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# Antibacterial Activity of Silver Nanoparticles against *Acinetobacter baumannii* Isolated from Different Clinical Specimens

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**Abstract**
In this study Six hundred samples were collected during the period from October 2018 to Mars 2019 from Medical city of Marjan Hospital in Province of Babylon. The clinical specimens included burn, blood, wound and urine. The samples stood principally cultured onto blood, MacConkey and Chrom agar plates used for isolation and they were incubated at 37C for 24-48 hours. Utilizing morphological characteristics, the bacterial isolates that developed as a pure and predominant growth from clinical samples were recognized. And biochemical tests in addition to Vitek2 system. In this study, a total of 20/600 (3.33%) isolates were identified as *Acinetobacter baumannii*, 485/ 600 (80.83%) isolates were recognized as a different bacterial species 95/600 (15.83%) of samples showed no bacterial growth. This training investigates the effectiveness of silver nanoparticles (AgNPs) against 20 *A.baumannii* isolates. Antibacterial activity was examined by disk diffusion assay using different concentrations of (500, 250, 125, 62.5, 31.2, 15.6 µg/ml) and each isolate's MIC and MBC values were established. With an increase in inhibition zone diameter that is directly inversely proportional to the rise in nanoparticle concentration, AgNPs demonstrated potent wide spectrum antibacterial action against the tested microorganisms. AgNPs had a MIC between 62.5 and 250 g/ml and an MBC between 125 and 500 g/ml.

**Keywords:** Nanoparticles, Anti-Bacterial Activity, MIC, MBC

## Introduction
Memberships of Acinetobacter genus ensure arisen from organisms of moot pathogenicity to worldwide pan resistant nosocomial pathogens in the ancient twofold or three periods meanwhile (2005-2006). Here further 30 genomic kinds of Acinetobacter recognized accordingly of which extra two third of Acinetobacter contagions are owing to Acinetobacter baumannii. A.baumannii inhabits fit humans transiently at a little stupidity on the whole-hearted also barrier, humid skin of axillae, generally assures that there won't be an infection between the toes, throat and intestinal tract (Jaggi *et al*., 2012) [15].
Multidrug resistant A.baumannii infection incline to arise in immune repressed person, in person with severe essential disease then in individuals exposed to hostile processes then cured with wide scale antibiotics (Garcia-Garmendia *et al*., 2001) [13]. Infections owing to intensive care units are where A.baumannii frequently first appears, where they are a common cause of ventilator -associated pneumonia, infections of urinary tract, then bacterem ia. A.baumannii too origins, albeit fewer often, complex skin besides central nervous system, soft tissue and abdominal (Fournier and Richet, 2006) [12]. A.baumannii eats develop a main pathogen create in fight related wounds (Aronson *et al*., 2006) [8].
The maximum worrying harms met through this dated are the bacterium's capability to store varied machines of resistance then the rise of resistant strains to wholly commercially accessible antibiotics attached with the absence of novel antimicrobic mediators. This has caused in a partial best of antibiotics for handling of multidrug resistant of A.baumannii.The greatest vigorous agents alongside to multidrug resistant A.baumannii are the polymyxin E (Colistin), polymixins-polymixin B and tigecycline (Lolans *et al*., 2006) [19].
Silver nanoparticles takes wide stood recognized to display a robust harmfulness to a diverse variety of microorganisms for this resolve silver nanoparticles compounds have been used widely in several bactericidal customs. Compounds of silver also used in the medical zone to treat burns and a variety infection (Wan *et al*., 2016) [27]. Good energies have been ready to realize this stuff by electron microscopy, which has presented dimension needy line of AgNPs with bacteria (Kaweeteerawat *et al*, 2017) [16].

## Materials and Methods

### Silver Nanoparticles and Media

From (US research nanomaterials inc USA, AgNPs 40nm were purchased. We purchased standardized (Mueller Hinton, MacConkey, Chrom, Nutrint and blood agar) from HIMEDIA in India.

### Isolation and Characterization

The bacterial isolates were obtained from patients who were admitted to the Medical City of Mirjan Hospital, in Babylon province. For bacterial isolation and purification, samples were subjected to bacteriological techniques such as growing on blood and MacConkey agar plates for 24-48 hours at 37°C. The Vitek 2 compact system was used for the antibiotic profile test and to confirm the isolates. (Macfaddin, 2000; Lahiri, *et al*, 2004) [20, 17], (AL Ajeeli, *et al*. 2013) [2].

### Microscopic Examination

Gram-stain was used to study morphology of bacterial cells in order to determine their size, organization and degree of response to stain. Bacteria appeared as conventional after staining. After stanining, bacteria seemed as traditional gram-negative, pairs coccobacilli (Lahiri, *et al*., 2004) [17].

