiotics and synthesized $TiO_2$ nanoparticles**

To determine combined effects, as described byRoy*et al*., [19]. Each standard paper disc was further impregnated with sub-inhibitory concentration of $TiO_2$ nanoparticles. A single colony of recurrent UTI bacterial isolates were grown over night in Muller-Hinton broth medium at 35C$^o$. The inoculums were prepared by diluting the overnight cultures with (0.9%) NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared disks containing of $TiO_2$ nanoparticles. After incubation at 37C$^o$ for 24 hour, the zones of inhibition were measured.

## Results and Discussion:

Forty four isolates (88%) were isolated and identified from fifty samples of urine, the results showed that 13 isolates (29.5%) belonged to *Escherichia coli* which was the most predominant and 6 isolates (13.6%) belonged to *Klebsiella pneumonia*, 5 isolates (11.3%) belonged to *Psudomonasaeruginosa*, 2 isolates (4.5%) belonged to *Acinetobacterbaumannii* and one isolare belonged to *M.morganii*, table (1). A ddditionally when using the card for gram positive bacteria, resultsshowed that 11 isolates (25%) belonged to *S.aureus*and 6 isolates (13.6%) belonged to *S.saprophyticus*, table (1).

Most of recurrent UTI are caused by gram-negative bacteria like *Escherichia coli, Proteusmirabilis*, *Proteus vulgaris Klebsiella*sp, *Pseudomonasaeruginosa*, *Acinetobacter*, *Serrati, and Morganellamorganii* and by Gram positive bacteria include *Enterococcus*, *Staphylococcus* especially coagulase-negative staphylococci, and *Streptococcus agalacticae* [20]. Found that from 510 patients could isolate *E. coli* from 464 (90%) patients, *Klebsiella spp*. from 27 (5%) patients. *E. coli* was the causative agent in 269 of 289 (93%) uncomplicated UTIs and in 195 of 221 (88%) complicated UTIs isolates. Furthermore [21]. Considered*Escherichia coli* was the most predominant uropathogen with (43.7%), followed by *KlebsiellaPneumoniae* (14.1%), *Pseudomonas. auruginosa*and *Proteusmirabilis* (9.4%), *Staphylococcus aureus* (7.8%), *Morganellamorganii*. moreover, Abdallah*et al.,* [22]. Confirmed that the most frequently isolated microorganisms were *Escherichia coli* (31.7%) followed by *Klebsiella* (15%); *Staphylococcus aureus*; *Pseudomonas* (6.7%) and the least common was *Enterobacter* (1.7%).

**Table (1):** Frequency of bacterial uropathogens isolated from recurrent UTI suspected patients.

| Isolates | Number | Percentage % |
|---|---|---|
| *S. aureus* | 11 | 25 |
| *S.saprophyticus* | 6 | 13.6 |
| *E.coli* | 13 | 29.5 |
| *K.pneumoniae* | 6 | 13.6 |
| *P.aeruginosa* | 5 | 11.3 |
| *A.baumanii* | 2 | 4.5 |
| *M. morganii* | 1 | 2.27 |
| Total | 44 | 100 |

## Antibiotic Susceptibility test

The results of Antibiotic Susceptibility test showed that all the isolates subjected to this study appeared varying resistance to the antibiotics were used, all isolates of *S.aureus* were resistant to (Ampicillin, Amoxicillin/ clavulanicacid, Aztreonam and Trimethoprim/ Sulphamethoxazol), while sensitive totally for two antibiotics (Amikacin and Imipenem) and varied sensitivity to other antibiotics at a different propotions, *S.saprohyticus* appeared sensitivity towards (Amikacin, Imipenem and Gentamicin (except isolate 1) and resistant against antibiotics (Ampicillin, Amoxicillin/

clavulanic acid, Aztreonam, Ceftriatone). On the other hand among gram negative uropathogen showed resist to most antibiotic compared with the gram positive bacterial isolates. *M.morganii* and *A.baumanii* have been the same ability to resist 9 antibiotic included (Ampicillin, Amoxicillin/ clavulanic acid, Aztreonam and Trimethoprim/ Sulphamethoxazol, Gentamicin, Tetracyclin, Nitrofurantoin, Ceftriatone, Azithromycin, and Nalidixic acid) but they were sensitive to Amikacin, Ciprofloxacin, and Imipenem.

Bacterial isolates of *E.coli* characterized by being sensitive to the Imipenem and Amikacin and resistant towards (Aztreonam, Tetracyclin, Ampicillin and Amoxicillin/ clavulanic acid, additionally the all isolates of *K.pneumoniae* were resistant toward (Ampicillin, Amoxicillin/ clavulanic acid, Aztreonam, Gentamicin, and Ceftriatone) appeared sensitive to Amikacin and Imipenem. In the same time clinical isolates of *P.aeruginosa* displayed ahighly resistant to (Tetracyclin, Ampicillin, Amoxicillin/ clavulanic acid, Azteroname, Nitrofurantoin, and Ceftriatone) and in contrast it was sensitive to (Amikacin, Gentamicin (except isolate 4), and Imipenem). Baral *et al*., [23]. Reported that the most prevalent MDR pattern shown by *E. coli* included resistance to amoxycillin, ciprofloxacin, cephotaxime, cefixime, co-trimoxazole, ofloxacin, norfloxacin, ceftazidime, gentamycin and ceftriaxone. Abdallah*et al*. [22]. Observed in his study that two antibiotic (Imipenem and Amikacin) were found the most useful antibiotics overall against gram-negative bacterial isolates. Al-Khafaji [24]. Mentioned that all clinical *A. baumannii* isolates were completely susceptible to imipenem, either. Sarathbabu*et al.*, [25]. Has resulted in his study that some bacterial isolates of *K.pneumoniae*were resistant to Nitrofurantoin and Ceftriaxone. Muvunyi*et al*., [26] noted that the bacterial isolates isolated from urinary tract infections characterized by being sensitive to the Imipenem, Imipenem and Amikacin were most effective antibiotics against gram-negative isolates while Vancomycin and Ciprofloxacin were most effective against gram positive isolates [23].

