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Extracellular, biosynthesis of silver nanoparticles using *Enterobacter cloacae* (mk163462) and their antibacterial activity against certain multidrug resistant pathogens

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Abstract
In the present investigation, the extracellular biosynthesis of silver nanoparticles using the culture filtrate of silver resistance isolate *Enterobacter cloacae* (MK163462**)** was reported. The biosynthesized silver nanoparticles were characterized by UV-Visible spectrophotometer, FT-IR, SEM and XRD analysis. The AgNPs were spherical, with a size ranged from 12 to 30 nm. Antibacterial activity and synergistic potential of synthesized silver nanoparticles were assessed against certain MDR strains. The silver nanoparticles alone and combination with antibiotics showed greater antibacterial activity against all the MDR strains used in the present study.

Keywords: Extracellular, biosynthesis, silver nanoparticles, *Enterobacter cloacae* (mk163462)

Introduction
Nanotechnology deals with the study of structures at 1-100 nanometers (nm) which have novel properties and functions attributable to their small size (Sharma *et al.,* 2009) [27]. The nanomaterials may give solutions to innovative and environmental challenges in the areas of solar energy generation, medicine and water treatment (Sharma *et al.,* 2009; Vigneshwaran *et al.,* 2009; Tripathy *et al.,* 2009) [27, 32, 30].
Silver is toxic to bacteria as it interferes with metabolism (Bragg & Rainnie 1974, Charley & Bull 1979) [4] and other functions. It forms complexes with membranes, enzymes and nucleic acids (Slawson *et al.,* 1992) [28]. Silver nanoparticles known to have versatile applications as bactericides, fungicidices, household appliances, cleaners, clothing, cutlery, toys and medical equipments (Nowack *et al.*,2010; Reidy *et al.*,2013; Marin *et al.*, 2015) [24, 18].
MDR pathogens could cause severe infections that increase the mortality and morbidity. The emergence of MDR strains in mainly due to improper use of antibiotics. In the current scenario, it is important to search for alternative to extenuate the problems caused by MDR strains. AgNPs have been considered as best alternative to antibiotics due to their several fascinating applications in biomedical fields as antimicrobial agents.
The AgNPs are the powerful agents against the MDR bacteria such as Methicillin-resistant *Staphylococcus aureus*, ampicillin-resistant *Pseudomonas aeruginosa*. (Hanh *et al.*, 2016) [10]. Multidrug-resistant pathogens such as Extended Spectrum Beta-Lactamase (ESBL) producing Gram-negative bacteria *E. coli* resistant to many antibiotics. (Manikprabhu and Lingappa, 2014) [17].
Pseudomonas aeruginosa is highly vulnerable to genetic changes leading to multidrug resistance. They can able to survive in extreme environments and acting as one of the important agents in hospital infections. *Klebsiella pneumoniae* is a Gram-negative bacterial pathogen which causes a large number of infections. Recently, multidrug-resistant strains of *Klebsiella pneumoniae* acquired resistance to majority of the antibiotics (Deshpande and Chopade, 1994). *Staphylococcus aureus* is a natural flora and sometimes with their multidrug resistance phenotype threatens our life as a notorious pathogen (Hiramatsu and Baba, 2014). The occurrence of Methicillin resistant *staphylococcus aureus* MRSA continues to increase as a nosocomial pathogen. The over use of various kinds of antibiotics has led to the emergence of multidrug resistant *Staphylococcus aureus* strains.
Conventional methods of silver nanoparticles synthesis involve the use of highly toxic chemicals, which inturn cause environmental pollution problems. In addition to the cost, the synthesis of silver nanoparticles controlled conditions, capping agents, solvents etc., for silver

nanoparticles synthesis (Gopinath *et al.*, 2012) [9]. Nowadays, microbial synthesis of silver nanoparticles is the most preferred way, because of low cost, nontoxic, ecofriendly approach. Hence, the present investigation aimed to synthesize and characterize silver nanoparticles using the culture filtrate of *Enterobacter cloacae* (MK163462**)** and assess their antimicrobial activity against multidrug resistant pathogens and the synergistic activity with standard antibiotics.

