ORIGINAL RESEARCH PAPER

Volume - 12 | Issue - 05 | May - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SYNERGISTIC EFFECT OF SILVER NANOPARTICLES AND DORIPENEM AGAINST CARBAPENEM RESISTANT ESBL PRODUCERS

Medical Microbiology

Pradnya Jadhav* Bharati Vidyapeeth (Deemed To be University) Medical College and Hospital, Sangli. *Corresponding Author

Shubhangi Gadgil Bharati Vidyapeeth (Deemed To be University) Medical College and Hospital, Sangli.

Shilpa Shah Bharati Vidyapeeth (Deemed To be University) Medical College and Hospital, Sangli.

ABSTRACT

Background: The indiscriminate use of carbapenem against ESBL producers resulted in developing resistance to carbapenems. To overcome this resistance, the use of silver nanoparticles (AgNPs) is of great interest. AgNPs have high antibacterial activity and also, they enhance the antibacterial activity of other antibiotics. So, this study aimed to evaluate the synergistic effect of AgNPs with doripenem against carbapenem resistant ESBL producers. **Aim and objectives:** To evaluate the synergistic effect of silver nanoparticles and doripenem against carbapenem resistant ESBL producers. **Materials and methods:** AgNPs were synthesized from *Pseudomonas aeruginosa* ATCC 27853. Characterization of AgNPs was done by visual inspection, UV spectroscopy and scanning electron microscopy. ESBL and CRE detection were done according to CLSI guidelines. Synergistic effect of AgNPs with doripenem against CRE was evaluated by Checkerboard assay. **Results:** The mixture containing *Pseudomonas aeruginosa* and $AgNO_3$, turned pale yellow to dark brown, after the incubation period. Maximum absorption of UV rays was seen at 423 nm. The mean size of AgNPs was 26.187 ± 9.109 nm and the shape was spherical as seen under scanning electron microscope. Among 575 isolated ESBL producers, 24% were CRE and *Klebsiella oxytoca* was the most predominant isolate (33.33 %). Combination of doripenem and AgNPs showed the synergistic effect for all the tested CRE isolates with FIC index < 0.5. **Conclusion:** *Pseudomonas aeruginosa* potentially reduces $AgNO_3$ to form AgNPs. These biosynthesised AgNPs enhanced the antibacterial activity of doripenem against CRE (synergistic effect). In conclusion, carbapenem-AgNPs combination can be used in near future to treat bacterial infections caused by carbapenem resistant ESBL producers.

KEYWORDS

Pseudomonas aeruginosa, AgNPs, doripenem, synergistic effect.

INTRODUCTION:

The emergence of multi-drug resistant organisms is due to the irrational use of various antibiotics. One of the mechanisms for this, is the production of Extended Spectrum beta lactamases (ESBLs).[1] For the treatment of infections caused by ESBL producers, carbapenems are used and the indiscriminate use of carbapenems leads to carbapenem resistance. Recently, the incidence of carbapenem resistance among Enterobacteriaceae is increasing.[2]

Carbapenem Resistant Enterobacteriaceae (CRE) can be defined as resistance to one or all of the carbapenems including imipenem, meropenem, ertapenem or doripenem; and resistant to all third generation cephalosporins including Ceftriaxone, Cefotaxime and Ceftazidime.[3]

When the carbapenems are used in combination with other compounds, they can play a major role to treat CRE infections.[4] Recently, nanotechnology plays an important role to overcome the antibacterial resistance. Silver nanoparticles (AgNPs) can be alternative to conventional antibiotics [5] due to high antibacterial activity and they can enhance the antibacterial activity of other antibiotics (synergy) by lowering the MIC of an antibiotic combined with AgNPs.[6]

So, the synergistic effect of AgNPs with doripenem against CRE was studied to evaluate the change in MIC of doripenem when it is used in combination with silver nanoparticles.

MATERIALS AND METHODS:

An experimental study was carried out in the Department of Microbiology, BVDU, MCH, Sangli from January 2020 to June 2022. This study was approved by Institutional Ethical Committee with the reference number BV(DU)/MCH/2146/20-21.