## Detection of Extended β –lactmase of bacterial isolates

Results of present study reported no production for ESBL enzyme from all gram negative isolates under. Briongos-Figuero*et al.*,[27] reported that 23,839 urine samples which collected from hospital in Spain in 2009, (60%) isolated was *E.coli* and just (6%) were ESBL producing strains.while in 2010 level of bacteria isolates reached to (59.4%) and just (7%) were ESBL producing strain Although ESBLs occur predominantly in *Klebsiella*spp. and *E. coli*, they have also been described in other genera of the Enterobacteriaceae family including *Citrobacter*spp., *Serratia*spp., *Proteus* spp., and *Enterobacter* spp.[28]. The prevalence of ESBL- producing strains of Enterobacteriaceae varies from country to country and from species to species in Asia [29].

However, the predominance of ESBL among clinical isolates varies among geographic areas with low rates of (3-8%) in Sweden, Japan and Singapore to much higher predominance prevalence rates reported from Portugal (34%), Latin America (30-60%), and Turkey (58%) [28].

## Detection of metalo β- lactmase of bacterial isolates

Our results exhibited that 2 isolates of *A.baumanii*, *M.morganii*, and only one isolate of *E.coli* had abililty to produce metallo beta lactmase enzyme as shown in table (2). Beta lactmases are enzmyes responsible for many failures of antimicrobial therapy by hydrolysis of β lactam ring of antibiotics (penicillin, cephalosporins, monobactam, carbapenems) and cause resistance to them. Most of enterobacteriae and *P.aeruginosa* produce chromosomally determined class 1 beta lactmase [30]. Resistance to βlactam antibiotics in *E. coli* and *K. pneumoniae*is typically mediated by beta-lactamases placed in the periplasma, hydrolyze the beta-lactam ring and prevent antibiotic binding to target sites on the bacterial cytoplasmic membrane. Multidrug-resistant *A. baumannii* isolates are increasingly reported worldwide and multidrugereistanceoccure due to produce beta lactmase [31]. Carbapenems have potent activity against *Acinetobacter*spp. and are usually the drugs of choice against multidrug resistant *Acinetobacterbaumannii* isolates. *Acinetobacter*spp. may develop resistance to carbapenems through variousmechanisms, including class B and D carbapenemase production decreased permeability, altered penicillin bindingproteins, and rarely, overexpression of efflux pumps [32] plasmids carrying resistance genes and/ or resistance determinants involved in horizontal gene transfer have been described in several *A. baumannii*strains [33].

**Table (2):** Detection of metalo beta-lactmase of recurrent bacterial isolates**.**

| Bacterial isolates | Inhibition zone(mm) | | Bacterial isolates | Inhibition zone(mm) | |
|---|---|---|---|---|---|
| | Imipenem | Imipenem+EDTA | | Imipenem | Imipenem+EDTA |
| *S.aureus1* | 0 | 0 | *K.pneumonia6* | 0 | 0 |
| *S.aureus2* | 0 | 0 | *A.baumani1* | 20 | 30 |
| *S.aureus3* | 0 | 0 | *A.baumani2* | 30 | 38 |
| *S.aureus4* | 0 | 0 | *M.morganii1* | 23 | 30 |
| *S.aureus5* | 0 | 0 | *P.aeruginosa1* | 20 | 24 |
| *S.aureus6* | 0 | 0 | *P.aeruginosa2* | 0 | 0 |
| *S.aureus7* | 0 | 0 | *P.aeruginosa3* | 0 | 0 |
| *S.aureus8* | 0 | 0 | *P.aeruginosa4* | 20 | 25 |
| *S.aureus9* | 0 | 0 | *P.aeruginosa5* | 0 | 0 |
| *S.aureus10* | 0 | 0 | *E.coli1* | 25 | 35 |
| *S.aureus11* | 0 | 0 | *E.coli2* | 30 | 33 |
| *S.saprophyticus1* | 0 | 0 | *E.coli3* | 0 | 0 |
| *S.saprophyticus2* | 0 | 0 | *E.coli4* | 30 | 33 |
| *S.saprophyticus3* | 0 | 0 | *E.coli5* | 10 | 15 |
| *S.saprophyticus4* | 0 | 0 | *E.coli6* | 15 | 17 |
| *S.saprophyticus5* | 0 | 0 | *E.coli7* | 0 | 0 |
| *S.saprophyticus6* | 0 | 0 | *E.coli8* | 23 | 24 |
| *K.pneumonia1* | 25 | 25 | *E.coli9* | 0 | 0 |
| *K.pneumonia2* | 37 | 40 | *E.coli10* | 0 | 0 |
| *K.pneumonia3* | 20 | 23 | *E.coli11* | 15 | 17 |
| *K.pneumonia4* | 0 | 0 | *E.coli12* | 15 | 20 |
| *K.pneumonia5* | 0 | 0 | *E.coli13* | 0 | 0 |

0=no inhibition zone, high light=enzyme production

## Antibacterial activity of $TiO_2$ nanoparticles synthesized by *L.crispatus*

Antibacterial activity of $TiO_2$ nanoparticles was determined on the basis of minimum inhibitory concentration (MIC) values. MIC at concentrations ranging to (4, 8, 16, 32, 64, 128) mg/ml was determined for all bacterial isolates causing recurrent UTI. Results showed that the MIC of $TiO_2$ nanoparticles was found to be 32 mg /ml for *E. coli*, *M.morganii*, *A.baumanii and S.aureus* isolates as seen in table (3), while the MIC for *K.pneumoniae*, *P.aeruginosa* and *S.saprophyticus*isolates were observed at 64 mg /ml. The differences in MIC of $TiO_2$ nanoparticles probably result from differences in the genus and species under tested.