Materials and Methods

Biosynthesis of silver nanoparticles

The silver resistant isolate *Enterobacter cloacae* (MK163462) obtained from jewellery waste contaminated soil was used in the present study. The culture filtrate from *Enterobacter cloacae* was added with filter sterilized $AgNO_3$ solution (1mM). The mixture was incubated on rotating shaker at room temperature for 72 hrs (Das *et al.*, 2014) [31]

Characterization of silver nanoparticles

The silver nanoparticles were characterized by visible color change; UV-visible spectroscopy and Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-ray diffraction methods.

UV- Visible spectroscopy

The formation silver nanoparticles were confirmed by a color change by visual observation and subsequent scan in UV-visible spectra, the wavelengths ranging from 200-800nm (SHIMAGU-UV 1800nm) to check the maximum absorbance (λ max). (Musarrat *et al.*, 2010).

Fourier Transform Infra-Red spectrometer (FT-IR) analysis

FTIR spectroscopy is used to determine the functional group present on the surface of the nanoparticles. These measurements were carried out using AVATAR-330 FTIR spectrophotometer (Thermo Nicolet) range at the 400-4000 at resolution of 4 cm^{-1}.

Scanning Electron Microscope (SEM)

The dried sample mounted on a sample holder and then coated with gold using sputter coater. The sample was kept on copper grid stained with uranyl acetate and lead citrate and was observed under SEM (JEOL-JSM-5610LV) to determine the surface morphology of the AgNPs.

X-Ray Diffraction analysis

Biosynthesized silver nanoparticles were freeze dried and used for X-ray diffraction measurement of AgNPs using Cu-Kα radiation (λ= 1.5406 Å) operated at a voltage of 40 kV and a current of 30 mA. The scanning range of Bragg angles 2θ at a scanning rate of 0.388/min in powder diffractometer (Philips X'Pert Pro X-Ray diffractometer).

Antimicrobial activity of silver nanoparticles against multiple drug resistant pathogens

The antibacterial efficacy of synthesized silver nanoparticles was tested against certain multidrug resistant clinical isolates such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The isolates were obtained from Raja Muthiah Medical College and Research centre, Annamalai University, Tamil Nadu.

MDR Strains Used

S. No	MDR Pathogens	Source	Drug resistance	Drug sensitive
1.	*E. coli*	Urine	Cefepime, Ciprofloxacin, Ceftazidime, Aztreonam, Ceftazidime, Ampicillin, Gentamicin, Meropenem, Cefoxitin, Amikacin, Amoxyclav, Piperacillin and Tazobactam.	Chloramphenicol
2.	*Klebsiella pneumoniae*	Pleural fluid	Imipenem, Cefepmie, Ciprofloxacin, Ceftazidime, Aztreonam, Ampicillin, Gentamicin, Meropenem, Cefoxitin, Amikacin, Amoxyclav, Piperacillin, Tazobactam.	Chloramphenicol
3.	*Pseudomonas aeruginosa*	Pus	Cefepime, Meropenem, Amikacin.	Ciprofloxacin
4.	*Staphylococcus aureus*	Blood	Cefepime, Ciprofloxacin, Linezolid, Clindamycin.	Gentamicin

The bacterial suspension was swabbed on the Muller Hinton Agar (MHA) plates using sterile cotton swab. The sterile disc at 6 mm dimension was impregnated with AgNPs in different concentrations (5µg, 10µg, 15µg, 20µg/ml). The disc impregnated with antibiotics like Chloramphenicol (*E. coli* and *Klebsiella pneumoniae*) Ciprofloxacin (*Pseudomonas aeruginosa*), and Gentamicin (*Staphylococcus aureus*) (5 µg/disc) placed on the plates as a control. These discs were placed in Muller- Hinton Agar plates and incubated for 24 hrs at 37°C. The susceptibility of the pathogens was determined by measuring the diameter of the zone of inhibition using Hi-Media zone scale (Prakash *et al.*, 2013).

Synergistic effect of silver nanoparticles with standard antibiotics against multidrug resistant pathogens

The antibacterial activity of AgNPs in combination with different antibiotics against four MDR bacterial pathogens was studied by following the method described by Patra and Baek (2017) [20]. The bacterial cultures were swabbed on the surface of sterilized Muller Hinton Agar (MHA) plates using sterile cotton swabs. Silver nanoparticles (20µg/ml) were combined with 4 different antibiotic discs such as Vancomycin, chloramphenicol, gentamicin and ciprofloxacin; they were placed at equal distance and incubated at 37˚C for 24 hrs. After incubation, synergistic antibacterial activity of silver nanoparticles + antibiotics was assessed by measuring the zone of inhibition formed around the disc.