### Antibacterial Activity of AgNPs

The antibacterial activity of AgNPs was examined against Acinetobacter baumannii. According to Clinical and Laboratory Standards Institute. Disk diffusion assay was used to determine the (bacterial sensitivity) to AgNPs. In sterile deionized water, duplicate AgNPs were utilized at concentrations of 500, 250, 125, 62.5, 31.2, and 15.6 g/ml. When a zone of inhibition was shown around the disk following the incubation period and the diameter was measured using a digital venire caliper, the test was deemed successful. (Raheem *et al*, 2018). Minimal Inhibitory and Minimal bactericidal concentration detect according to (Al-Jassani and Raheem, 2017).

## Results and Discussion

### Isolation and Identification of A. baumannii isolates

Six hundred samples from Hilla Teaching Hospital and Medical City of Mirjan in Babylon Province were calm from October 2018 to June 2019.The medical samples include traces of blood, burns wounds and urine. The samples were grown primarily on Blood, Chrom agar and agar plates of MacConkey, which were isolated and incubated for 24-48 At 37 Co. In addition to the Vitek2 system and molecular detection, bacterial isolates obtained from clinical samples were identified through microscopically, macroscopically properties and biomedical test. A total of 20/600 (3.33%) isolates were also identified as Acinetobacter baumannii, 485/600 (80.83%) isolates as other bacterial spp and 95/600 (15,83%) isolates showed no bacterial growth as shown in Fig 1.

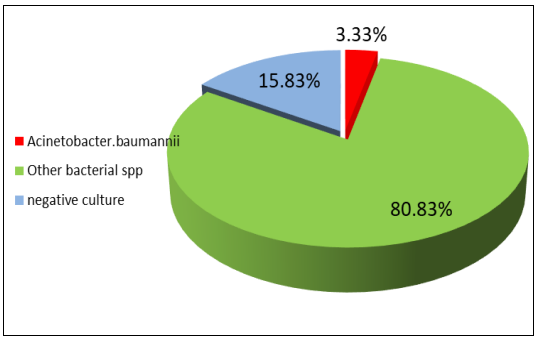


**Fig 1:** Total sample and Percentages

The identification of these isolates depends on the microscopic characteristics. Bacteria appeared later as Gram-negative, coccobacilli and pairs and morphological characteristics such as colony size, shape, colour, natural pigment, clearness, elevation, texture, lactose fermentation on MacCon key agar and the formation of hemolysin on blood agar. On blood agar the A.baumannii colonies show circular, convex, all nonhemolysis, smooth, opaque, raised, fluffy and smaller than Enterobacteriaceae, whereas on MacConkey agar nonlactose is fermented, but colonies display a purplish that may cause the organism to be wrong with the fermented lactose, whereas on nutrient agar colonies tend to be oval, smooth, convex, translucent to slightly opaque and unpigmented with complete margin and appear to be red, circular on chromium agar. As shown in Fig 2, (MacFaddin, 2000; Lahiri *et al*., 2004) [20, 17].

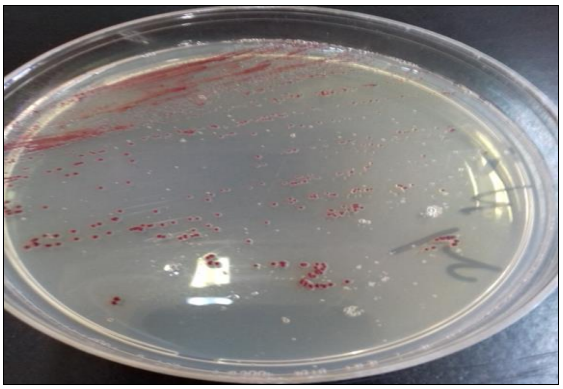

**Fig 2:** Acinetobacter baumannii colony on Chrom agar

### Distribution of *Acinetobacter baumannii* isolates Among Clinical Specimens:

*Acinetobacter baumannii* isolates have been mended with various percentages from medical samples, as shown in Table 1.

**Table 1:** Numbers and Percentages of *A. baumannii* between Clinical Samples

| Clinical samples type | No. of sample | No. (%) of *A. baumannii* isolates |
|---|---|---|
| Burn swab | 300 | 10 (3.33%) |
| Wound swab | 190 | 5 (2.63%) |
| Urine | 65 | 3 (4.61%) |
| Blood | 45 | 2 (4.44%) |
| Total | 600 | 20 (3.33%) |

The disparity in isolation levels for whole studies may be due to numerous factors such as collection place and date and collection period (Al-Hilali (2019) [5]. The percentage of isolation could be diverse rendering to difference in nearby patients levels of contamination and environmental factors. Many infections of the Acinetobacter have a Seasonal variation of 50 percent higher rates of infection from July to October than at other times of the year. This variation has been defined by warmer, more humid ambient air, favoring Acinetobacter production, and potentially stopping potential environmental pollutants, such as air-conditioner condensate (Lahiri *et al*., 2004) [17].