Bacterial resistant to current antibiotics have become serious public health problems that elevated the need to develop new bactericidal materials. Metal oxide nanoparticles $TiO_2$ and $Ag_2O$ nanoparticles exhibited significant antibacterial activity [34] approved that $TiO_2$ nanoparticles with MIC value (200 µg/ml) inhibited bacterial growth of *E. coli* at20 h of incubation. Huang *et al*., [35] reported that $TiO_2$ increased the permeability of cell membrane and cell death happened by leaking out of vital components in bacterial cell. While Liu *et al*., [36]. Investi [37] observed the antibacterial effects of $TiO_2$ nanoparticles against different bacteria included *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Bacteroidesfragilis*and *Enterococcus* under UV light. $TiO_2$ nanoparticles saturated on to woven and knitted textiles exhibited high antibacterial activity against *S.aureus* and *K.pneumoniae.*

Titanium nanoparticles has more efficient antibacterial agent compared to CdO nanoparticles and can be useful in the treatment of infectious diseases caused by *E. coli* [38]. The bactericidal effect of $TiO_2$ has been attributed to the decomposition of bacterial outer membranes by reactive oxygen species (ROS), hydroxyl radicals (OH), which leads to phospholipid peroxidation and finally cell death [39]. $TiO_2$ appeared antimicrobial activity due to its strong oxidizing property [40].

Strong binding of $TiO_2$ nanoparticles to the outer membrane of *E. coli* caused the inhibition of active transport dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis. According to zhang and chen [41]. Exhibited that metal oxides carry the positive charge while the microorganism carry negative charge, this causing electromagnetic attraction between microorganism surface and the metal oxides which leads to oxidization and finally death.

Nanomaterials are known to inactivate cellular enzyme and DNA by binding to electron donating groups such as carboxylates, Amides, Indoles, Hydroxyles, Thiols etc., they cause little pores in bacterial cell walls leading to increased permeability and cell death[42].

Our result under RMRC analysis as shown in figure (3-9) appeared the presence of carbon element containing $TiO_2$ nanoparticles which may be explained the antibacterial activity towards bacterial isolates causing recurrent UTI. This explaination agree with Cheng *et al.*[43] who found that $TiO_2$ nanoparticles which contain carbon showed much higher antimicrobial activity than commercial $TiO_2$ nanoparticles.

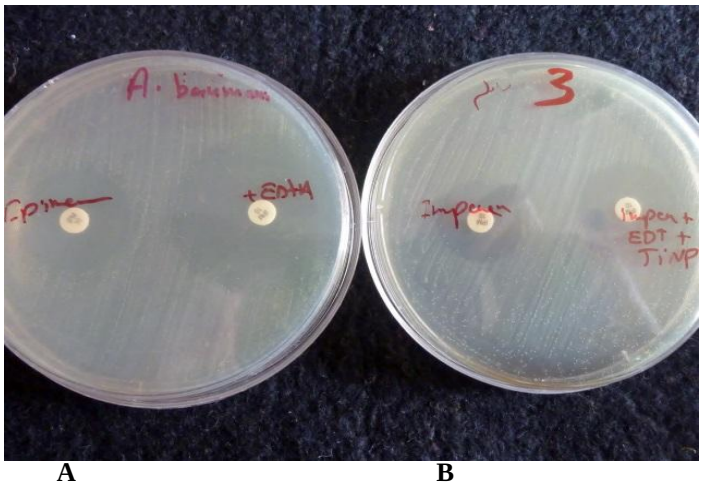
**Table (3):** MIC (mg/ml) of $TiO_2$ nanoparticles against bacterial isolates causing recurrent UTI.

| No. of isolate | MIC (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *P.aeroginoa* | *K.pneumona* | *E.coli* | *M.morganii* | *S.aureus* | *S.saprophyticus* | *A.baumannii* |
| 1 | 64 | 64 | 32 | 32 | 32 | 64 | 32 |
| 2 | 64 | 64 | 32 | | 32 | 64 | 32 |
| 3 | 64 | 64 | 32 | | 32 | 64 | |
| 4 | 64 | 64 | 32 | | 32 | 64 | |
| 5 | 64 | 64 | 32 | | 32 | 64 | |
| 6 | | 64 | 32 | | 32 | 64 | |
| 7 | | | 32 | | 32 | | |
| 8 | | | 32 | | 32 | | |
| 9 | | | 32 | | 32 | | |
| 10 | | | 32 | | 32 | | |
| 11 | | | 32 | | 32 | | |
| 12 | | | 32 | | | | |
| 13 | | | 32 | | | | |