Results and Discussion

Enterobacter cloacae are ubiquitous organisms present in terrestrial and aquatic environments. Due to their ubiquitousness, they could grow in metal contaminated soils. In the present study, a silver resistant bacteria *Enterobacter cloacae* derived from jewellery waste contaminated soil was used to reduce Ag+ ions into elemental form. The culture filtrate of *Enterobacter cloacae* (MK163462) supposed to contain extracellular enzymes liberated due to cellular activities might be responsible for the biosynthesis of silver nanoparticles. Silver nanoparticles synthesis was confirmed through the color change of culture filtrate from pale yellow to brown color. Characterization studies viz, UV-Visible spectrophotometer, FTIR, SEM and XRD analysis revealed that the silver nanoparticles were spherical in shape, crystalline with a size range from 12-30 nm. The absorption peak of silver nanoparticles found at 430 nm, this was due to surface plasmon resonance of silver nanoparticles (Fig-1). FT-IR spectra indicated the presence of protein in silver nanoparticles. The study revealed the presence of NH stretching 3303 cm^{-1} secondary amide, the absorption peaks 1366 cm^{-1} and 1041 cm^{-1}, -C-N stretching primary amide 1651 cm^{-1}. The overall observation confirmed the presence of proteins in samples of silver nanoparticles (Fig-2). The SEM image showed the small agglomeration with spherical shape and the size range from 12 to 30 nm (Fig-3).

XRD analysis confirmed the presence of crystalline nature of the silver nanoparticles. The XRD analysis shows four intense peak in the whole spectrum of 2ɵ values 33.28ºC, 46.38 ºC,67.54 ºC and 77.64 ºC corresponding to (111),(200),(220) and (311) respectively (Fig-4).

Similar results are observed by many researchers (Aziz *et al.*, 2016; El-Baghdady *et al.*, 2018; Karthick and Radha 2012; Sudha *et al.*, 2012; Rahimi *et al.*, 2016) [14, 13, 29]. Globally, there is an increasing prevalence of MDR pathogens especially, in the hospital environment infections with multidrug resistant bacteria are a major threat to all fields of medical science as they are hard to treat with antibiotics.

