



## Comparative Analysis of Antibacterial activity of PEG coated and uncoated Biogenic Silver Nanoparticles on Multi-Drug Resistant Pyogenic bacteria isolated from Oral Lesions.

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### KEYWORDS

Phytogenic, antibacterial activity, silver nanoparticles, multi drug resistance, TEM.

### ABSTRACT:

The aim of the present study is to evaluate the antibacterial activity of silver nanoparticles that were biogenically synthesized from *Klebsiella pneumoniae* (BNP) and Neem leaves aqueous extract (NLNPs), followed by Polyethylene glycol capping (PEG). Characterization by UV-Vis spectroscopy, XRD and TEM revealed the topology and morphology of these biosynthesized silver nanoparticles. Average diameter of these nanoparticles was found to be 19.02 nm for BNP and 15 nm for NLNP. These nanoparticles were assessed for their antibacterial activity on the bacterial pathogens that were isolated from oral lesions (*S. aureus*, *E. coli* and *K. pneumoniae*). On the analysis of antibiotic susceptibility, these isolates were found to resist multiple antibiotics and hence they were categorized as MDRs. Antibacterial sensitivity testing by well diffusion method of PEG coated and uncoated BNP and NLNP revealed the strong inhibition potential of these silver nanoparticles on the pyogenic oral isolates *S. aureus*, *E. coli* and *K. pneumoniae*. It can be concluded from this study that coating of the BNP and NLNP by PEG has a poor impact on the antibacterial properties as compared to uncoated BNP and NLNP.

### 1. Introduction:

Multi-drug resistant bacteria are now a serious threat to public health and contemporary medicine, and their rise has been deemed a worldwide health disaster [1]. These adaptable microorganisms have quickly developed in recent years, becoming able to withstand the effects of many antibiotics, rendering previously effective therapies useless. This issue, also known as antimicrobial resistance (AMR), has a big impact on how patients are treated, how healthcare is organized, and how society as a whole is doing [2]. The common isolates obtained from the clinical setup includes a diverse group of both Gram positive and Gram negative bacterial pathogens like *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* that are responsible for various infections related to vital organs of cardiovascular system, respiratory system,

gastrointestinal tract and genitourinary system [3–7]. Patients suffering from infections caused by these group of pathogens are difficult to be treated by antibiotics [8]. The persistent adaptability of these pathogens and their propensity to propagate diseases highlight the urgent need for a deeper knowledge of the mechanisms causing their genesis as well as the creation of cutting-edge tactics to counter this expanding threat. The overuse and abuse of antibiotics, both in clinical settings and in agriculture, are the primary causes of this issue [9,10]. In clinical practice, antibiotics are sometimes recommended for viral infections needlessly or used incorrectly with insufficient doses and durations, which can unintentionally select for antibiotic-resistant bacterial strains [11,12]. Numerous variables, such as patient demand, uncertainty about the diagnosis, and a desire to immediately relieve symptoms, can contribute to overprescribing and other inappropriate usage of



medications. The effects of microorganisms that are resistant to many drugs are severe. The arsenal of viable antibiotics is declining, and infections that could once be treated are now potentially fatal [13].

In order to combat the emergence of AMR strains, novel strategies which includes the use of silver nanoparticles as an alternative substitute for antibiotics are being explored [14–16]. These nanoparticles have been reported to effectively target pathogens of various entities like bacteria, fungi and viruses [17–20]. Silver nanoparticles can interact with the cell membranes of bacteria, causing disruption and also harm the cell membrane structurally, thereby increasing the permeability and causing the loss of vital ions and cellular elements [21]. When in contact with microorganisms, silver nanoparticles can produce reactive oxygen species such superoxide ions and hydroxyl radicals. Oxidative stress brought on by ROS can harm biological components like DNA, proteins, and lipids and result in cell death [22–24]. When silver nanoparticles bind to microorganisms DNA, the genetic material undergoes structural modifications which results in DNA damage, disruption of transcription and replication processes [25]. The silver ions ( $\text{Ag}^+$ ) that are released by silver nanoparticles are extremely poisonous to microorganisms as they interfere with vital cellular functions including electron transport chains and energy synthesis leading to the death of microorganism [26,27]. Despite the fact that silver nanoparticles have a lot of potential as antibacterial agents, it's important to take into account any potential toxicity to human and environmental cells as well as the emergence of silver-resistant microbes [28–30]. In order to use silver nanoparticles safely and effectively while minimizing any potential downsides, research is still being done in this area. One of the key factors that correlates the toxicity of the nanoparticles lies in their mode of synthesis. Previous researches have also cited the role of the chemically synthesized nanoparticles to be more cytotoxic as compared to those that are synthesized biologically [31,32]. There are various routes for the synthesis of silver nanoparticles biologically which involves the use of various plant sources and microorganisms like bacteria, fungi and yeast [33–37]. The nanoparticles derived from these sources have shown to display enhanced level of antimicrobial, antioxidant and other properties that makes their use to be highly compatible for biomedical applications. Yet there is a constant contradiction for the identification of the best methods out of phytogetic and bacteriogenic modes of nanoparticles synthesis that can be used for the formation of highly effective and biomedically compatible nanoparticles. The present article is an attempt to identify the best suitable method for the synthesis of the silver nanoparticles by using the

extracellular extract of bacteria and aqueous extract of plant leaves. The nanoparticles derived from these sources were then evaluated for their antibacterial abilities against the pyogenic group of bacteria that were isolated from pyogenic oral lesions.