**Effect of $TiO_2$ nanoparticles synthesized by *L.crispatus* on metalobetalactmase**

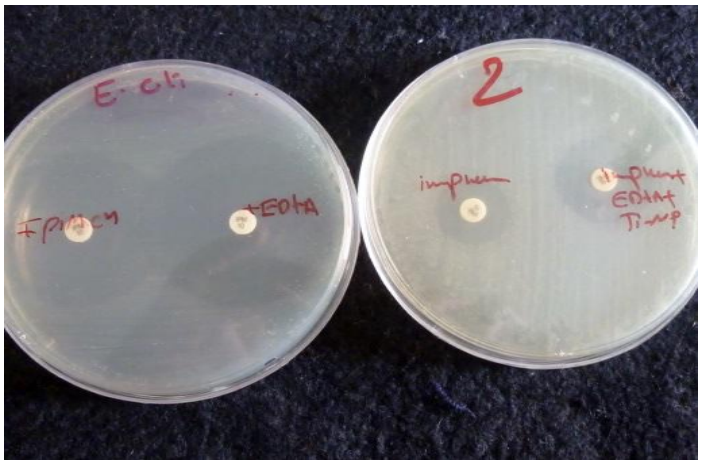
Effect of $TiO_2$ nanoparticles synthesized by *L.crispatus* on metalobetalactmase produced by bacterial isolates included *E.coli* (1), *M.morganii*, *A.baumanii* (1) and *A.baumanii* (2). Results showed in figure (1, 2) thereduction in inhibition zone diameter of MBL positive isolates of *A.baumanii* (1) and *E.coli*from (2o to 25) mm after treatment with $TiO_2$ nanoparticles compared to control (25-35, 20-30) mm respectively which composed of (Imipenem, EDTA+ Imipenem) without treatment with $TiO_2$ nanoparticles, while inhibition zone diameters of *M.morganii*and *A.*baumanii (2) as shown in figure (3) were (15, 20) (10, 20) mm respectively after treatment compared to control without treatment with inhibition zone diameters (23, 30), (30, 38) respectively as in figure(3).

The present study clearly indicate that sub-MIC of $TiO_2$nanoparticles were found to be very effective in $MBL_S$ enzyme activityproduced by *E.coli*, *M.morganii* and *A.baumanii.* So no data is available till now to our knowledge on inhibitory effect of biological or physical or chemical synthesized $TiO_2$nanoparticles that caused impossibility to compare our results with other researchers.




**A** **B**

**Figure (1):** Effect of $TiO_2$ nanoparticles synthesized by *L.crispatus* on metalobetalactmase of *A.baumanii*(1)**.**
**(A) before treatment (B) after treatment**




A B

**Figure (2):** Effect of $TiO_2$ nanoparticles synthesized by *L.crispatus* on metalobetalactmase of *E.coli* (1). **(A) before treatment (B) after treatment.**

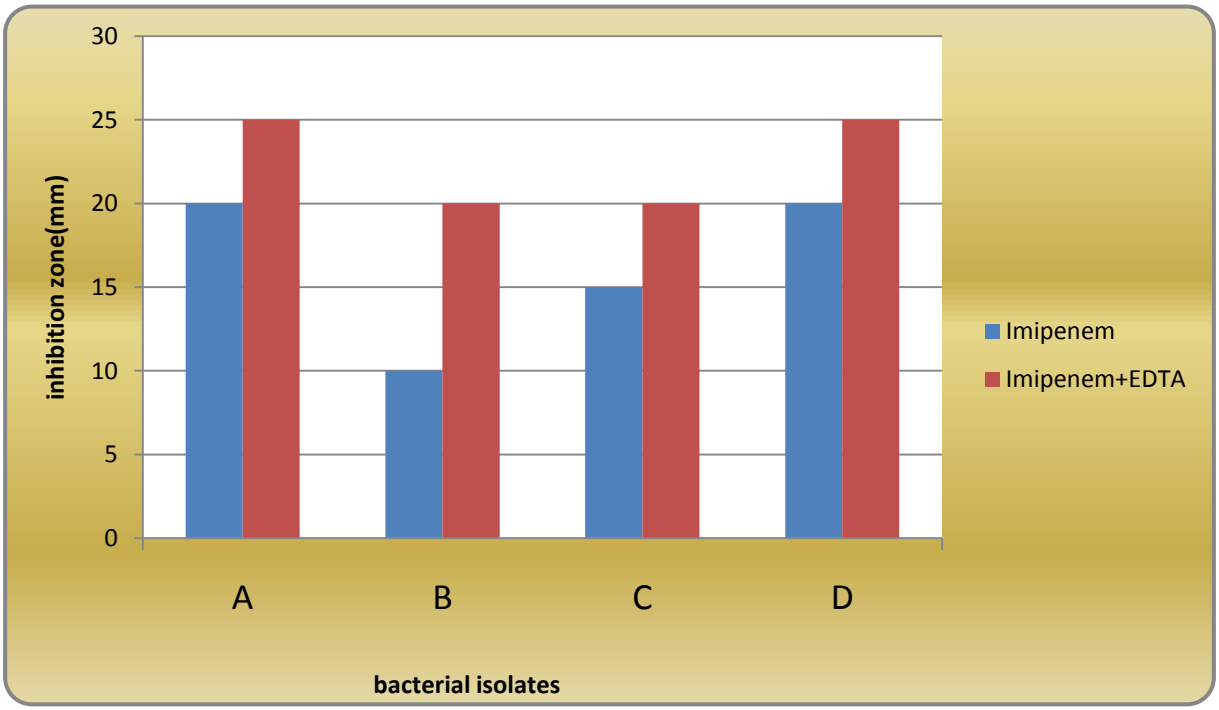



**Figure (3):** Effect of $TiO_2$ nanoparticles synthesized by *L.crispatus* (LV20) on metalobetalactmase. **A=*A.baumanii*$_1$, B=*A.baumanii*$_2$, C=*M.morganii*, D=*E.coli*$_1$**