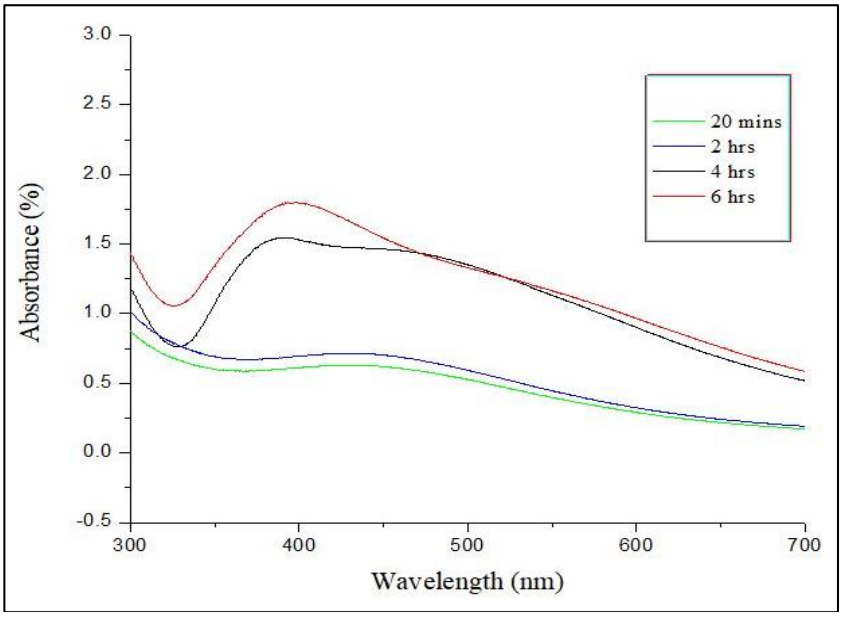
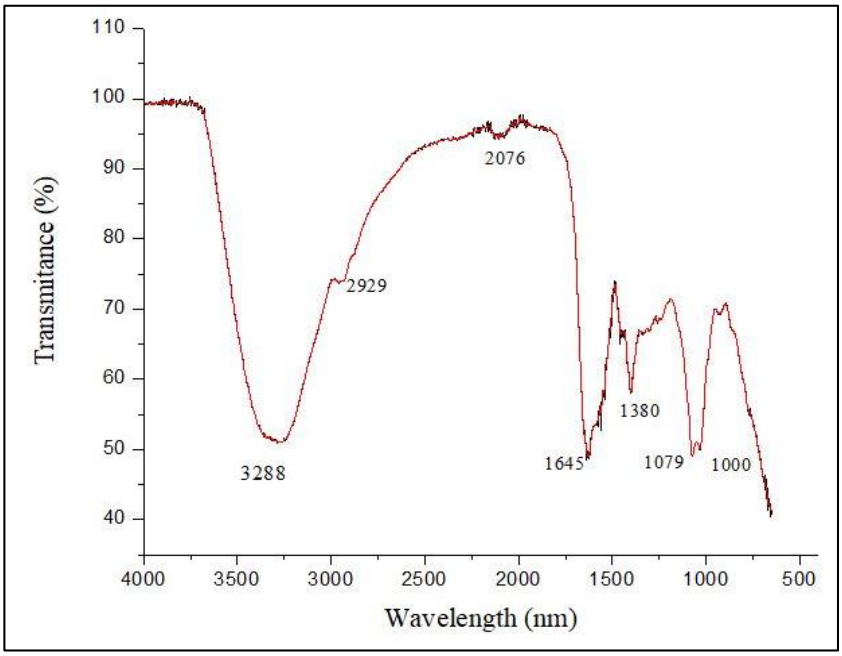
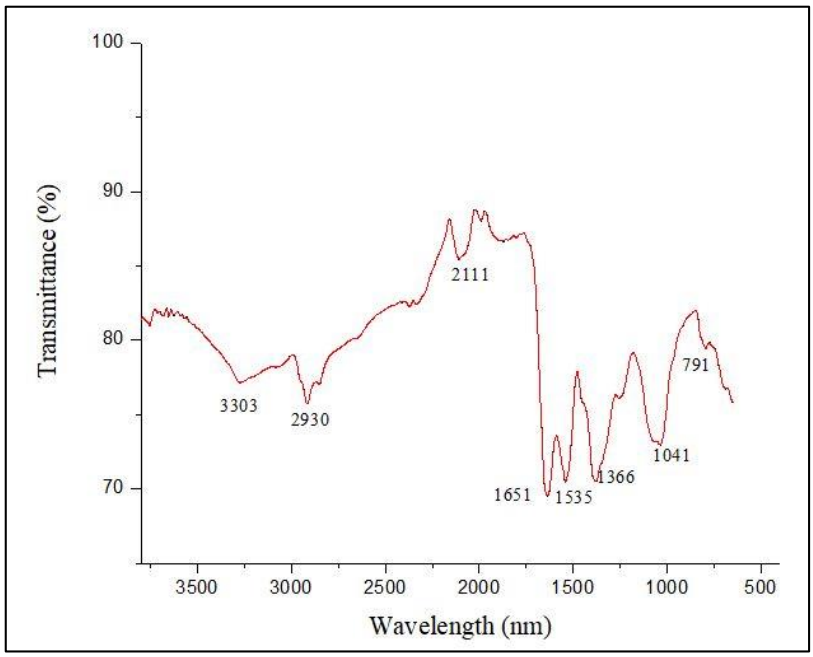


Fig 1: The UV–Vis absorption spectrum of AgNPs synthesized by supernatant of *Enterobacter cloacae*. JWS-1 isolate.



(a)



(b)

Fig 2: The Fourier Transform Infra-Red spectroscopy of (a) Culture supernatant alone; (b) AgNPs. Absorption peaks located at 3288, 2929, 2076, 1645, 1380, 1079 and 1000 cm^{-1} were observed upon culture supernatant, whereas absorption peaks located at 3303, 2930,2111,1651,1535,1366,1041 and 791 cm^{-1} were observed for the AgNPs.