AgNPs were synthesized from *Pseudomonas aeruginosa* ATCC 27853 under the optimized physicochemical parameters (silver nitrate concentration- 0.4 g/L pH- 9, incubation temperature- 70 ^{0}C and incubation time- 96 hours).[7,8,9,10] Characterization of AgNPs were done by visual inspection, UV spectroscopy and Scanning electron microscopy (SEM).

ESBL and CRE detection in gram negative bacilli from different clinical specimens was done according to CLSI guidelines.[11] Synergistic effect of AgNPs with doripenem against CRE was studied by Checkerboard assay.[12] In this method, 96 wells microtiter plate was used. 2-fold serial dilutions of AgNPs and doripenem were done. Column 1 contained serial dilution of doripenem alone, while row A contained a serial dilution of AgNPs alone. These controls were used to determine the MIC for each test compound. This experiment was done in triplicates and the average was considered for calculation of FIC index. To calculate the FIC index, the following equation was used:

$$\frac{A}{MIC_A} + \frac{B}{MIC_B} = FIC_A + FIC_B = \text{FIC Index}$$

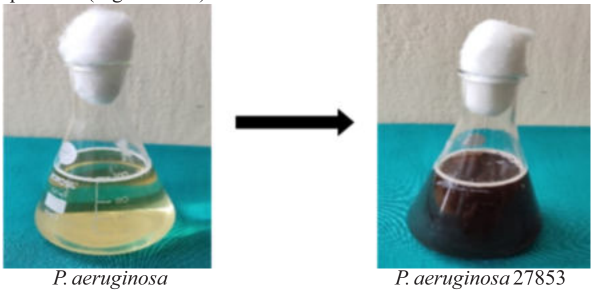
where A and B are the MIC of AgNPs and doripenem in combination, and MIC_A and MIC_B are the MIC of AgNPs and doripenem individually. When the combination of compounds results in FIC value of <0.5, it is synergistic effect. When the FIC value is in between 0.5 – 4, it is additive or indifference effect and when the FIC value is >4, the effect is antagonistic.

RESULTS:

The mixture containing *Pseudomonas aeruginosa* ATCC 27853 and $AgNO_3$ solution, turned yellow to brown, after the incubation period. As shown in FigureNo:1.

In the present study, the instrument used for UV spectroscopy was UV visible spectrophotometer (Agilent technologies, Cary 60). The maximum absorption of UV rays was seen at 423 nm. (Figure No: 2).

The shape and size of AgNPs was studied by Scanning Electron Microscope (SEM), Model: JEOL JSM – 6360, JAPAN. The mean size of biosynthesized AgNPs was 26.187 ± 9.109 nm and the shape was spherical. (Figure No: 3)