## Evaluation of combined effect between Antibiotics and synthesized $TiO_2$ nanoparticles

The effect of $TiO_2$ nanoparticles combined with different antibiotics was investigated against multi resistant recurrent UTI causing bacteria using disk diffusion method. The diameter of inhibition zones (mm) around the different antibiotic discs included (Ampicillin, Amoycillin/ CLavulanic, Trimethoprim-sulphamethaxazol, Azithromycin, Aztreonam, Ceftriaxone, Gentamicin, Tetracyclin, Nitrofurantoin) with and without $TiO_2$nanopatrticles against test isolates were measured. The antibacterial activities of some antibiotics have been increased in the presence of $TiO_2$ nanoparticle (subMIC)

against all isolates while others didn't affect. The highest antibacterial activities of antibiotics combined with $TiO_2$ were observed against *E.coli* isolates for Ampicillin, Gentamycin, Amoycillin/ CLavulanic acid, Trimethoprim-sulphamethaxazol, Azithromycin, Tetracyclin, Nitrofurantoin. While *A. baumanii* appeared sensitive to Ampicillin, Gentamycin, Trimethoprim sulphamethaxazol and Azithromycin compared with antibiotics alone. *K.pneumonia* became sensitive to Ampicillin, Gentamycin, tetracyclin and *P.aeruginosa* to Ampicillin, Trimethoprimsulphamethaxazol, Azithromycin and Gentamycin.

The more antibacterial activities of exhibited against *M.morganii* for all antibiotics except Azithromycin figure (4). $TiO_2$nanoparticle shows no effect on antibacterial activities of all antibiotics except Ampicillin and Gentamicin against gram positive bacteria (*S.aureus* and *S.saprophyticus*) table (4). The present study showed the good effect of $TiO_2$ nanoparticles synthesized by *L.crispatus*on antibiotics activity as a strong and effective bactericidal agent.

There is great need of agents to kill bacteria and other microorganisms due to the antibiotic resistance developed by the bacteria [(44)].

reported that sub-inhibitory concentration of $TiO_2$ nanoparticle significantly improved antibiotic efficacy against *S. aureus* when combined with beta lactums, cephalosporins, aminoglycosides and suggested the mechanisms involving the interaction of nanomaterials with biological molecules and believed that microorganisms carry anegative charge while metal oxides carry a positive charge, this cause attraction between microorganism and treated surface leads to oxidizing of microbe and finaly dead. The combination effect of Ag nanoparticles and ampicillin has become more potential compared to the otherantibiotics due to the DNA binding action of the silver nanoparticles and the cell wall lysis action of the ampicillin [(45)].

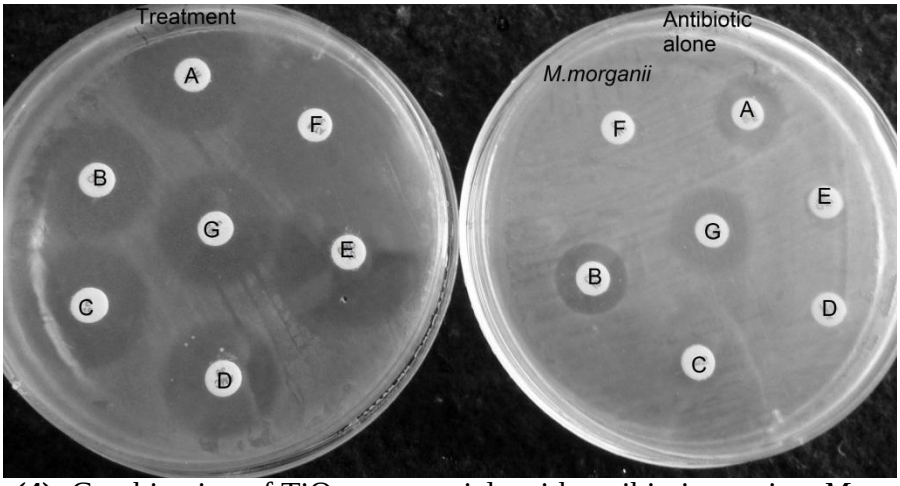



**Figure (4):** Combination of $TiO_2$ nanoparticle with antibiotics against *M.morganii*.
A=Ampicillin, B=Gentamicin, C=Teteracyclin, D=Amoxicillin/ clavulanicacid, E=Ceftriatone, F= Azteroname, G=Nitrofurantoin.

Nanomaterials release ions, which react with the thiol group (-SH) of the proteins display on the bacterial surface due to inactivated proteins resulted decreasing the membrane permeability then cell death [41].

Moreover, Hwang *et al*., [46]. Studied the Synergistic effect of silver nanoparticles and ampicillin were noticed against *E. faecium, Streptococcus mutans*and *E. coli* ATCC 25922 while Synergistic effects of Ag nanoparticles and chloramphenicol were found only against *E. faecium*and *P. aeruginosa*the explaination of synergistic combination of nanoparticles and chloramphenicol exhibited increased hydroxyl radical formation which is might be an important cause of the synergism. Other studies reported that antibacterial activity of nanoparticle along with antibiotics ampicillin and gentamicin against *S. typhi* increased in the presence of silver nanoparticles [47]. While other studies Naqvi *et al*., [48] hypothesized that nanoparticles creat a complex with ampicillin and disrupt peptidoglycan in the cell wall. Being positively charged, they attack negative charges of transmembrane proteins and can damage the cell membrane and block the transport channels. It might be possible that they enter inside the bacteria and damage cellular activities like transportation, nucleic acid functioning and protein synthesis. In the other hand, Namasivayam*et al*., [49].