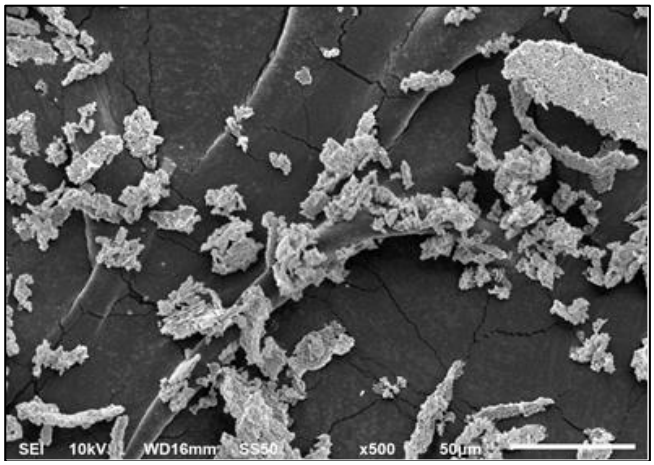


Fig 3: SEM Image indicating the shape, size and form of silver nanoparticles synthesized from *Enterobacter cloacae*

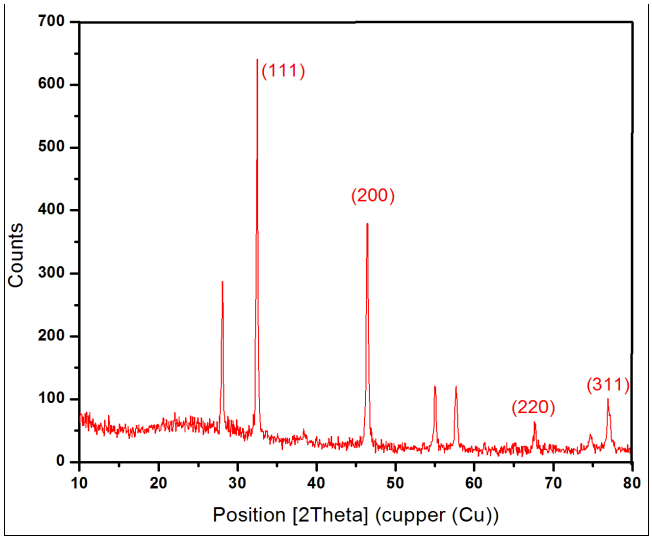


Fig 4: XRD patterns of silver nanoparticles synthesized from *Enterobacter cloacae*.

Rai *et al*., (2012) stated that silver nanoparticles could be best alternative to antibiotics to control infections caused by MDR strains.

In the present study, antibacterial activity of AgNPs against MDR clinical isolates were tested using disc diffusion method and results were depicted in Table-1. The AgNps had highest activity against *E. coli* (13.6±0.5mm) at 20 µg/ml concentration. The *Staphylococcus aureus* showed a mild growth inhibitory effect (10.1±0.1 mm) even at high concentration. Similar results observed by Kim *et al.*, (2007) [15], they reported that silver nanoparticles showed mild growth inhibitory effect against MDR *Staphylococcus aureus* and *E. coli*.

Prakash *et al.* (2013) studied the antibacterial activity of phytosynthesized silver nanoparticles against MDR pathogens namely *Klebsiella pneumoniae*, *Micrococcus luteus* and *Staphylococcus aureus*. The study concluded that maximum antibacterial efficacy observed against gram negative multidrug resistant viz., *Klebsiella pneumoniae* and moderate activity against gram positive multidrug resistant *staphylococcus aureus* and *Micrococcus luteus*.

Amirulhusni *et al.* (2012) [2] demonstrated the antibacterial effect of silver nanoparticles on MDR *Pseudomonas aeruginosa*. The antibacterial activity of silver nanoparticles synthesized from the fungus *Penicillum* was assessed against two MDR clinical isolates. The results revealed that the MDR *E. coli* sowed better growth inhibition at 80µl concentration of silver nanoparticles. The MDR *Staphylococcus aureus* showed a mild growth inhibition when compared with *E. coli* (Datter Singh *et al.*, 2014) [19]. Similar results were observed by many authors (Kim *et al.*, 2007, Ninganagouda *et al.*, 2013) [15].