The current results comparable with local study by Alsehlawi, *et al*., (2015) [6] who establish that isolation rate of A.baumannii in Al-Najaf city was (3.5%) and in AL-Hillah city by (Al-Hilali (2019) [5], they establish that isolation rates of A.baumannii were (5.5%) while studies in Al- Diwaniya city by Al-Garaawi, (2012) [3] and in Hillah city by (Al-Harmoosh, 2015 [4]; Al-Hindawi, 2018), they establish that isolation rates of A.baumannii were (0.76%) and (1.5%) respectively, however Sahar and AL-Yasseen, (2014) [25] who noted that A.baumannii isolates were (18%)from clinical samples, these results became divergence with the current study.

**Antibacterial Activity of Silver Nanoparticles Against *A.baumannii* Isolates Detected by Minimum Inhibition Concentration an Minimum Bactericidal Concentration:**

Silver nanoparticles have potent and natural antibacterial agents showing antibacterial properties against A.baumannii by disk and agar well diffusion method and antibiotic sensitivity has been measured depending on the inhibition (mm) rendering diameter zone (CLSI, 2019; Raheem *et al*, 2019) [11, 23].

Different concentrations of AgNPs were tested against A.baumannii (500, 250, 125, 62.5, 31.2, 15.6 µg/ml).The findings showed antibacterial activity of AgNPs against A.baumannii in response to increased concentration of nanoparticles as shown in Table 2 and Fig 3 increased in in inhibition areas. The AgNP MIC ranged from (62,5µg/ml to 250µg/ml) and the MBC ranged from (125µg/ml to 500µg / ml) as show in Table 3.

This results of study are approves with results of (Chauhan *et al*., 2015; Wan *et al*., 2016) [10, 27] which establish that the silver nanoparticles has antibacterial activity against A.baumannii, and a Babylon study by AL-Tameme and AL-Hasnawy., (2017), which found that the sliver nanoparticles (200 µg/ml) while Raheem *et al*., (2018) established the MIC of AgNPs ranged (16-125 µg/ml) and MBC (125-500 µg/ml) against E.coli, K.Pneumoniae, S.typhi, S.saureus, P.smirabilis and P.aeruginosa.

**Table 2:** Antibacterial activity of Silver Nanoparticles against of 20 *A.baumannii* Isolates by disk diffusion test

| Isolates No. | (Inhibition zone diameter in mm) | | | | | |
|---|---|---|---|---|---|---|
| | 500µg/ml | 250µg/ml | 125µg/ml | 62,5µg/ml | 31,2µg/ml | 15,6µg/ml |
| 1 | 20 | 17 | 15 | 12 | 10 | **7** |
| 2 | 19 | 16 | 14 | 11 | 9 | 6 |
| 3 | 21 | 19 | 16 | 12 | 9 | 7 |
| 4 | 19 | 17 | 16 | 12 | 9 | 6 |
| 5 | 20 | 18 | 15 | 11 | 9 | 6 |
| 6 | 20 | 17 | 15 | 10 | 9 | 7 |
| 7 | 19 | 17 | 15 | 10 | 8 | 5 |
| 8 | 20 | 18 | 16 | 11 | 9 | 6 |
| 9 | 21 | 18 | 16 | 11 | 9 | 6 |
| 10 | 19 | 17 | 15 | 10 | 8 | 5 |
| 11 | 20 | 18 | 15 | 9 | 8 | 6 |
| 12 | 18 | 16 | 14 | 9 | 7 | 5 |
| 13 | 22 | 18 | 16 | 11 | 8 | 6 |
| 14 | 20 | 17 | 15 | 10 | 8 | 5 |
| 15 | 21 | 18 | 16 | 11 | 9 | 6 |
| 16 | 20 | 17 | 14 | 10 | 8 | 5 |
| 17 | 21 | 18 | 16 | 12 | 9 | 6 |
| 18 | 19 | 16 | 13 | 9 | 7 | 5 |
| 19 | 18 | 15 | 12 | 10 | 6 | 5 |
| 20 | 19 | 18 | 16 | 12 | 9 | 6 |

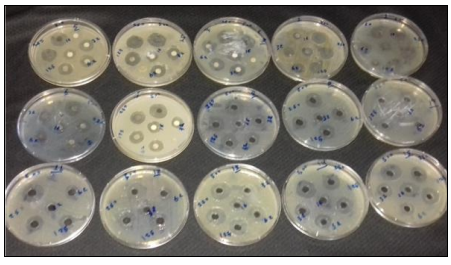

**Fig 3:** Antibacterial activity of Silver Nanoparticles against 20 *A.baumannii* isolates detected by disk diffusion test

