

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2017.602.089>**Antibacterial Activity of Biogenic Platinum Nanoparticles: An *invitro* Study**

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The aim of this study was to screen the antibacterial activity of biogenic platinum nanoparticles. During this study platinum nanoparticles prepared from marine actinobacteria (*Streptomyces* sp.) were used to access antibacterial activity against *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus*, coagulase negative *Staphylococcus* sp. and *Pseudomonas aeruginosa*. During this study, biogenic platinum nanoparticles exhibited broad spectrum antibacterial activity against coagulase negative *Staphylococcus* sp. (20.33±1.52), followed by *Proteus vulgaris* (17.66±2.08), *Staphylococcus aureus* (16.33±1.52), *E. cloacae* (14.00±2.64) and *E. coli* (12.33±1.52), while poor antibacterial activity against *P. aeruginosa* (08.33±0.57) and *K. pneumoniae* (07.66±1.52).

Introduction

A nanoparticle is small molecules or objects that behaves as a whole unit in terms of its transport and properties. Nanoparticles have one dimension that measures 100 nanometers or less. In recent past, nanotechnology has emerged as a prominent field of science which involves production, development and application of various nanomaterials. Currently a variety of metallic and non-metallic nanoparticles such as gold, alginate, silver, copper, zinc, titanium, magnesium, platinum and silica etc., are emerged. These nanoparticles are used in various fields such as optical devices (Anderson and Moskovits, 2006), catalytic (Jiang *et al.*, 2005), bactericidal (Sekar *et al.*, 2011), antibiofouling (Agarwal *et al.*, 2014), dye degradation (Priyragini *et al.*, 2014), electronic (Rao *et al.*, 2000), sensor technology

(Sharpe and Andreescu, 2015), treatment of some cancers (Praetorius and Mandal, 2007) and malaria (Karthik *et al.*, 2013). This wide range of application justifies the research and development inputs required in the field of nanotechnology.

Nanoparticles are commonly synthesized using several physical and chemical methods (Mohanraj and Chen, 2006), however the production cost, man power requirements and their non-eco-friendly nature pose a great concern. Therefore scientists have develop the methods of biosynthesis of nanoparticles, which allows the rapid, low cost, non-toxic, eco-friendly approach to synthesized nanoparticles. Most recently, biosynthesis of nanoparticles using plants (Ravichandran *et al.*, 2016) and microorganisms (Veena *et al.*,

2016) have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain nanomaterials.

Actinobacteria are a group of gram positive, fungi like bacteria, represents a valuable source of industrially important metabolites. The actinobacteria isolated from marine atmosphere are much more valuable, as they can grow in stress conditions by producing certain chemicals. In addition to metabolites, in recent past marine actinobacteria have been reported to synthesize various nanoparticles viz, silver (Prakasham *et al.*, 2012) and gold (Karthik *et al.*, 2013) etc. these marine actinobacteria are usually nontoxic, and does not produce any harm to humans and animals, thus providing a safe way for the synthesis of nanoparticles.

Platinum is a very important rare metal present on earth. Platinum can be reduced to nanoparticles using various physical, chemical and biological methods; however these nanoparticles may vary in their shape and size depending upon the method of synthesis and production conditions. In recent past, platinum nanoparticles have been extensively studied for their antioxidant properties (Kim *et al.*, 2008). Platinum nanoparticles contain possible applications in the development of fuel cells and for developing hydrogen storage materials (Wen *et al.*, 2008; Li *et al.*, 2007). They can be used as coating material, and for the development of nanofibers and polymer membranes. Therefore this study is designed to study the antibacterial activity of biogenic platinum nanoparticle.

Materials and Methods

Biosynthesis of platinum nanoparticles

Platinum nanoparticles were previously biologically synthesized using marine

actinobacteria *Streptomyces* sp. isolated from soil sediments samples collected from coastal areas of Chennai, India, while chloroplatinic acid hexahydrate was used as substrate. These nanoparticles were characterized using, UV-visible spectrophotometer, FTIR Spectroscopy, XRD and TEM analysis (Data not shown).