Singh *et al.*, 2014 [19] studied the antibacterial activity of silver nanoparticles from *Phyllanthus amarus* against 15 clinical isolates of MDR *Pseudomonas aeruginosa*. The silver nanoparticles showed dose dependent inhibitory activity against all the MDR isolates tested.

In our study also, the silver nanoparticles showed dose dependent inhibitory activity against MDR isolate. The interaction of two antimicrobial agents is called as synergistic action (Berenbaum *et al.,* 1978) [3], in which the impact caused by the two drugs in combination is greater than their individual effects (Jawetz, 1968) [12]. The study about synergistic interaction between silver nanoparticles and antibiotics is highly essential to control outbreaks of MDR pathogens (Aziz *et al*., 2016). In the present study, the synergistic effect of *Enterobacter cloacae* synthesized silver nanoparticles with standard antibiotics was studied against MDR pathogens. As expected, there was sustainable increase in the antimicrobial activity was noticed when the antibiotics mixed with biosynthesized silver nanoparticles.

The results obtained for synergistic action of AgNPs with antibiotics were tabulated in the Table-2. The antibiotic chloramphenicol in combination with AgNPs recorded greater inhibition against all the MDR pathogens used in the study. Among the different antibiotics, gentamicin showed antibacterial activity against all the studied bacteria. Chloramphenicol+ 20µg/ml silver nanoparticles yielded maximum zone of inhibition against *E. coli* (32.2±0.5mm) and *K. pneumoniae* (30.1±0.5mm). The *S. aureus* and *E. coli* had acquired resistance against Ciprofloxacin; *E. coli* had showed resistance against Vancomycin**,** whereas *Pseudomonas aeruginosa* had developed resistance against chloramphenicol. But when AgNPs were applied simultaneously with antibiotics all the MDR isolates were inhibited by some extent. Our results can be correlated with the studies of Hwang *et al.,* 2012 [11]. They reported that the synergistic interaction of silver nanoparticles and chloramphenicol effective against *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*.

Fayaz *et al.* (2010) [8] reported that the antibacterial activity of different antibiotics like ampicillin, kanamycin, erythromycin and chloramphenicol increase in the presence of silver nanoparticles from *Trichoderma* sp. against pathogens. According to them, the increase in synergistic activity might be due to bonding reaction between antibiotic and nanosilver. The active groups such as hydroxyl and aminogroups in the antibiotic molecules react easily with nanosilver by chelation. Silver nanoparticles induced reactive oxygen species generation enhances the cell permeability, thereby stimulates the antibiotic action.

Abd-elraby *et al.* (2016) studied the combined effect of silver nanoparticles with six different standard antibiotic discs (Ciprofloxacin, Ampicillin, Streptomycin, Gentamicin, Tetracycline and lincomycin) against MDR bacteria. The results indicated that the antibacterial activity of tested antibiotics increased in the presence of silver nanoparticles

Naqvi *et al.,* (2013) [25] the susceptibility of bacterial isolates increased from 20-35%, when antibiotics used (Imipenem, gentamicin, vancomycin and ciprofloxacin) in conjugation with mycosynthesized silver nanoparticles from *Aspergillus flavus.* The pathogens like *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus* sp. and *Micrococcus luteus* were highly inhibited by the combined effect of silver nanoparticles and antibiotics (Imipenem, gentamicin and ciprofloxacin).

Antibacterial activity of silver nanoparticles against MDR enteric human pathogens were studied by Payal agarval and Nikhilesh kulkarni (2017) [21]. Among the pathogens tested susceptibility of the pathogens *viz salmonella*, *klebsiella* and *E. coli* were increased when silver nanoparticles and antibiotics applied simultaneously.

Table 2: Antimicrobial activity of silver nanoparticles synthesized by the isolate JWS-1 (*Enterobacter cloacae*) against multidrug resistant pathogens.

Multi Drug Resistant Pathogens	Zone of inhibition (mm)				Standard Ciprofloxacin, Chloramphenicol, Gentamycin (5µg/disc)
	Concentrations used (µg/ml)				
	5	10	15	20	
Staphylococcus aureus	-	7.3± 0.5	8.1± 0.5	10.1± 0.1	33.4± 0.4 (Gentamicin)
Klebsiella pneumoniae	7.1± 0.4	8.5± 0.4	10.8± 0.5	12.6± 0.3	31.2±0.7 (Chloramphenicol)
E. coli	7.3± 0.5	9.8± 0.3	10.7± 0.6	13.6± 0.5	16.3±0.4 (Chloramphenicol)
Pseudomonas aeruginosa	7.5± 0.3	8.5± 0.4	10.7± 0.7	11.5± 0.5	27.4± 0.5 (Ciprofloxacin)

Table 2: Synergistic action of AgNPs with standard antibiotics against multidrug resistant pathogens.