## 2. Materials and methods:

### 2.1 Isolation and identification of pyogenic bacteria:

Pyogenic pathogens were isolated from the pus samples which was procured from a tertiary health care unit. The isolates were screened for their morphological, biochemical and molecular characterization. These isolates were also assessed for their resistance against different antibiotics and also for their ability to produce biofilms.

### 2.2 Preparation of neem leaves aqueous extract:

50 gm of fresh neem leaves were selected for the preparation of the aqueous extract. The leaves were collected, washed thrice with sterile double distilled water. The leaves were then added to 100 ml of sterile distilled water and heated at 85°C for 30 minutes, followed by cooling at room temperature and filtered through filter paper. The filtrate thus obtained was stored at 4°C till further use.

### 2.3 Isolation and identification of soil isolate for the synthesis of silver nanoparticles:

*Klebsiella pneumoniae* subsp. *quasipneumoniae*, isolated from the previous study [33], was used for the biosynthesis of the silver nanoparticles.

### 2.4 Synthesis of silver nanoparticles from bacteria:

The isolates thus obtained was inoculated in 50 ml of sterile nutrient broth and incubated at 37°C for 48 hours. The growth thus obtained was centrifuged at 5500 rpm for 30 minutes and the filtrate thus obtained was used for the synthesis of silver nanoparticles. 10 ml of the extracellular extract was then added to the 1 mM  $\text{AgNO}_3$  solution followed by incubation in dark conditions at room temperature for 24 hours. Change in the color of the solution from pale yellow to brown color indicated the formation of silver nanoparticles. Nanoparticles thus formed was purified by centrifugation at 14,000 rpm for 30 minutes with autoclaved distilled water followed by redispersion in sterile autoclaved distilled water till further use.

### 2.5 Synthesis of silver nanoparticles from neem leaves:

10 ml of the neem leaves aqueous extract was added to 1 mM  $\text{AgNO}_3$  solution. The reaction mixture was then incubated at room temperature under dark conditions for 24 hours. Change in the color of the solution from pale yellow to brown color indicated the formation of silver



nanoparticles. Nanoparticles thus formed was purified by centrifugation at 14,000 rpm for 30 minutes with autoclaved distilled water followed by redispersion in sterile autoclaved distilled water till further use.

## 2.6 Capping of silver nanoparticles by Polyethylene glycol:

Polyethylene glycol (PEG) solution was made by adding 10 gm of PEG powder to 90 ml of sterile distilled water followed by continuous stirring for 24 hours at room temperature. The PEG solution thus formed, was divided into two flasks each containing 50 ml of BNP and NLNP respectively. The reaction mixture was then stirred on magnetic stirrer for 48 hours at room temperature.

## 2.7 Characterization of silver nanoparticles:

For the assessment of the surface plasmon resonance, the nanoparticles were screened under the spectrum ranging from 200-800 nm by using the UV-Vis spectrophotometer. Powdered silver nanoparticles were exposed to the Cu-K $\alpha$ 1 X Ray Diffractometer with wavelength 1.5046 Å and operated at 30 mA and 40 kV with 2 $\theta$  (30- $^{\circ}$ 140 $^{\circ}$ ) for the assessment of crystallinity. For the topological identification of the synthesized silver nanoparticles, the nanoparticles were sonicated with ethanol and placed on copper grid, followed by drying and observed under Transmission electron microscope.

## 2.8 Isolation and identification of pyogenic isolates obtained from oral lesions:

Pus samples from the patients were collected and inoculated in Brain Heart Infusion broth followed by incubation at 37 $^{\circ}$ C for 24 hours. The growth thus obtained was then examined for their morphology by Gram staining and were also streaked on Mc Conkey agar, Blood agar and Mannitol salt agar for their biochemical characterization. Identification of the pyogenic bacterial isolates was also confirmed by analysis on the VITEK-2 system. The isolates were then screened for susceptibility to various antibiotics by Kirby Bauer disc diffusion method.