Antimicrobial activity of the platinum nanoparticles

Test organisms

Escherichia coli, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus*, *Coagulase negative Staphylococcus* sp. and *Pseudomonas aeruginosa*. Bacterial cultures were obtained from Dhanwantri Hospital and Research Centre, Jaipur, Rajasthan, India. All bacterial cultures were maintained on nutrient agar medium and stored at 4°C. For antibiogram and antibacterial activity testing, organisms were inoculated in MHB and incubated overnight at 37°C to make a uniform suspension.

Antibiogram

All the clinical isolates of bacteria were screened for their sensitivity towards standard antibiotics. Antibiotics included Amikacin (30 µg/disc), Amoxicillin+clavulanic acid (30 µg/disc), Ampicillin+sulbactam (10 +10 µg/disc), Aztreonam (30 µg/disc), Cefepime (30 µg/disc), Cefuroxime (30 µg/disc), Cefpodoxime (10 µg/disc), Ciprofloxacin (30 µg/disc), Colistin (10 µg/disc), Cloxacillin (05 µg/disc), Clarithromycin (15 µg/disc), Doxycycline (30 µg/disc), Gentamycin (10 µg/disc), Levofloxacin (05 µg/disc), Linezolid (30 µg/disc), Moxifloxacin (05 µg/disc), Meropenem (10µg/disc), Ofloxacin (05µg/disc), Piperacillin +tazobactam (30 µg/disc), Polymixin-B (10 µg/disc),

Norfloxacin (10 µg/disc) and VA: Vancomycin (30 µg/disc). Drug sensitivity test was performed by disc diffusion method on Muller Hinton agar (MHA) plates. Bacterial isolates were inoculated in to nutrient broth for 8 hours. The concentration of the suspensions was adjusted to 0.5 using a spectrophotometer. Isolates were seeded on Mueller Hinton agar plates by using sterilize cotton swabs. The standard antibiotic discs were placed on the agar surface using a sterilize forceps. Plates were incubated at 37°C for 48 hours. Plates were observed for zone of inhibition. The experiment was performed in triplicates (Iqbal *et al.*, 2004).

Antibacterial assay

Antimicrobial activity of the biologically synthesized platinum nanoparticles was checked by agar well diffusion method on MHA plates. The concentrations of both suspensions were adjusted to 0.5 using a spectrophotometer and were lawn cultured on MHA plates by using sterilised cotton swabs. In each of these plates, three wells were cut out using a standard cork borer (7 mm diameter). Using a micropipette, 100 µl of chloroplatinic acid hexahydrate solution (100µg/ml), 100µl of platinum nanoparticle (100µg/ml) and 100µl of distilled water was added to separate wells. Plates were incubated for 24 hours at 37°C. Anti-bacterial activity was evaluated by measuring the zone of inhibition. Experiment was performed in triplicates (Kumar *et al.*, 2010a).

Determination of relative percentage inhibition

The relative percentage inhibition of the biologically synthesized platinum nanoparticles with respect to positive control was calculated by using the following formula (Kumar *et al.*, 2010). Relative percentage inhibition of the biologically synthesized platinum nanoparticles = $[100 \times (x-y)] / (z-y)$

Where,

x: total area of inhibition of the biologically synthesized platinum nanoparticles

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using $\text{area} = \pi r^2$; where, r = radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

MIC of the biologically synthesized platinum nanoparticles was performed by modified agar well diffusion method. Sample was diluted in sterilized distilled water to make a concentration range from 0.01-10 mg/ml. Test cultures were inoculated in MHB and seeded on MHA plates using sterilized cotton swabs. In each of these plates four wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100 µl of each dilution was added in to wells. Plates were incubated at 37°C for 24 hours and fungal plates were incubated at 28°C for 72 the results were recorded. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC (Okunji *et al.*, 1990; Rios *et al.*, 1988).