Multidrug resistant pathogens	Zone of inhibition (mm)							
	Antibiotics (5 µg/disc)				Antibiotics +AgNPs (5µg + AgNPs 20µg)			
	Vancomycin	Chloramphenicol	Gentamicin	Ciprofloxacin	Vancomycin	Chloramphenicol	Gentamicin	Ciprofloxacin
Staphylococcus aureus	9.6 ± 0.3	18.2 ± 0.5	27.3 ± 0.3	-	11.1 ± 0.5	20.1± 0.5	28.2± 0.5	11.1± 0.5
Klebsiella pneumoniae	15.2 ± 0.3	30.1± 0.5	19.6 ± 0.3	23.3 ± 0.3	17.2 ± 0.3	30.1± 0.5	22.2± 0.5	24.2± 0.3
E. coli	-	31.1 ± 0.5	10.1 ± 0.5	-	12.2 ± 0.5	32.2± 0.5	12.2± 0.5	11.1± 0.5
Pseudomonas aeruginosa	10.1 ± 0.5	-	14.2 ± 0.3	19.6 ± 0.3	13.3± 0.3	11.1± 0.5	16.2± 0.5	23.3± 0.3

He combination of antibiotics and nanoparticles could increase the antibiotics' efficacy against resistant pathogens (Fayaz *et al.,* 2010; Li *et al.,* 2005) [8, 16]. In addition, nanoparticle–antibiotic conjugates lower the in the dosage, which reduces unwanted side effects and increases antimicrobial properties. Furthermore, due to this conjugation, the concentrations of antibiotics were increased at the place of antibiotic–microbe contact and facilitate the binding between microbes and antibiotics (Allahverdiyev *et al.,* 2011) [1]. Synergistic interaction of nanoparticles and chloramphenicol effective against *Enterococcus faecium* and *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*.

Conclusion

In conclusion, the silver resistant isolate *Enterococcus cloacae* (MK163462) has shown to be potential for extracellular synthesis of silver nanoparticles in the size range of 12-30 nm. The extracellular synthesis is advantageous than intracellular from the technical point of view. Since the intracellular synthesis require cell lysis to release silver nanoparticles. In addition, the silver nanoparticles displayed antimicrobial activity against certain MDR strains. The synergistic potential of silver nanoparticles with antibiotics revealed greater microbicidal activity against all the MDR strains. The silver nanoparticles could act as better alternative to antibiotics, which has been confirmed on our study.