## 2.9 Identification of antibiotic resistant pyogenic strains:

The pyogenic bacterial isolates were inoculated in 10 ml of sterile Muller Hinton Broth, followed by incubation at 37 $^{\circ}$ C for 18 hours. Post incubation, the bacteria from the broth culture were then transferred to sterile Muller Hinton Agar Plates by using sterile cotton swab and antibiotic discs comprising of antibiotics against Gram Positive and Gram negative bacteria was incorporated to it. Assessment of the antimicrobial activities was carried out by the Kirby -Bauer method against the antibiotic discs comprising of antibiotics Ampicillin (20 mcg), Cotrimoxazole (25 mcg), Tazobactam (110 mcg),

Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Ceftriaxone (30 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amikacin (30 mcg) and Levofloxacin (5 mcg), was used against the gram-negative isolates, while antibiotics such as Ampicillin (20 mcg), Cotrimoxazole (25 mcg), Cephalexin (30 mcg), Tetracyclin (30 mcg), Ciprofloxacin (5 mcg), Levofloxacin (5 mcg), Linezolid (30 mcg), Cloxacilin (5 mcg), Roxithromycin (15 mcg), Lincomycin (2 mcg) and Gentamicin (10 mcg) were used against Gram positive isolates. The plates were then incubated at 37 $^{\circ}$ C for 24 hours after which the zone of inhibition was measured (in mm).

## 2.10 Antibacterial activity of silver nanoparticles:

Pyogenic bacterial isolates were transferred to sterile Muller Hinton (MH) Broth followed by incubation at 37 $^{\circ}$ C for 18 hours. The bacterial growth thus obtained after the incubation was then transferred on the sterile MHA plates and wells of 6 mm diameter was punched with the help of sterile well borer. 50  $\mu$ l of the PEG coated and uncoated silver nanoparticles was then added to each well and incubated at 37 $^{\circ}$ C for 24 hours. Broad spectrum antibiotic Streptomycin sulphate was used as the positive control while 1 mM AgNO $_3$  was used as a negative control. The experiment was done in triplicate and the result thus obtained was evaluated in the form of Mean  $\pm$  SD. Statistical analysis of the result was done on the basis of the one-way ANOVA followed by Tukey's multiple comparison test to assess the significance.

## 2.11 MIC evaluation of silver nanoparticles:

Assessment of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of BNP and NLNP was done by broth dilution method. Overnight cultures of the pyogenic bacterial isolates which were grown in sterile MH broth. Two-fold serial dilution of BNP and NLNP in the concentrations ranging from 50-1.56  $\mu$ g/ml was added to the broth containing the bacterial concentration of 10 $^6$  CFU/ml. Streptomycin sulphate was used as a positive control while sterile distilled water was used as a negative control. The setup was then incubated at 37 $^{\circ}$ C for 24 hours and the MIC was evaluated by analysis of visual turbidity of the tubes.

## 2.12 MBC evaluation of silver nanoparticles:

50  $\mu$ l aliquot was taken from the tubes which showed no growth and was seeded on the sterile BHI agar plates followed by incubation at 37 $^{\circ}$ C for 24 hours. The minimal concentration at which 99.9% of the bacterial population failed to develop colony on the agar plate was considered as the endpoint for MBC.

## 3. Result

### 3.1 Isolation and identification of pyogenic bacteria:



20 pus samples were collected from the patients visiting various departments at I.T.S. Dental college, Greater Noida for the treatment of oral conditions. Out of the 20 pus samples *S. aureus* was isolated from 12 samples while *K. pneumoniae* was isolated from 5 and *E. coli* was obtained from 3 pus samples respectively. Confirmation of these pathogens was carried on the basis of morphological, biochemical and automated analysis.

### 3.2 Identification of drug resistance in the isolated pyogenic bacteria:

On the basis of the antibiotic sensitivity tests, as shown in **Table-1 (a)** and **(b)**, the isolates were considered as sensitive and resistant. It was observed that all the pyogenic bacterial isolates were resistant to multiple antibiotics.

| S. No. | Antibiotics             | Zone of inhibition (mm) |           |                      |              |
|--------|-------------------------|-------------------------|-----------|----------------------|--------------|
|        |                         | <i>E. coli</i>          | Category  | <i>K. pneumoniae</i> | Category     |
| 1.     | Ampicillin (20 µg)      | 0±0                     | Resistant | 0±0                  | Resistant    |
| 2.     | Cotrimoxazole (25 µg)   | 0±0                     | Resistant | 0±0                  | Resistant    |
| 3.     | Cefotaxime (30 µg)      | 0±0                     | Resistant | 0±0                  | Resistant    |
| 4.     | Tazobactam (110 µg)     | 8±1.1                   | Resistant | 19±0.5               | Intermediate |
| 5.     | Chloramphenicol (30 µg) | 0±0                     | Resistant | 19±1.1               | Intermediate |
| 6.     | Ciprofloxacin (5 µg)    | 0±0                     | Resistant | 0±0                  | Resistant    |
| 7.     | Ceftriaxone (30 µg)     | 0±0                     | Resistant | 0±0                  | Resistant    |
| 8.     | Tetracycline (30 µg)    | 12±0.5                  | Resistant | 0±0                  | Resistant    |
| 9.     | Ofloxacin (5 µg)        | 0±0                     | Resistant | 0±0                  | Resistant    |
| 10.    | Gentamicin (10 µg)      | 0±0                     | Resistant | 17±0.5               | Intermediate |
| 11.    | Amikacin (30 µg)        | 0±0                     | Resistant | 15±0.5               | Intermediate |
| 12.    | Levofloxacin (5 µg)     | 11±0.5                  | Resistant | 0±0                  | Resistant    |