Statistical analysis

The results of the antimicrobial activity of biologically synthesized platinum nanoparticles are expressed as mean \pm standard deviation of the response of 3 replicates determinations per sample. Results were analyzed by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

Antibiogram study

Antibiogram studies provides a systemic information about the drug resistant pattern of microorganisms, therefore it can be used as an important tool to access the drug resistance in

microorganism and to implement corrective actions to outcome this. During this study, *E. coli*, *E. cloacae*, *K. pneumoniae*, *Proteus vulgaris*, *S. aureus*, coagulase negative *Staphylococcus* sp. and *P. aeruginosa* were subjected to antibiogram study. Among these bacterial cultures, except *E. coli* all exhibited multiple drug resistance toward commonly clinical drugs, while *P. aeruginosa* exhibited maximum resistance (Table 1).

Antibacterial activity of biologically synthesize platinum nanoparticles

In this study, biologically synthesize platinum nanoparticles was screened for antibacterial activity against *E. coli*, *E. cloacae*, *K. pneumoniae*, *Proteus vulgaris*, *S. aureus*, coagulase negative *Staphylococcus* sp. and *P. aeruginosa* isolated from the clinical samples. Biologically synthesized platinum nanoparticles exhibited highest antibacterial activity against coagulase negative *Staphylococcus* sp. (20.33 ± 1.52), followed by *Proteus vulgaris* (17.66 ± 2.08), *Staphylococcus aureus* (16.33 ± 1.52), *E. cloacae* (14.00 ± 2.64) and *E. coli* (12.33 ± 1.52), while poor antibacterial activity against *P. aeruginosa* (08.33 ± 0.57) and *K. pneumoniae* (07.66 ± 1.52) (Table 2).

During this study, antimicrobial activity of biologically synthesize platinum nanoparticles was compared with the antimicrobial activity of standard drugs for evaluating relative percentage inhibition (Table 3). The biologically synthesize platinum nanoparticles exhibited maximum relative percentage inhibition against coagulase negative *Staphylococcus* sp. (88.09%) followed by *P. vulgaris* (77.96%), *S. aureus* (71.36%), *E. cloacae* (41.77%), *E. coli* (40.68%), *P.*

aeruginosa (30.71%) and *K. pneumoniae* (12.67%). MIC values of the biologically synthesize platinum nanoparticles against bacterial strains were range between 6 and 50 µg/ml. Result of MIC are reported in Table 4.

Bacterial infections are one of the major causes of health problems around the globe. Microbes results in significant number of deaths worldwide. According to World Health Organization (WHO), microbial infections collectively resulted in approximately 20-25% of death worldwide (WHO, 1999). Applications of antibiotics have significantly reduced the number of death rate however failed to stop it, in addition, incidence of drug resistance among the microbes are reported worldwide (Suller and Russell, 2000; Poole, 2005).

Contagious research for the development of antimicrobial compounds can help to counter this problem. In recent past nanoparticles have shown promising results to inhibit microorganisms and drug resistant microorganisms. Several studies conducted around the globe are reporting the antimicrobial properties of various nanoparticles such as silver, gold and zinc against a broad range of nanoparticles (Salem *et al.*, 2015; Zhang *et al.*, 2015; Thomas *et al.*, 2014). However the activity of the particles can variety based on the method they are prepared and because of particle size. Therefore in this study, biogenic platinum nanoparticles exhibited a broad spectrum antibacterial activity. The results of this study are in agreement with previous studies where platinum nanoparticles have been reported to exhibit strong antimicrobial activity (Elhusseiny and Hassan, 2013; Rajathi and Nambaru, 2014).