Reported the synergistic effect of antibiotics choloroamphenicol with silver nanoparticles against biofilm of clinical isolate of *Pseudomonas aeruginosa*. Combined use of nanoparticle–antibiotic conjugates towards decreasing antibiotic resistance currently observed for specific bacteria and conventional antibiotics. Alloy of (Ag-Au) nanoparicles together with antibiotic observed a higher enhancement of antibacterial activity kanamycin on *E. coli* and betalactam antibiotic on *S. aureus* that have been reported bySantos *et al*., [50].

A synergistic effect of antibiotics (imipenem, gentamycin, vancomycin, and ciprofloxacin) in conjugation with biologically synthesized Ag nanoparticles increased the susceptibility against *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, Bacillus* spp., and *M. luteus* from (20–35)%. Combination ciprofloxacin and imipenem with nanoparticles were found to be most efficient in inhibiting bacteria, if bacteria improve resistance to one of them, the other bactericidal agent would kill the bacteria [51]. The combination of antibiotics and metal nanoparticles could increase the antibiotics' efficacy against resistant pathogens. In addition, nanoparticle–antibiotic conjugates lower the amount of both agents in the dosage, which reduces harmfulness and increases antimicrobial properties. Additionally, due to this conjugation, the concentrations of antibiotics were increased at the location of antibiotic–microbe contact and thus accelerate the binding between microbes and antibiotics [45].

**Table (4):** The comparative activities of various antibiotics and antibiotic with $TiO_2$ nanoparticles against recurrent UTI causative bacteria**.**

| Bacterial isolate | (AM) | | (AMC) | | (SXT) | | (AZM) | | (ATM) | | (CRO) | | (CN) | | (TE) | | (F) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ab | Ab+ $TiO_2$ | Ab | Ab+ $TIO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ | Ab | Ab + $TiO_2$ |
| *E.coli2* | R | S | R | S | R | S | R | S | R | R | R | R | R | S | R | S | R | S |
| *E.coli3* | R | S | R | S | R | S | R | S | R | R | R | R | R | S | R | R | R | S |
| *A.baumanii1* | R | S | R | R | R | S | R | S | R | R | R | R | R | S | R | R | R | R |
| *A.baumanii2* | R | S | R | R | R | S | R | S | R | R | R | R | R | S | R | R | R | R |
| *K.pneumonia5* | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| *K.pneumonia6* | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| *M.morganii* | R | S | R | S | R | S | R | R | R | S | R | S | R | S | R | S | R | S |
| *S.aureus 2* | R | S | R | R | R | R | R | R | R | R | R | R | R | S | R | R | R | R |
| *S.aureus 4* | R | S | R | R | R | R | R | R | R | R | R | R | R | S | R | R | R | R |
| *S.saprophyticus1* | R | S | R | R | R | R | R | R | R | R | R | R | R | S | R | R | R | R |
| *P.aeruginosa4* | R | S | R | R | R | S | R | S | R | R | R | R | R | S | R | R | R | R |

AM=Ampicillin, CRO=Ceftriatone, AMC=Amoxicillin/ clavulanic acid, AZM=Azithromycin F=Nitrofurantoin, CN=Gentamicin, TE=Teteracyclin, SXT =Trimethoprim/ Sulphamethoxazol, ATM= Azteroname, R=Resistant, S=sensitive, Ab:Antibiotic

## References:


[1]. Klaus-Joerger, T.;Joerger, R.; Olsson, E.andGranqvist, C.(2001). Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends Biotechnol., 19(1):15-20.

[2]. Li,X., Xu, H., Chen, Z. and Chen,G. (2011). Biosynthesis of nanoparticles by microorganisms and their applications. J. Nanomaterials .,16:

[3]. Malarkodi C.; Chitra,K.; Rajeshkumar,K.; Gnanajobitha,K.; Paulkumar, M .;Vanaja and G Annadurai.G.(2013) Novel eco-friendly synthesis of titanium oxide nanoparticles by using *Planomicrobium*sp. and its antimicrobial evaluation. Der Pharmacia Sinica, 4(3):59-66.

[4]. Stamm, W. E. &Norrby, S. R. (2001). Urinary tract infections: disease panorama and challenges. J Infect Dis 183 (Suppl. 1), S1–S4.

[5]. Ronald, A.R. (2002).Infections of upper urinary tract .Ch.34,In: Disease of the kidney, 6th ed.,Vol.1,eds:Robert W. Schriery and Carl W. Gottschalk.permission of Harper and Row publishers ,Inc. U.S.A.

[6]. Al-Haidari C H S. (2010). Microbiological study of urinary tract infection, Antibiotics susceptibility pattern and Extend Spectrum Beta Lactamase prevalence among children in Erbil city. M.Sc. thesis. Erbil: Hawler Medical University. College of Medicine. Iraq.

[7]. Charles, M.; Kodner, Emily, K.; and Thomas (2010) Recurrent Urinary Tract Infections in Women: Diagnosis and Management *Am Fam Physician.* 15; 82(6):638-643.