**Table-1 (a)** Antibiotic sensitivity test of Gram negative pyogenic isolates *E. coli* and *K. pneumoniae*.

| S. No. | Antibiotics            | Zone of inhibition (mm) |              |
|--------|------------------------|-------------------------|--------------|
|        |                        | <i>S. aureus</i>        | Category     |
| 1.     | Ampicillin (20 µg)     | 0±0 (R)                 | Resistant    |
| 2.     | Co-Trimoxazole (25 µg) | 0±0 (R)                 | Resistant    |
| 3.     | Cephalexin (30 µg)     | 0±0 (R)                 | Resistant    |
| 4.     | Tetracycline (30 µg)   | 0±0 (R)                 | Resistant    |
| 5.     | Cefotaxime (30 µg)     | 0±0 (R)                 | Resistant    |
| 6.     | Ciprofloxacin (5 µg)   | 0±0 (R)                 | Resistant    |
| 7.     | Levofloxacin (5 µg)    | 14±0.3 (R)              | Resistant    |
| 8.     | Linezolid (30 µg)      | 26±1.4 (S)              | Sensitive    |
| 9.     | Cloxacillin (5 µg)     | 15±0 (R)                | Resistant    |
| 10.    | Roxithromycin (15 µg)  | 20±0.8 (I)              | Intermediate |
| 11.    | Lincomycin (2 µg)      | 10±0.6 (R)              | Resistant    |
| 12.    | Gentamicin (10 µg)     | 0±0 (R)                 | Resistant    |

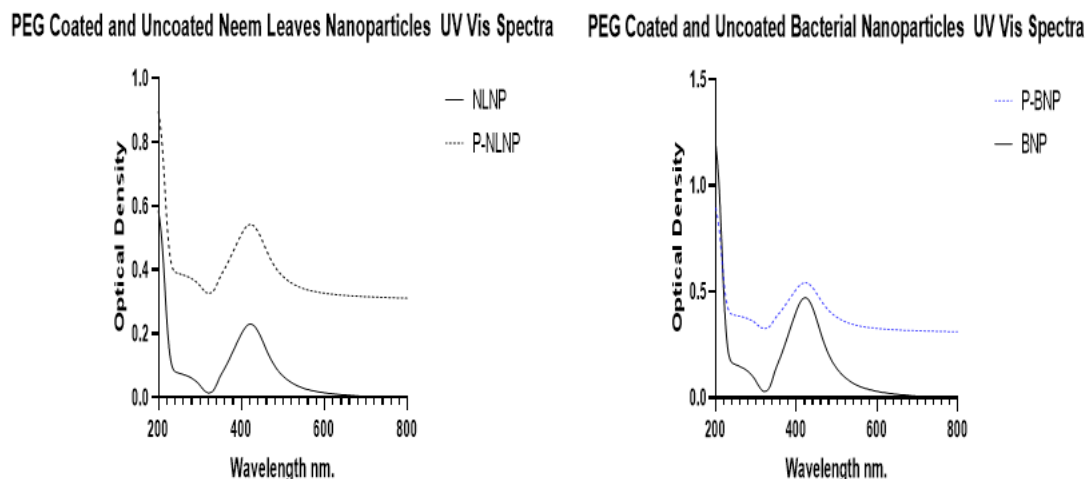
**Table-1 (b)** Antibiotic sensitivity test of Gram positive pyogenic isolate *S. aureus*.

### 3.3 Characterization of silver nanoparticles:

#### 3.3.1 UV-Vis:

3.3.2 The addition of cellular extracts from *Klebsiella* spp. to a 1mM AgNO<sub>3</sub> solution triggered surface plasmon resonance activity in BNP. This led to a colour shift from pale yellow to brown during the incubation period, aligning with findings from prior studies. A comparable response occurred when ethanolic extracts from Neem leaves were introduced to the 1mM AgNO<sub>3</sub> solution. UV-Visible spectroscopy disclosed a surface

plasmon resonance peak at 422.5 nm for BNP and 432 nm for NLNP. The alteration in colour, as observed through spectrophotometry, was ascribed to the bio-reductive attributes of the ethanolic extracts, serving as a catalyst in the conversion of Ag<sup>+</sup> to Ag<sup>0</sup> for both NLNPs and Bacterial cellular extract. The peak intensity in the UV-Visible spectra affirmed the successful formation of silver nanoparticles in both BNP and NLNPs.