Table.1 Antibigram study of bacterial isolates

Test organisms	Antibiotics used						
	EC	ECI	KP	PV	SA	CNSA	PA
AK	S	S	S	S	S	S	S
AMC	S	S	I	R	R	I	R
A/S	S	S	R	I	R	S	R
AZM	S	S	R	R	S	S	R
AT	S	I	R	R	n.a.	n.a.	R
CPM	S	R	I	R	S	R	I
CXM	I	S	R	R	R	I	R
CPD	I	S	R	R	R	R	R
CPZ	S	S	S	R	S	S	R
CAZ	S	S	S	R	R	R	R
COT	I	S	R	R	S	S	R
CIP	S	S	S	I	R	S	R
CL	S	S	S	S	n.a.	n.a.	S
COX	n.a.	n.a.	n.a.	n.a.	I	S	n.a.
CLR	n.a.	n.a.	n.a.	n.a.	R	S	n.a.
DO	S	S	I	R	S	S	S
GNM	S	S	S	I	R	R	R
LE	S	S	S	R	S	I	R
LZ	n.a.	n.a.	n.a.	n.a.	S	S	n.a.
MO	S	R	S	I	R	S	R
MRP	S	S	S	S	S	S	S
OF	S	R	I	R	R	S	R
PIT	S	S	S	S	S	S	I
PB	S	S	S	S	n.a.	n.a.	S
NX	I	S	S	R	R	S	R
TEI	n.a.	n.a.	n.a.	n.a.	S	S	n.a.
VA	n.a.	n.a.	n.a.	n.a.	S	S	n.a.

Here, S: Sensitive, I: Intermediate, R: Resistant, n.a.: not applied,
 EC: *Escherichia coli*, ECI: *Enterobacter cloacae*, KP: *Klebsiella pneumoniae*, PV: *Proteus vulgaris*, SA: *Staphylococcus aureus*, CNSA: coagulase negative *Staphylococcus sp.*, PA: *Pseudomonas aeruginosa*
 AK: Amikacin, Amc: Amoxicillin+clavulonic acid, AS: Ampicillin +sulbactam, AT: Aztreonam, CPM: Cefepime, CXM: Cefuroxime, CPD: Cefpodoxime, CIP: Ciprofloxacin, CL: Colistin, COX: Cloxacillin, CLR: Clarithromycin, DO: Doxycycline, GNM: Gentamycin, LE: Levofloxacin, LZ: Linezolid, MO: Moxifloxacin, MRP: Meropenem, OF: Ofloxacin, PIP: Piperacillin+tazobactam, PB: Polymixin-B, NX: Norfloxacin and VA: Vancomycin.

Table.2 Antibacterial activity of biologically synthesized platinum nanoparticles

Test organism	Zone of inhibition (mm)		
	Pt NPs	PC	NC
<i>Escherichia coli</i>	12.33±1.52	19.33±1.52	0.0±0.0
<i>Enterobacter cloacae</i>	14.00±2.64	21.66±0.57	0.0±0.0
<i>Klebsiella pneumoniae</i>	07.66±1.52	19.66±0.57	0.0±0.0
<i>Proteus vulgaris</i>	17.66±2.08	20.00±1.00	0.0±0.0
<i>Staphylococcus aureus</i>	16.33±1.52	19.33±1.52	0.0±0.0
<i>Coagulase negative Staphylococcus sp.</i>	20.33±1.52	21.66±.577	0.0±0.0
<i>Pseudomonas aeruginosa</i>	08.33±0.57	18.33±1.52	0.0±0.0

Here, PC: positive control, NC: negative control

Values are expressed as mean ± standard deviation of the three replicates,

Zone of inhibition not include the diameter of the well.