P. aeruginosa ATCC27853 + AgNO and   *P. aeruginosa* 27853 + AgNO after incubation

Figure No: 1 showing formation of AgNPs

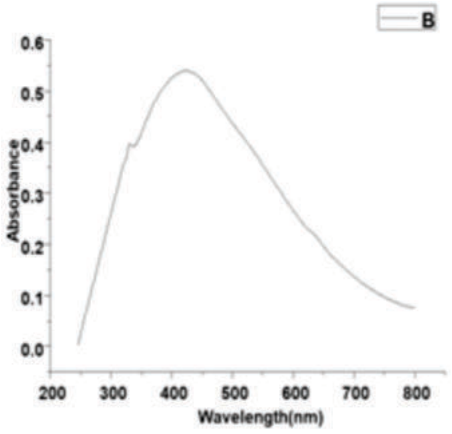


Figure No: 2 Showing Absorbance Peak At 423 nm under UV Vis Spectroscopy

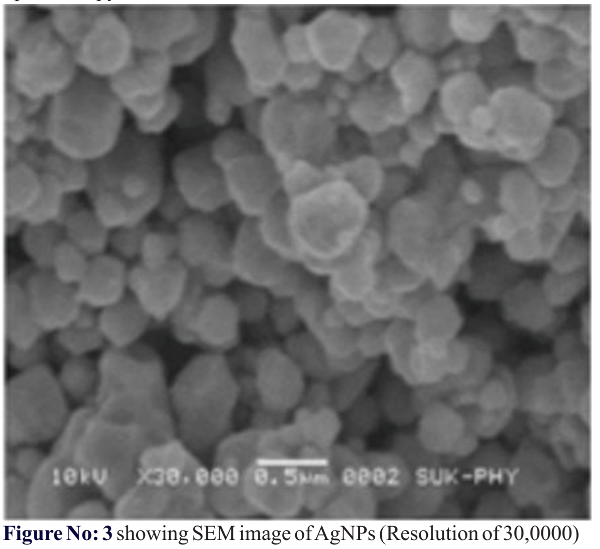


Figure No: 3 showing SEM image of AgNPs (Resolution of 30,0000)

Among 575 isolated ESBL producers, 138 (24%) were resistant to doripenem (Table No: 1). *Klebsiella oxytoca* was the most predominant isolate (33.33 %) followed by *Klebsiella pneumoniae* (32.18 %) and *E.coli* (19.32 %).

Table No: 1 Microbiological Profile Of Carbapenem Resistant ESBL Producers

Isolate	No of ESBL producer (n= 575)	No of carbapenem resistant ESBL producer (n= 138)	Percentage (%)
E.coli	295	57	19.32
Klebsiella pneumoniae	233	75	32.18
Proteus mirabilis	29	0	0
Klebsiella oxytoca	18	6	33.33
Total	575	138	24

In checkerboard assay, the MIC of doripenem was found less when combined with AgNPs. i.e., MIC changed from >4 to <1 (resistant to sensitive) with FIC value ranges from 0.10 to 0.22 (Figure No:4, Table No: 2). Furthermore, combination of doripenem and AgNPs showed the synergistic effect for all the tested CRE isolates with FIC index < 0.5.

Table no: 2 FIC Indices Of Carbapenem Resistant ESBL Producers (checkerboard Assay)

Isolate	FIC Index (FIC dor+FIC AgNPs)	Combinational effect (combine FIC Index)
Klebsiella pneumoniae (N=75)	0.22 ± 0.07	<0.5 = synergy
E.coli (N=57)	0.17 ± 0.09	<0.5 = synergy
Klebsiella oxytoca (N=6)	0.10 ± 0.05	<0.5 = synergy
Proteus mirabilis (N=0)	-	-

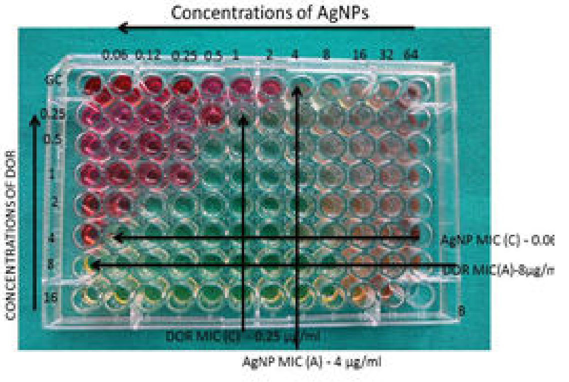


Figure No: 4 Showing Checkerboard Assay

DISCUSSION:

Previous research studies, [13, 14] stated that NADH and NADH-dependent nitrate reductase enzyme secreted by *Pseudomonas aeruginosa,* acts as the reducing agent of silver ions and subsequent formation of AgNPs.

In the present study, the maximum absorption of UV rays was seen at 423 nm confirming the biosynthesis of AgNPs. Similar to our result, in some studies [13, 15] the absorption peak of UV rays was seen at 420-430 nm with a good nanoparticle dispersion without aggregation.

In our study, 26.187 ± 9.109 nm was the mean size of AgNPs according to SEM and the shape was spherical. These results are in accordance with the other previous studies. [7, 8] They have found the spherical shaped AgNPs synthesized from *Pseudomonas aeruginosa*, and the size varies from 20-50 nm.

A total of 575 ESBL producers were isolated in the present study from different clinical specimens (Table No: 1). Among these, 24% were carbapenem resistant ESBL producers. The prevalence of CRE in India ranges from 13-51 %.[2] According to various studies,[2, 16, 17] prevalence of CRE was 24.2%, 12.26% and 71.25%, respectively. Our results are in accordance with the results of some studies [2, 18] where *Klebsiella pneumoniae* was the predominant isolate followed by *E.coli* and *Proteus spp.* According to some studies, [16, 19] *E.coli* was more in number (60%) followed by *Klebsiella pneumoniae* (33%).