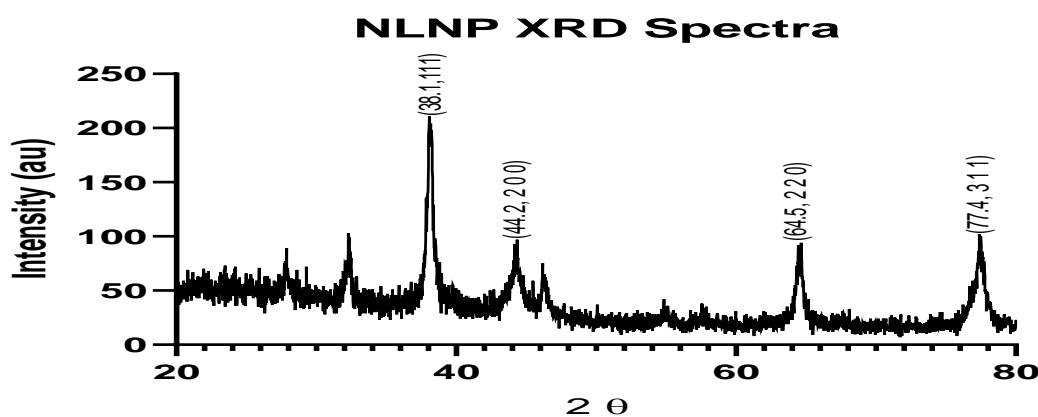


**Fig.1. UV-Visible Spectroscopy analysis of PEG coated and uncoated NLNP and BNP Silver Nanoparticles:** P-NLNP =PEG coated Neem Leaf Nanoparticles, P-BNP= PEG coated Bacterial Nanoparticles, NLNP = Neem Leaf Nanoparticles and BNP = Bacterial Nanoparticles.

### 3.3.2 XRD:

After conducting XRD analysis on the powdered AgNPs, various angular diffraction patterns were obtained and subsequently examined using the X'Pert Highscore Plus software (Version 3.0, PANalytical, Netherlands). The results were then utilized to assess the crystalline nature of the nanoparticles, including

parameters such as peaks, Full Wavelength Half Maxima (FWHM), and Miller's indices for determining the crystallinity of both BNPs and NLNPs. The diffraction peaks, identified at  $2\theta$  values of  $38.1^\circ$  (1 1 1),  $44.2^\circ$  (2 0 0),  $64.5^\circ$  (2 2 0), and  $77.4^\circ$  (3 1 1), were observed in the case of NLNPs on specific crystallographic planes, as depicted in Fig. 5 (a).



**Fig. 3. (a) XRD Spectra of NLNP**



## Bacterial Silver Nanoparticles XRD

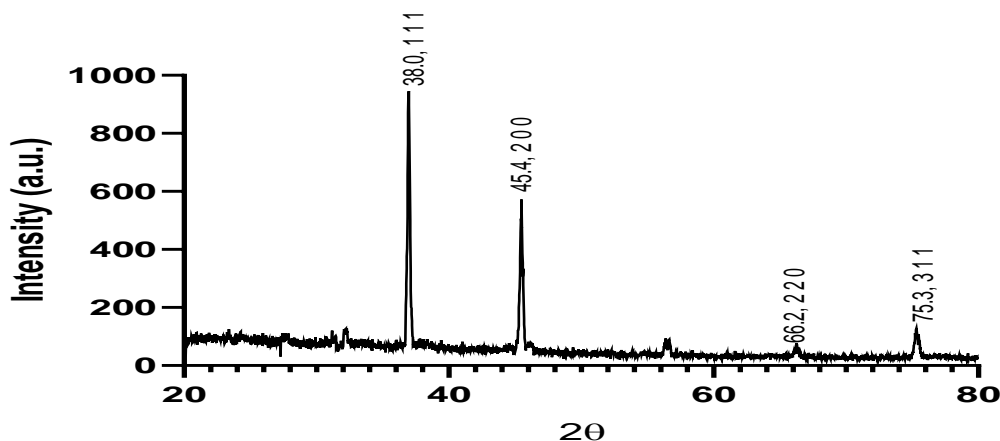


Fig. 3. (b) XRD Spectra of BNP

For BNPs, the diffraction peaks were identified at  $2\theta$  values of  $38.0^\circ$  (1 1 1),  $45.4^\circ$  (2 0 0),  $66.2^\circ$  (2 2 0), and  $75.3^\circ$  (3 1 1), as illustrated in **Fig. 5 (b)**. Examining the data derived from the crystal patterns of both BNP and NLNP indicated a resemblance in the Face-centered Cubic shape.

### 3.3.3 TEM:

The TEM analysis of uncoated BNPs revealed the existence of spherical silver nanoparticles, with a mean diameter of approximately 19.02 nm, determined using ImageJ software (Version 1.80, USA). In case of uncoated NLNPs, the size of the nanoparticle was found to be 15.02 nm. PEG coated BNP and NLNP had an averages size of 20 and 16.04 nm respectively as illustrated in **Fig. 2 (a-d)**.