Table.3 Relative percentage inhibitions of biologically synthesized platinum nanoparticles

Test organism	RPI (%)
<i>Escheriachia coli</i>	40.68
<i>Enterobacter cloacae</i>	41.77
<i>Klebsiella pneumonia</i>	12.67
<i>Proteus vulgaris</i>	77.96
<i>Staphylococcus aureus</i>	71.36
<i>Coagulase negative Staphylococcus sp.</i>	88.09
<i>Pseudomonas aeruginosa</i>	20.71

RPI: Relative percentage inhibition

Table.4 Minimum inhibitory concentrations of biologically synthesized platinum nanoparticles

Test organism	MIC (µg/ml)
<i>Escheriachia coli</i>	10
<i>Enterobacter cloacae</i>	8
<i>Klebsiella pneumonia</i>	20
<i>Proteus vulgaris</i>	6
<i>Staphylococcus aureus</i>	20
<i>Coagulase negative Staphylococcus sp.</i>	10
<i>Pseudomonas aeruginosa</i>	50

MIC: Minimum inhibitory concentration

In conclusion, in this study biogenic platinum nanoparticles exhibited a significant antimicrobial activity against a broad range of bacterial cultures. It could be concluded that marine actinobacteria can effectively produce platinum nanoparticles with broad spectrum antibacterial properties which could be used to develop new antimicrobial drugs.

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References

- Agarwal, A., Mehra, A., Karthik, L., Kumar, G., Rao, K.V.B. 2014. Antibiofouling property of marine actinobacteria and its mediated nanoparticle. *Int. J. Nanoparticles*, 7(3-4): 294-306.
- Anderson, D.J., Moskovits, M. 2006. A SERS-active system based on silver nanoparticles tethered to a deposited silver film. *J. Phys. Chem. B.*, 110(28): 13722-13727.
- Elhousseiny, A.F., Hassan, H.H. 2013. Antimicrobial and antitumor activity of platinum and palladium complexes of novel spherical aramides nanoparticles containing flexibilizing linkages: structure–property relationship. *Spectrochimica Acta Part A: Mol. Biomol. Spectroscopy*, 103: 232-245.
- Iqbal, M., Ali, K.M., Daraz, B., Siddique, U. 2004. Bacteriology of mastitic milk and *in vitro* antibiogram of the isolates. *Pak. Vet. J.*, 24(4): 161-164.
- Jiang, Z.J., Liu, C.Y., Sun, L.W. 2005. Catalytic properties of silver nanoparticles supported on silica spheres. *J. Phys. Chem. B.*, 109(5): 1730-17305.
- Karthik, L., Kumar, G., Keswani, T., Bhattacharyya, A., Reddy, B.P., Rao, K.V.B. 2013. Marine Actinobacterial mediated Gold nanoparticles synthesis and their antimalarial activity. *Nanomed.*, 9(7): 951-960.
- Kim, J., Takahashi, M., Shimizu, T., Shirasawa, T., Kajita, M., Kanayama, A., Miyamoto, Y. 2008. Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of *Caenorhabditis elegans*. *Mech. Ageing Dev.*, 129(6): 322-331.
- Kumar, G., Karthik, L., Rao, K.V.B. 2010a. Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – an *in vitro* study. *Int. J. Pharmaceutical Sci. Rev. Res.*, 4(2): 141-144.
- Kumar, G., Karthik, L., Rao, K.V.B. 2010b. *In vitro* anti-Candida activity of *Calotropis gigantea* against clinical isolates of *Candida*. *J. Pharmacy Res.*, 3(3): 539-542.
- Li, Y., Yang, R.T., Liu, C.J., Wang, Z. 2007. Hydrogen storage on carbon doped with platinum nanoparticles using plasma reduction. *Ind. Eng. Chem. Res.*, 46: 8277-8281.
- Mohanraj, V.J., Chen, Y. 2006. Nanoparticles – A Review. *Trop. J. Pharmaceutical Res.*, 5(1): 561-573.
- Okunji, C.O., Okeke, C.N., Gugnani, H.C., Iwu, M.M. 1990. An antifungal saponin from fruit pulp of *Dracaena mannii*. *Int. J. Crude Drug Res.*, 28(3): 193-199.
- Poole, K. 2005. Aminoglycoside Resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, 49(2): 479-487.
- Praetorius, N.P., Mandal, T.K. 2007. Engineered nanoparticles in cancer therapy. *Recent Pat. Drug Deliv. Formul.*, 1(1): 37-51.
- Prakasham, R.S., Buddana, S.K., Yannam, S.K., Guntuku, G.S. 2012. Characterization of silver nanoparticles synthesized by using marine isolate *Streptomyces albidoflavus*. *J. Microbiol. Biotechnol.*, 22(5): 614-621.
- Priyragini, S., Veena, S., Swetha, D., Karthik, L., Kumar, G., Rao, K.V.B. 2014. Evaluating the effectiveness of marine actinobacterial extract and its mediated titanium dioxide nanoparticle in the degradation of azo dyes. *J. Environ. Sci.*, 26(4): 775-782.
- Rajathi, F.A.A., Nambaru, V.R.M.S. 2014. Phytofabrication of nano-crystalline platinum particles by leaves of *Cerbera manghas* and its antibacterial efficacy. *Int. J. Pharm. Bio Sci.*, 5(1): 619-628.
- Rao, C.N.R., Kulkarni, G.U., Thomas, P.J.,