[8]. Miriagou, V., Cornaglia,G., Edelistin , M., Galani,I., Giske, C.G., Gniadkowski,M., Malamou-Lada, E., Martinez-Martinez,L., Navarro,F., Nordmann,P., Pexi,L.,Papournaras,S., Rossolini,G.M., Tsakaris,A., Vatopoulose,A., and Canton, R. 2010.Acquired carbapenemase in Gram negative bacterial pathogenes: detection

and surveillence issues. Clin Microbial infect. 16: 112-122.
[9]. Sungkaworn T, Triampo W, nalakarn P, Triampo D, tang IM, Lenbury Y.(2007). The effects of Tio2nanoparticles on tumor cells colonies: fractal dimension and morphological properties, Int J biomed Sci; 2(1):67-74.
[10]. Allaker, R, P.(2010)"The use of nanoparticles to control oral biofilm formation," *Journal of Dental Research*, vol. 89, no. 11, pp. 1175
[11]. Carr, F.J.; Chill, D. and Maida, N. (2002). The lactic acid bacteria: A literature survey. Critical Reviews in Microbiology, 28(4): 281-370.
[12]. Forbes, B.A.;Sahm, D.F. and Weissfled, A.S.(2002).Baily and Scott's Diagnostic Microbiology, 11th ed. Mosby company, Baltimore, USA.
[13]. CLSI. (2011). Performance standard for antimicrobial susceptibility testing; Twenty-First informational supplement. M100-S21., l 31(1).
[14]. Jarlier, V.; Nicolas, M. H.; Fournier, G. and Philippon, A. (1988). Extended broad-spectrum_-lactamases conferring transferable resistance to newer B-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis., 10:867–878.
[15]. Bashir, D.; Thokar, M.A.; Fomda, B.A.; Bash, G.; Zahoor, D.; Shabir Ahmad, A. and Toboli, A.S. (2011). Detection of metallobeta-lactamases (MBL) producing *Pseudomonas aeruginosa* at atertiary care hospital in Kashmir. African J. Microbiol. Res., 5: 164-172.
[16]. Azhar, A.M., BehtasLadan, S.A., AjabadiEbrahimi, M.T. and Heydari,M. ( 2011). Lactobacillus-mediated biosynthesis of titanium nanoparticles in MRS broth medium. Brno, Czech Republic, EU., 9: 21– 23.
[17]. Salman, J.A.S.; Ibrahem, K.H.; Ali, F.A. (2012).Effect of culture media on biosynthesis of titanium dioxide nanoparticles using *Lactobacillus crispatus.*, Intern. J. of Advanced Research, 2(5): 1014-1021.
[18]. Morello, J.A.; Granato, P.A. and Mizer, H.E. (2003). Laboratory Manual and Workbook in Microbiology: Applications to patient Care. 17thed. The Mc Grow-Hill Companies: 97-99.
[19]. Roy, A.S.; Parveen, A.; Kuppakar, A.R. and Prasad, M.N.N.A.(2010). Effect of Nano - Titanium Dioxide with Different Antibiotics against Methicillin-Resistant *Staphylococcus aureus*. J. Biomaterials and Nanobiotech., 1:37-41.
[20]. Azap, K.; Arslan, H.; erefhanog, K.S.; lu1, S. C.; olakog, I.; Erdog, H.; Timurkaynak, F. and Senger, S.S.(2010) Risk factors for extended-spectrum b-lactamase positivity in uropathogenic*Escherichia coli* isolated from community-acquired urinary tract infections European Society of Clinical Microbiology and Infectious Diseases.,16: 147–151.
[21]. Mohamed T.; Shaaban, H. A.; Ghozlan; Marwa, M. E.andMaghraby (2012). Susceptibility of Bacteria Infecting Urinary Tract to Some Antibiotics and essential Oils.
[22]. Abdallah, N.M.A.; Elsayed, S.B.; Yassin, M.M; Mostafa, and El-gohary, G.M. (2011) Biofilm forming bacteria isolated from urinary tract infection, relation to catheterization and susceptibility to antibiotics. Int. J. for Biotechnol. and Molecular Biology Research., 2(10):172-178.
[23]. Baral, P.; Neupane, S.; Marasini, B.P.; Ghimire, K.R.; Lekhak, B. and Shrestha, B. (2012) High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal.BMC Research Notes., 5:38.
[24]. AL-Khafaji, S.M.S. (2006). Study on capsule of *Acinetobacterbaumannii* and its effect on Immune Response. Ph.D. Thesis. Biology department. College of Science. AL- Mustasiriyah University.
[25]. Sarathbabu, R.; Ramani, T.V.; Bhaskararao, K. and Panda, S. (2012) Antibiotic susceptibility pattern of Klebsiella pneumonia isolated from