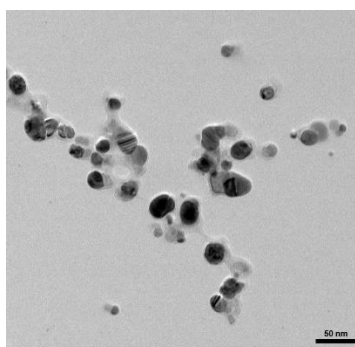


Fig. 2. (a) PEG uncoated BNPs

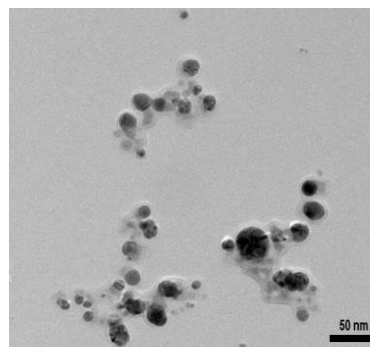


Fig. 2. (b) PEG coated BNPs

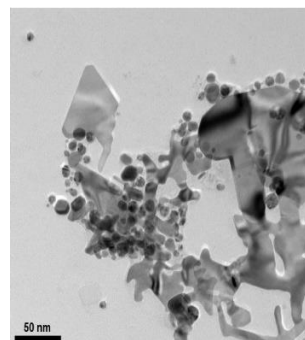
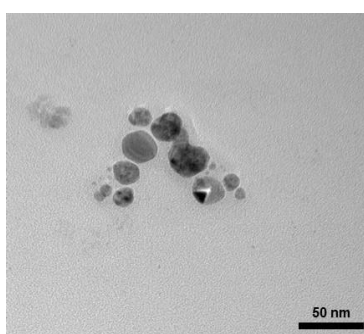




Fig. 2. (c) PEG uncoated NLNPs

Fig. 2. (d) PEG coated NLNPs

### 3.4 Antibacterial activity of silver nanoparticles:

The antimicrobial activity of BNP and NLNP was assessed by the well diffusion method as shown in Fig.6 (a-c), against multi-drug resistant *S. aureus*, *K. pneumoniae* and *E. coli*, the result of which is summarized in Table-1. It was observed that the zone of inhibition in case of non-coated BNP was 22, 21 and 19 mm, while the zone of inhibition in case of NLNP was

20, 18 and 19 mm against *S. aureus*, *K. pneumoniae* and *E. coli* respectively. PEG coated P-BNP and P-NLNP were 16, 14 and 12 mm against *S. aureus*, *K. pneumoniae* and *E. coli*. The results were compared with the standard antibiotic Streptomycin sulphate, with the concentration of 50 µg/ml, which showed the zone of inhibition of 23, 20 and 18 mm against *S. aureus*, *K. pneumoniae* and *E. coli*.

| S. No. | Pyogenic pathogens   | Zone of inhibition (mm) |      |       |        |              |
|--------|----------------------|-------------------------|------|-------|--------|--------------|
|        |                      | BNP                     | NLNP | P-BNP | P-NLNP | Streptomycin |
| 1.     | <i>S. aureus</i>     | 22                      | 20   | 19    | 18     | 22           |
| 2.     | <i>E. coli</i>       | 21                      | 18   | 18    | 18     | 24           |
| 3.     | <i>K. pneumoniae</i> | 19                      | 19   | 20    | 17     | 26           |

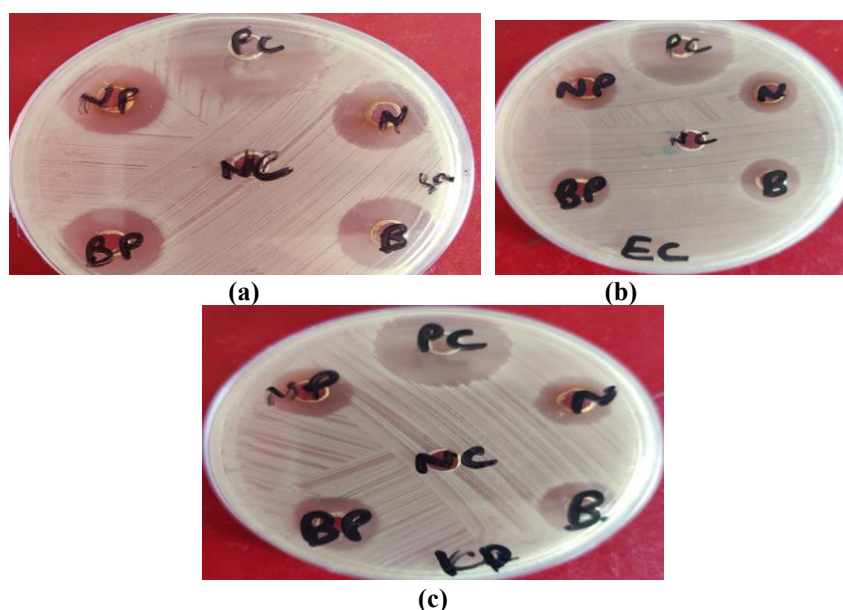


Fig. 6. Antibacterial activity of PEG Coated Bacterial and Neem Leaf Nanoparticles (BP and NP) and uncoated nanoparticles (B and N) against (a) *S. aureus* (Sa) (b) *E. coli* (EC) and (c) *K. pneumoniae* (KP). PC= Positive Control, Streptomycin sulphate (50 µg/ml).