The prevalence of CRE varies due to various factors like irrational use of antibiotics, their dosing regimens and the different levels of hospital infection control practices.[2,3]

Carbapenem resistance is due to production of enzyme carbapenemases which hydrolyze all penicillins, cephalosporines, monobactams and carbapenems. The other mechanisms for carbapenem resistance are over production of ESBLs and over expression of efflux pumps.[2,3]

In the present study (Table No:2), the MIC of doripenem alone and in combination with the AgNPs against CRE was calculated by Checkerboard assay and it is found that MIC of doripenem was less when combined with AgNPs. i.e., MIC changed from >4 to <1 (resistant to sensitive) with FIC value ranges from 0.10 to 0.22. Furthermore, combination of doripenem and AgNPs showed the synergistic effect for all the CRE isolates with FIC index < 0.5.

Our results are in accordance with the studies, [20,21] which demonstrated the synergistic effect of AgNPs in combination with imipenem against gram negative bacteria. Another study [22] showed, the MIC of imipenem against *Klebsiella pneumoniae* was 32 µg/ml and when imipenem was combined with AgNPs, a synergistic effect was observed at a concentration 1 µg/ml with FIC index 0.07.

This synergistic effect is due to the bonding reaction between antibiotic and AgNPs. The active groups of an antibiotic molecule like hydroxyl and amido group reacts easily with AgNPs by chelation.[20, 21]

Again, imipenem causes the cell wall lysis of bacteria which leads to increase in AgNPs penetration into the cells.[20]

AgNPs can be used in combination with the carbapenem antibiotics against CRE. Such a combinational therapy should be further studied to form new combinations of AgNPs in synergy with other antibiotics.

CONCLUSION:

Along with demonstrating antibacterial activity, biosynthesized AgNPs are capable of increasing antibacterial activity of carbapenem antibiotic through synergism. Furthermore, antibacterial compounds have lower MICs when used in combination, which reduces their harmful effects. In general, the results indicate that the use of carbapenem- AgNPs combination can treat bacterial infections caused by carbapenem resistant ESBL producers.

REFERENCES:

[1] Lavanya Segar, Shailesh Kumar, Noyal Mara Joseph, Umadevi Sivaraman. Prevalence of Extended spectrum beta lactamases among Enterobacteriaceae and their antibiogram pattern from various clinical samples. Asian journal of Pharmaceutical and clinical research. 2015; vol 8; (5).
[2] Sukanya Verma, Suyasha Thorat, Kishor Bhisure. Prevalence of carbapenem resistant Enterobacteriaceae- A study in a tertiary care hospital in Mumbai. Journal of Evolution Medical and dental sciences. 2018: vol 7; 45; 4909-4912.
[3] Pravin K. Nair, Michelle S. vaz. Prevalence of Carbapenem Resistant Enterobacteriaceae from a tertiary care hospital in Mumbai. Journal of Microbiology and infectious diseases. 2013; 3 (4): 207-210.
[4] Chau-chyan sheu, Ya- Ting Chang, Shang-Yi Lin, Yen- Hsu Chen. Infections caused by carbapenem Enterobacteriaceae: An update on therapeutic options. Frontiers- Microbiology. 2019: Vol 10
[5] Mahsa Shahbandeh, Anoosh Eghdami, Mehrdad Moosazadeh Moghaddam, Mohammadreza Jalali Jadoushan, Ali salimi, Mahdi Fasihi-ramandi. Conjugation of imipenem to silver nanoparticles for enhancement of its antibacterial activity against multidrugresistant isolates of Pseudomonas aeruginosa. J Biosci (2021) 46:26
[6] Maha Abd El Fattah K, Nanis Gamal A, Fatma Ibrahim S. Investigation of the efficacy of synthesized silver and zinc oxide nanoparticles against multidrug resistant gram negative bacterial clinical isolates. Archives of clinical Microbiology. 2017: vol.8 (5):67
[7] M.M.K. Peiris, S.S.N. Fernando, P.M. Jayaweera, N.D. H. Arachchi, T.D.C.P. Guansekara. Comparison of antimicrobial properties of silver nanoparticles synthesized from selected bacteria. Indian journal of microbiology. 2018: 58 (3); 301-311.
[8] Mudra K Peiris, Chinthika P Gunasekara, Pradeep Jayaweera, Nuwan Dharachchi. Biosynthesized silver nanoparticles: are they effective antimicrobials? Mem Inst Oswaldo Cruz, Rio DE Janeiro. 2017;112(8):537-543
[9] Anand Kumar Keshari, Ragini Shrivastava, Payal Singh, Virendra Yadav. Antioxidant and antibacterial activity of silver nanoparticles synthesized by cestrum nocturnum. Journal of ayurveda and integrative medicine. 2020: 11; 37-44
[10] Soumya Soman, J. G. Ray. Silver nanoparticles synthesized using leaf extract of ziziphus oenoplia (l.) mill: Characterization and assessment of antibacterial activity.journal of photochemistry and photobiology, b: biology2016: 163; 391-402.
[11] CLSI. 2018. Performance standards for antimicrobial susceptibility testing; 28 Th informational supplement. CLSI document M100. Wayne, PA: clinical and laboratory standards institute.
[12] Lorian 5th edition, Chapter 9. Antimicrobial Combinations, in Antibiotics In Laboratory Medicine, 2005; pp. 365-441. Lippincott Williams and Wilkins, Philadelphia, PA.
[13] Jinmei Yanga, Qiang Wangb , Chunxiao Wangc , Ruiyi Yanga , Mukhtar Ahmedd , Subramanian Kumarane , Periyannan Veluf and Bo Lic. Pseudomonas aeruginosa synthesized silver nanoparticles inhibit cell proliferation and induce ROS mediated apoptosis in thyroid cancer cell line (TPC1). 2019; Artificial cells, nanomedicine, and biotechnology; vol. 48, no. 1, 800–809
[14] Bahareh Khodashenas, Hamid Reza Ghorbani. Optimization of nitrate reductase enzyme activity to synthesize silver nanoparticles. IET Nanobiotechnology.2015; pp. 1-4
[15] Hina Singh, Juan Du, Priyanka Singh, Tae Hoo Yi. Extracellular synthesis of silver nanoparticles by Pseudomonas spp. THG-LS1.4 and their antimicrobial application. Journal of pharmaceutical analysis. 2018; (8); 258-264
[16] Nair PK, Vaz MS. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. J of Microbiology & Infectious Diseases (JMID) 2013;3(4):207-10.
[17] Kumar S, Bahdauria S. Leading trend of carbapenem resistance in enterobacteriaceae in India. Int J Basic Appl Med Res 2014;4(2):205-8.
[18] Chirag Modi, Suman Singh, Yagnesh Pandya, Chirag Patel, Rupal Patel. Prevalence of carbapenem resistant Enterobacteriaceae in a tertiary care hospital of Gujrat, India. JCDR. 2021;47332.
[19] Nagaraj S, Chandran SP, Shamanna P, et al. Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. Indian J Med Microbiol 2012;30(1):93-5.
[20] S. Hendiani, A. Ahya Abdi, P. Mohammadi, Sh. Kharazi. Synthesis of silver nanoparticles and its synergistic effects in combination with imipenem and two biocides against biofilm producing *Acinetobacter baumannii*. Journal of Nanomedicine. 2015. 2(3): 291-298.
[21] Sulaiman A. Al Yousuf. In vitro bactericidal and Imipenem synergistic effect of nano-silver against multiple drug resistant *Pseudomonas aeruginosa.* Journal of King saud university-Sciences. 2022: 34;101706.
[22] Marwa M. Abdel-Aziz, Mohamed Yosri, Basma H. Amin. Control of Imipenem Resistant – *Klebsiella pneumoniae* pulmonary infection by oral treatment using a combination of mycosynthesized Ag-nanoparticels and Imipenem. Journal of radiation research and applied sciences.2017; (10);353-360.