- Edwards, P.P. 2000. Metal nanoparticles and their assemblies. *Chem. Soc. Rev.*, 29: 27-35.
- Ravichandran, S., Paluri, V., Kumar, G., Karthik, L., Rao, K.V.B. 2016. A novel approach for the biosynthesis of silver oxide nanoparticles using aqueous leaf extract of *Callistemon lanceolatus* (Myrtaceae) and their therapeutic potential. *J. Experimental Nanosci.*, 11(6): 445-458.
- Rios, J.L., Recio, M.C., Vilar, A. 1990. Screening methods for natural products with antimicrobial activity: A review of literature. *J. Ethnopharmacol.*, 23(2-3): 127-149.
- Salem, W., Leitner, D.R., Zingl, F.G., Schratte, G., Prassl, R., Goessler, W., Reidl, J., Schild, S. 2015. Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. *Int. J. Med. Microbiol.*, 305(1): 85-95.
- Sekar, D.K., Karthik, L., Kumar, G., Rao, K.V.B. 2011. Biosynthesis of silver nanoparticles from marine yeast and their antimicrobial activity against multidrug resistant pathogens. *Pharmacol. online Newslett.*, 3: 1100-1111.
- Sharpe, E., Andreescu, S. 2015. Portable nanoparticle based sensors for antioxidant analysis. *Methods Mol. Biol.*, 1208: 221-231.
- Suller, M.T., Russell, A.D. 2000. Triclosan and antibiotic resistance in *Staphylococcus aureus*. *J. Antimicrob. Chemother.*, 46(1):11-18.
- Thomas, R., Janardhanan, A., Varghese, R.T., Soniya, E.V., Mathew, J., Radhakrishnan, E.K. 2014. Antibacterial properties of silver nanoparticles synthesized by marine *Ochrobactrum* sp. *Brazilian J. Microbiol.*, 45(4):1221-1227.
- Veena, S., Swetha, D., Karthik, L., Kumar, G., Rao, K.V.B. 2016. Antibiofouling activity of marine actinobacterial mediated titanium dioxide nanoparticles. *Indian J. Geo-Marine Sci.*, 45(4): 583-590.
- Wen, Z., Liu, j., Li, J. 2008. Core/shell Pt/C nanoparticles embedded in mesoporous carbon as a methanol-tolerant cathode catalyst in direct methanol fuel cells. *Adv. Materials*, 20(4): 743-747.
- WHO. Leading Cause of death. 1999.
- Zhang, Y., Dasari, T.P.S., Deng, H., Yu, H. 2015. Antimicrobial activity of gold nanoparticles and ionic gold. *J. Environ. Sci. Health, Part C*. 33(3): 286-327.

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