### 3.4.2 Assessment of MIC and MBC:

MIC and MBC values of PEG coated and uncoated BNP and NLNP displayed a strong inhibition of all the pyogenic pathogens at the lowest concentrations as displayed in Table 2 (a) and (b) and Fig. 2 (a) and (b). The uncoated BNP had the MIC values of 1.56 µg/ml against *S. aureus* and 3.12 µg/ml against *E. coli* and *K. pneumoniae* respectively. Uncoated NLNP had the MIC values of 3.12 µg/ml against *S. aureus* and *K. pneumoniae* and 6.25 µg/ml against *E. coli*. PEG coated BNP had a MIC of 12.5 µg/ml against *S. aureus*, while against *E. coli* and *K. pneumoniae* it had a value of 25.0

µg/ml respectively, whereas PEG coated NLNP had a MIC value of 25 µg/ml for *S. aureus* and *E. coli* followed by 50 µg/ml for *K. pneumoniae* respectively.

MBC values obtained for the PEG coated and uncoated BNP and NLNP also suggested the strong antibacterial activity. MBC of uncoated BNP was seen at the concentration of 3.12 µg/ml against *S. aureus* and 6.25 µg/ml against *E. coli* and *K. pneumoniae* respectively. Uncoated NLNP had the MBC values of 6.25 µg/ml against *S. aureus* and *K. pneumoniae* and 12.5 µg/ml against *E. coli*. PEG coated BNP had MBC of 25 µg/ml

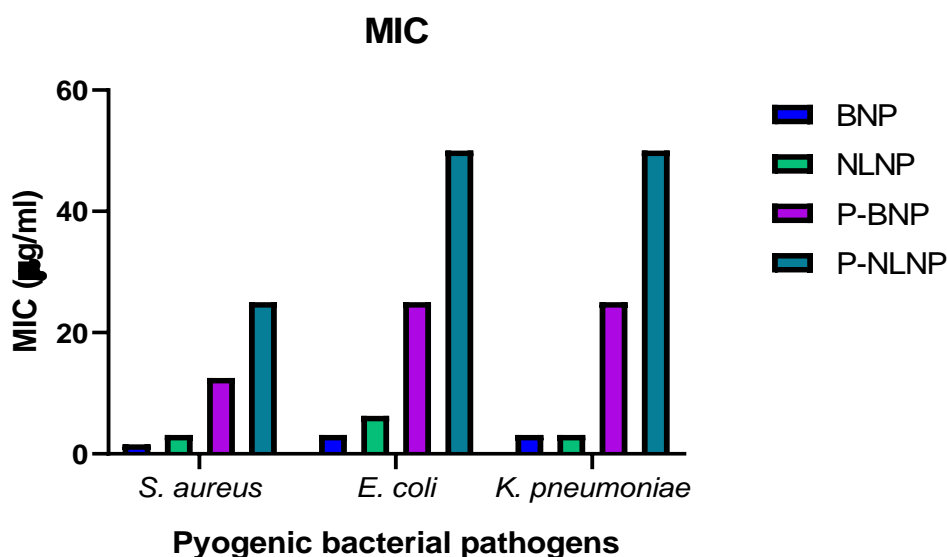


against *S. aureus*, while against *E. coli* and *K. pneumoniae* it had a value of 50 µg/ml respectively, whereas PEG coated NLNP had an MBC value of 25

µg/ml followed by 50 µg/ml for *E. coli* and *K. pneumoniae* respectively.

| S. No. | Pyogenic pathogens   | BNP  | NLNP | P-BNP | P-NLNP |
|--------|----------------------|------|------|-------|--------|
| 4.     | <i>S. aureus</i>     | 1.56 | 3.12 | 12.5  | 25     |
| 5.     | <i>E. coli</i>       | 3.12 | 6.25 | 25    | 50     |
| 6.     | <i>K. pneumoniae</i> | 3.12 | 3.12 | 25    | 50     |