References

1. Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: combining nanoparticles with anti- biotics and other antimicrobial agents. Expert Rev Anti Infect Ther, 2011, 91035–1052.
2. Athirah Nur Amirulhusni, Navindra Kumari Palanisamy, Zaini Mohd-Zain, Liew Jian Ping. Antibacterial Effect of Silver Nanoparticles on Multi Drug Resistant *Pseudomonas aeruginosa* International Journal of Medical and Health Sciences. 2012: 6:7.
3. Berenbaum MC. A method for testing for synergy with any number of agents. J Infect Dis. 1978; 137:122-130.
4. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli.* Can J Micro- boil. 1974; 20:883-889.
5. Charley RC, Bul, AT. Bioaccumulation of silver by a multispecies community of bacteria. Arch Microbiol. 1979; 123:239-244.
6. Deshpande LM, Chopade BA. Plasmid-mediated silver resistance in *Acinetobacter baumannii*. Biometals; 1994; 7:49-56.
7. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine. 2010; 6:103-109.
8. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against Gram positive and Gram-negative bacteria. Nanomedicine. 2010; 6:103-109.
9. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: a novel biological approach. Colloids Surf. B. 2012; 96:69-74.
10. Hanh TT, Phu DV, Thu NT, Quoc LA, Duyen DNB, Hien NQ. Gamma irradiation of cotton fabrics in $AgNO_3$ solution for preparation of antibacterial fabrics. Carbohydr. Polym. 2014; 101:1243-1248.
11. Hwang IS, Lee J, Hwang JH, Kim KJ, Lee DG. Silver nanoparticles induce apoptotic cell death in Candida albicans through the increase of hydroxyl radicals. FEBS J. 2012; 279:1327–1338.
12. Jawetz E. The use of combinations of antimicrobial drugs, Annual Review of Pharmacology. 1968; 8:151-170,
13. Karthik C, Radha KV. Biosynthesis and characterization of silver nanoparticles using *Enterobacter aerogenes*: a kinetic approach digest. Journal of nanomaterials and biostructures. 2012; 7:1007-1014.
14. Khaled Z, El-Baghdady Einas H, El-shatoury Omnia M, Abdullah Mostafa MH, Khalil. Biogenic production of silver nanoparticles by enterobacter *cloacae* ism26. turk j boil. 2018; 42:319-328.
15. Kim JS, Kuk E, Yu *et al*. Antimicrobial effects of silver nanoparticles, Nanomedicine. 2007; 31:95-101.
16. Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects of b-lactam antibiotic combined with silver nanoparticles. Nanotechnology. 2005; 16:1912-1917.
17. Manikprabhu D, Lingappa K. Synthesis of silver nanoparticles using the *Streptomyces coelicolor* klmp 33 pigment: An antimicrobial agent against ex-tended-spectrum beta-lactamase (ESBL) producing Escherichia. Mater. Sci. Eng. 2014; 45:434-437.
18. Marin S, *et al*. Applications and toxicity of silver nanoparticles: A recent review. Current Topics in Medicinal Chemistry. 2015; 15:1596-1604.
19. Ninganagouda S, Rathod S, Jyoti H, Singh D, Prema D, Haq MU. Int. J Pharm. Bio. Sci. 2013; 4:222.
20. Patra JK, Baek KH. Antibacterial Activity and Synergistic Antibacterial Potential of Biosynthesized Silver Nanoparticles against Foodborne Pathogenic Bacteria along with its Anticandidal and Antioxidant Effects. Front. Microbiol. 2017; 8:167.
21. Payal N, Agrawal Nikhilesh S, Kulkarni. Biosynthesis of Silver Nanoparticles from Silver Resistance Bacteria Isolated From Metal Contaminated Soil. Sch. Acad. J Biosci. 2017; 5:187-191.
22. R Du Abd-Elnaby H, Abo-Elala G, Abdel-Raouf U, Abdelwahab A, Hamed M. Antibacterial and anticancer activity of marine *Streptomyces parvus*: optimization and application. Biotechnol. Equip. 2016; 30:180-191.
23. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 2009; 27:76-83.
24. Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. Mat. 2013; 6:2295-2350.
25. SMM Raza Naqvi. Impact of Corporate Social responsibility on Brand image in Different FMCGs of Pakistan. Ijcrb. 2013; 5:1.
26. Sahar Rahim, Nadeem Javaid, Ashfaq Ahmad Shahid, Ahmed Khan Zahoor, AliKhan Nabil Alrajeh, Umar Qasim. Exploiting heuristic algorithms to efficiently utilize energy management controllers with renewable energy sources. Energy and Buildings. 2016.
27. Sharma VK, Yngard RA, Lin Y. Silver Nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science. 2009; 145:83-96.
28. Slawson RM, Trevors JT, Lee H. Silver accumulation and resistance in *Pseudomonas stutzeri*. Arch Microbiol. 1992; 158:398-404.
29. Sudha SS, Rajamanickam K, Rengaramanujam J. Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. Indian J Exp Biol. 2013; 52:393-399.
30. Tripathy A, Raichur AM, Chandrasekaran N, Prathna TC, Mukherjee A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta*

indica (Neem) leaves. Journal Nanoparticles Research, 2009.
31. Vidhya lakshmi Das, Roshmi Thomas, Rintu T, Varghese E, Soniya V, Jyothis Mathew, *et al*. Extracellular synthesis of silver nanoparticles by the *Bacillus* strain cs 11 isolated from industrialized area. 3 Biotech 2014; 4:121-126.
32. Vigneshwaran N, Nachane RP, Vardarajan PV, Balasubramanya RH. A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohydrate research. 2009; 341:2012-2018.