**Table 3:** Minimum Inhibition Concentration and Minimum Bactericidal Concentration of Silver Nanoparticles for *A.baumannii* isolates

| Bacteria isolate NO | MIC | MBC |
|---|---|---|
| 1 | 62.5µg/ml | 250µg/ml |
| 2 | 125µg/ml | 250µg/ml |
| 3 | 62.5µg/ml | 250µg/ml |
| 4 | 62.5µg/ml | 125µg/ml |
| 5 | 125µg/ml | 250µg/ml |
| 6 | 6.25µg/ml | 250µg/ml |
| 7 | 125µg/ml | 250µg/ml |
| 8 | 125µg/ml | 250µg/ml |
| 9 | 62.5µg/ml | 125µg/ml |
| 10 | 250µg/ml | 500µg/ml |
| 11 | 125µg/ml | 250µg/ml |
| 12 | 62.5µg/ml | 250µg/ml |
| 13 | 125µg/ml | 250µg/ml |
| 14 | 250µg/ml | 500µg/ml |
| 15 | 62.5µg/ml | 250µg/ml |
| 16 | 125µg/ml | 250µg/ml |
| 17 | 62.5µg/ml | 125µg/ml |
| 18 | 62.5µg/ml | 250µg/ml |
| 19 | 125µg/ml | 500µg/ml |
| 20 | 62.5µg/ml | 125µg/ml |

AgNPs exhibit exceptional physiochemical and biological influences. AgNPs has been said to be able anchor to cell wall of bacteria and thus infiltrate it. This act would base physical alterations in the bacterial mem brane similar to the destruction of the membrane which container contribute to the cell's then bactericidal leakage contents. It has also been established that AgNPs have antibacterial action on (Gm-ve) bacteria was more robust than (Gm+v)e bacteria. This occurrence can be described in the current variance in cell wall width among (Gm+ve 30 nm) and (Gm-ve 3–4nm), mostly composed of peptidoglycan (Chatterjee *et al*., 2015) [9]. In addition, it was shown that the bacterial membrane has a negative charge owed to phosphate, carboxyl and amino groups, the positive charge combines electrostatic attraction among AgNPs and cell membrane negative bacteria charge, thereby alleviating AgNPs affection on cell membranes (Abbaszadegan *et al*., 2015) [1].

It is possible to find improved antibacterial action later by modifying the charge of the AgNP surface to obtain stronger attractive force (Mandal *et al*., 2016) [21]. AgNPs can moreover infiltrate the membrane after adhesion to the bacterial wall, and join the bacteria. There is a size-dependent antibacterial influence, which is that smaller nanoparticles have a excessive area in interaction with the bacterial cells and can enter the cytoplasm extra frequently than bigger nanoparticles (Chatterjee *et al*., 2015) [9]. Once AgNPs enter the microbial cell, it may cooperate with cellular constructions and biomolecules for example lipids, proteins and DNA. Interface among AgNPs and cellular or biomolecular structures may result in bacterial dysfunction and ultimately death. More, AgNP ribosomal interactions contribute to their denaturation producing translation inhibition and synthesis of protein. It is also hypothesized that AgNPs effectually cooperate with the β-galactosidase carboxyl and thiol groups, obstruct intra cellular biological jobs and lead cell death. (You, *et al*., 2015) [28]. In addition, Ag NPs antibacterial function is also attributable to their high production potential of ROS and free radical contaminants for example superoxide anion, hydrogen peroxide, hypochloric acid hydroxyl radical, and single oxygen (Siritongsuk *et al*, 2016; Zhao *et al*., 2017) [26, 29]. In normal conditions, ROS created in cells is inadequate and can be removed by system of antioxidant (Ramalingam *et al*., 2016) [24]. AgNPs exert antibacterial effect by inactivating dehydrogenases in the respiratory chain and eventually gene rating excess ROS, which inhibited respiration and cell growth (Quinteros *et al*., 2016) [22]. AgNPs can down-regulate antioxid expression for example superoxide dismutase, glutathione (GSH) and catalase that can increase ROS increase. Increased ROS results in apoptosis such as reaction, lipid peroxidation, GSH depletion and DNA damage (Lee *et al*., 2014) [18]. Furthermore, AgNPs antibacterial activity was too influenced by metabolism associated with (ATP) adenosine triphosphate and ROS) (Hwang *et al*., 2012) [14].

## Conclusion

The results of this study showed that AgNPs have considerable inhibitory and anti-bacterial effect against A.baumannii. It is greatly commended that using AgNPs as alternative anti-bacterial agent without taking risk of developing resistant bacterial isolates as with antibiotics.

## Ethical Approval

Scientific Research Study Ethical Commission by ethical approval of ministries of ecological/health and higher education / scientific research in Iraq together.

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