**Table-2 (a):** MIC values of PEG coated and uncoated BNP and NLNP against pyogenic pathogens.

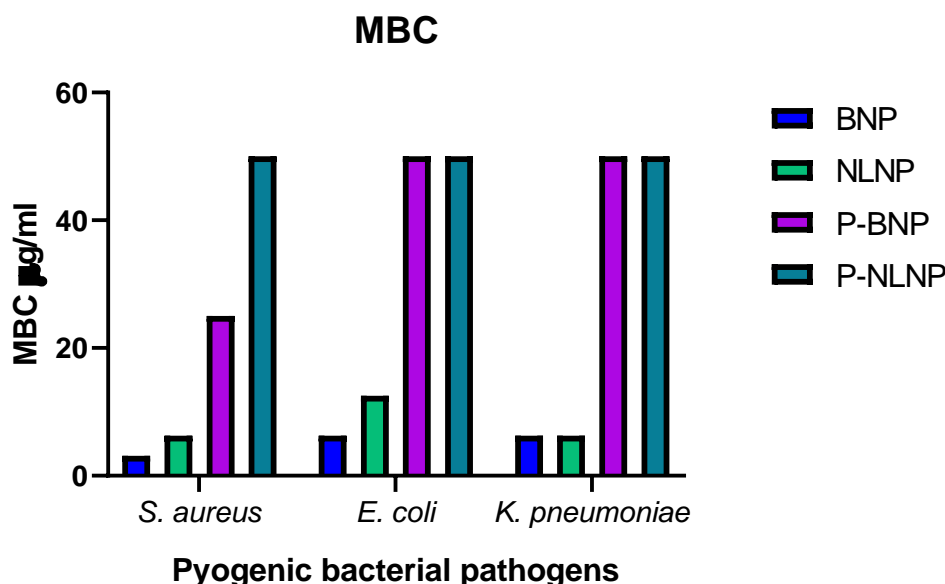


**Fig. 2 (a)** MIC values of PEG coated and uncoated BNP and NLNP against pyogenic bacterial isolates.

| S. No. | Pyogenic pathogens   | BNP  | NLNP | P-BNP | P-NLNP |
|--------|----------------------|------|------|-------|--------|
| 1.     | <i>S. aureus</i>     | 3.12 | 6.25 | 25    | 50     |
| 2.     | <i>E. coli</i>       | 6.25 | 12.5 | 50    | 50     |
| 3.     | <i>K. pneumoniae</i> | 6.25 | 6.25 | 50    | 50     |

**Table-2 (b):** MBC values of PEG coated and uncoated BNP and NLNP against pyogenic pathogens.





**Fig. 2 (b)** MBC values of PEG coated and uncoated BNP and NLNP against pyogenic bacterial isolates.

#### 4 Discussion:

The present study emphasizes on the effect of capping agent Polyethylene glycol on the antibacterial effect of the silver nanoparticles that were synthesized from the extracellular extract of bacteria and plant extracts. These nanoparticles were then tested for their antibacterial activity on the pyogenic multi drug resistant bacteria that were isolated from the oral lesions.

Nanoparticles that are synthesized from the biological sources like bacteria and plant are called as biogenic nanoparticles [38–40]. In the present study the silver nanoparticles were biosynthesized by using *K. pneumoniae* which was isolated from soil sample. This strain is one of the rarely used bacterial strain that has been utilized for the biosynthesis of silver nanoparticles [33,41]. The other source used for the biosynthesis of the silver nanoparticles included was aqueous extract of neem leaves, which has been used in previous studies for the biosynthesis of the silver nanoparticles [32,38,42,43].

Surface plasmon resonance peaks of bacteria derived silver nanoparticles (BNP) and neem leaf derived silver nanoparticles (NLNP) was estimated by UV-Visible spectroscopy. It was observed that the peaks of the BNP was at 422.5 nm which is in accordance with the previous study [44], while in case of neem leaf derived silver nanoparticles (NLNP) the peak was seen at 432 nm which is similar to the result of Avinash et al and Madhav et al [45,46]. In case of PEG coated BNP and NLNP the surface plasmon peaks were found to be in the similar range as that of the uncoated ones, although a change in the absorbance values were seen. For the PEG uncoated BNP and NLNP, the absorbance values were

0.562 and 1.526 respectively. While in case of PEG coated the absorbance value of these nanoparticles increased to 0.86 in case of BNP while the absorbance decreased to 1.498 in case of NLNP. XRD analysis revealed the crystalline morphology of BNP and NLNP. The peaks were observed at 38.0° (1 1 1), 45.4° (2 0 0), 66.2° (2 2 0), and 75.3° (3 1 1) in case of BNPs while in case of NLNPs, the peaks were observed at 38.1° (1 1 1), 44.2° (2 0 0), 64.5° (2 2 0), and 77.4° (3 1 1). In both of the cases of the XRD results of BNP and NLNP the results were found similar to the studies conducted previously [47,48]. Topological analysis of the nanoparticles by TEM revealed the spherical morphology of both PEG coated and uncoated BNP and NLNP.

Assessment of the antibacterial activity of the silver nanoparticles by the well diffusion of technique revealed the potential of the PEG coated and uncoated silver nanoparticles as an alternate to the traditionally used antibacterial agents. The result highlights the potential of the uncoated silver nanoparticles to be more as compared to the PEG coated silver nanoparticles on the multi drug resistant pyogenic isolates. The result thus obtained was similar to the study conducted by Kasim et al. [49]. It was also seen that the PEG coated BNP and NLNP had MIC and MBC values more than the PEG uncoated BNP and NLNP.

#### 5 Conclusion:

The present study highlights the implication of the stabilizing agent Polyethylene glycol or PEG, which is used as the capping agent of nanoparticles and its effect on the antibacterial properties.



Based on the results, it can be concluded that PEG capping can interfere the antibacterial properties and other physiological characteristics of the biologically synthesized silver nanoparticles to some extent. As the decrease in the antibacterial activity of the nanoparticles is minute, PEG coated silver nanoparticles can still be used as an alternative source to the traditional approach to control the ailment causing source.

### 6 Acknowledgements:

The authors would like to thank the Indian Institute of Technology- Delhi and All India Institute of Medical Sciences- New Delhi for providing the technical support and facilities for the identification and characterization of silver nanoparticles. The author would also like to express their kind gratitude to the Principal, Heads and faculty members of Department of Periodontics and Oral Medicine of I.T.S Dental college, Greater Noida, for their assistance in the study.

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