sputum,urine and pus samples. J. Pharma. and Biol.Sci.,1:4-9.
[26]. Muvunyi, M.; Masaisa, F.; Bayingana, C.; Mutesa, L. and Musemakweri, A. (2011). Decreased susceptibility to commonly used antimicrobial agents in bacterial pathogens isolated from urinary tract infections in Rwanda: need for new antimicrobial guidelines. American J. tropical medicine and hygiene.
[27]. Briongos-Figuero, L.S.; Gomez-Traveso, T.; Bachiller-Luque, P.; Dominguez-Gil, G.M. and Gomez-Nieto, A. (2012) Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing enterobacteria. Int J ClinPract., 66: 891–896.
[28]. Paterson, D. L. and Bonomo, R. A. (2005). Extended-Spectrum β- Lactamases: a clinical update. Clin. Microbio. Review., 18: 657-686.
[29]. Bell, J.M.; Turnidge, J.D. and Gales, A.C.(2002). Prevalence of extended spectrum β- lactamase (ESBL) producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY antimicrobial surveillance program (1998- 1999). Diagnosis Microbiol. Infec. Dis. J.; 42: 193-198.
[30]. Lakshmi, R.; Nusrin, K.S.; Ann, G.S. and Sreelakshmi, K.S. (2014) Role of beta lactmase in antibiotic resistance:A review.Int.Res.J.Pharm.,5(2):37-40.
[31]. Poirel, L.; Bonnin, R.A. and Nordmann, P. (2011) Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life, 63:1061–1067.
[32]. Heritier, C.; Poirel, L.; Lambert, T. and Nordmann, P. (2005) Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacterbaumannii*. Antimicrob. Agents Chemother., 49:3198–3202.
[33]. Mugnier, P.D.; Poirel, L.andNordmann, P. (2009) Functional Analysis of Insertion Sequence ISAba1, Responsible for Genomic Plasticity of *Acinetobacterbaumannii*. J Bacteriol ., 191: 2414-2418.
[34]. Allahverdiyev, A.; MAbamor, E.S.; Bagirova1, M. and Rafailovich, M. (2011). Antimicrobial effects of $TiO_2$ and $Ag_2O$ nanoparticles against drug-resistant bacteria and leishmania parasites Future. Microbiol., 6(8): 933–940.
[35]. Huang, Z.; Maness, P.C.and Blake, D.M. (2000). Bactericidal mode of titanium dioxide photocatalysis. J. Photochem. Photobiol. A Chem., 130: 163–170.
[36]. Liu, P.; Duan, W.; Wang, Q.and Li, X. (2010). The damage of outer membrane of Escherichia coli in the presence of $TiO_2$ combined with UV light. Colloids Surf. B Biointerfaces., *78: 171–176* .
[37]. Tsuang, Y.H.; Sun, J.S.; Huang, Y.C.; Lu, C.H., Chang, W.H., and Wang, C.C. (2008). Studies of photokilling of bacteria using titanium dioxide nanoparticles. Artif Organs., 32(2):167-74.
[38]. Rezaei-Zarchi, S.; Javed, A.; Ghani, M.J.; Soufian, S.; Firouzabadi, F.B.; Moghaddam, A.B. and Mirjalili, S.H. (2010). Comparative Study of Antimicrobial Activities of TiO2 and CdO Nanoparticles against the Pathogenic Strain of *Escherichia coli*. Iran J Pathology., 5 (2):83 -89.
[39]. Cho, M.; Chung, H.; Choi, W. and Yoon, G. (2005). Different Inactivation Behaviors of MS-2 Phage and *Escherichia coli* in $TiO_2$Photocatalytic Disinfection. Appl Environ Microbiol. , 71(1): 270–275.
[40]. Jayaseelan, C.; Rahuman, A.A.; Roopan, S.M.; Kirthi, A.V.; Venkatesan, J.; Kim, S.K.; Iyappan, M.; Siva, C.(2013). Biological approach to synthesize $TiO_2$ nanoparticles using *Aeromonashydrophila*and its antibacterial activity. SpectrochimActaAMolBiomolSpectrosc., 15(107):82-9.
[41]. Zhang, H.andPotent, C. (2009). Antibacterial activities of Ag/ $TiO_2$nanocomposite powders synthesized by a one-pot sol-gel method. Environ Sci Technol., 43(8):2905-10.

[42]. Ahmad, R., Khatoon, N. and Meryam, S. (2013). Biosynthesis, characterization and application of $TiO_2$ nanoparticles in biocayalysis and protein floding. J. of proteins and proteomics., 4(2): 115-121.
[43]. Cheng, C.L.; Sun, D.S. and Chu, W.C. (2009). The effects of the bacterial interaction with visible-light responsive titaniaphotocatalyst on the bactericidal performance. J. Biomed. Sci., 16(7): 1-10.
[44]. Roy, A.S.; Parveen, A.; Kuppakar, A.R. and Prasad, M.N.N.A. (2010). Effect of Nano - Titanium Dioxide with Different Antibiotics against Methicillin-Resistant *Staphylococcus aureus*. J. Biomaterials and Nanobiotech., 1:37-41.
[45]. Fayaz, A.M.; Balaji, K.; Girilal, M.; Yadav, R.; Kalaichelvan, P.T. and Venketesan, R. (2010) Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomed. Nanotechnol. Biol. Med., 6(1):103–109.
[46]. Hwang, I.S.; Hwang, J.H.; Choi, H.; Kim, K.J. and Lee, D.G. (2012) Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved. J. Med Microbiol., 9:1719–1726.
[47]. Parameswari, E.; Udayasoorian, C.S.; Sebastian, P. and R.M. Jayabalakrishnan, R.M. (2010). The bactericidal potential of silver nanoparticles. Int. Res.J, of Biotechnol., 1(3).44-49.
[48]. Naqvi, S.Z.; Kiran, U.; Ali, M.I.; Jamal, A.; Hameed, A.; Ahmed, S. and Ali, N. (2013). Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. Int J Nanomedicine., 8:3187-3195.
[49]. Namasivayam, S.K.R.; Beninton, B.; Christo, B. S. M.; Karthigai A. K.; Kumar A.M. and Deepak K. ( 2013). Anti Biofilm Effect of Biogenic Silver Nanoparticles Coated Medical Devices against Biofilm of Clinical Isolate of *Staphylococcusaureus*., Global Journal of Medical research Pharma, Drug Discovery, Toxicology and Medicine ., 13(3).
[50]. Santos, M.M; Queiroz, M.J. and Baptista, P.V. (2012). Enhancement of antibiotic effect via gold:silver-alloy nanoparticles. J Nanopart Res., 14:859-864.
[51]. Syed Z.; Haider N.; UroojK.; Muhammad, Ali, I; Jamal, A.; Hameed, A.; Ahmed, S. and Ali.N. (2013). Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. Int. J. of Nanomedicine., 8: 3187–3195.