



## Combating antibiotic resistance in gram-negative bacteria through synergistic effect of silica nanoparticles and antibiotics.

1. M.Phil (Biotechnology)  
International Islamic University  
Islamabad, Pakistan.
2. MBBS, M.Phil  
Assistant Professor Biochemistry  
HITEC-IMS Texila Pakistan.
3. Ph.D  
Associate Professor Biological  
Science  
Faculty of Basic and Applied  
Sciences  
International Islamic University  
Islamabad, Pakistan.
4. BDS, M.Phil  
Lecturer Biochemistry  
HITEC-IMS Texila Pakistan.
5. Ph.D  
Assistant Professor Bioscience  
COMSATS Islamabad Pakistan.
6. Ph.D  
Lecturer  
Women College, University,  
Faisalabad.

### Correspondences Address:

Dr. Lubna Khurshheed  
International Islamic University  
Islamabad, Pakistan.  
lubna.mughal109@gmail.com

### Article received on:

07/08/2020

### Accepted for publication:

18/11/2020

## INTRODUCTION

The present-day increase of multidrug-resistant (MDR) and pan-drug-resistant pathogens poses major challenges in the management of infections.<sup>1</sup> Global statistics show a growing resistance among UTI pathogens to conventional drugs. Resistance has occurred even to more advanced antimicrobial agents.<sup>2</sup> There are almost 150 million urinary tract infections annually worldwide.<sup>3</sup> There is an abundant incidence of multidrug resistance to Gram-negative pathogens *Pseudomonas aeruginosa* (*P. aeruginosa*) and *E. coli* which are the common nosocomial pathogens globally.<sup>4,5</sup>

Nanotechnology-based combination therapy is becoming more common, as it produces synergistic anticancer and antimicrobial effect.<sup>6,7,8</sup> Inorganic mesoporous silica nanoparticles (SiNPs)<sup>9</sup> are proving to be exceptional among other nanoparticles<sup>10</sup>, their great loading ability, appropriateness for an easy functionalization,

**Lubna Khurshheed<sup>1</sup>, Raheela Yasmin<sup>2</sup>, Naveeda Riaz<sup>3</sup>, Rabbia Shabbir<sup>4</sup>, Kaleem Imdad<sup>5</sup>, Seemab Iqbal<sup>6</sup>**

**ABSTRACT... Objective:** Multidrug-resistant and pan-drug-resistant pathogens pose major challenges in the management of infections. Nanotechnology-based combination therapy is becoming more common, as it produces a synergistic antimicrobial effect. **Study Design:** Cross Sectional Study. **Setting:** Institute of Biogenetic Engineering (IBGE) Islamabad. **Period:** September 2015 to October 2017. **Material & Methods:** Silica nanoparticles were prepared by three different modifications using general Stober Method and the synthesized silica nanoparticles SiNPs were named as S1, S2 and S3. The synergism of Ciprofloxacin and Piperacillin-Tazobactam with a combination of silica nanoparticles was evaluated in LB grown culture to determine the sensitivity of *Escherichia coli* and *Pseudomonas aeruginosa*. **Results:** The combined application of Ciprofloxacin and S1, S2 and S3 respectively retarded the growth of *P. aeruginosa* almost completely whereas *E. coli* showed minimal growth inhibition. Collective therapy of Piperacillin-Tazobactam with S1, S2 and S3 inhibits the normal growth pattern of both *E. coli*, *P. aeruginosa* as compared to the control. **Conclusion:** Combined application of silica nanoparticles and antibiotics inhibited the growth of MDR gram-negative bacteria in vitro.

**Key words:** Antibiotic Resistance, Gram-Negative Bacteria, Silica Nanoparticles.

**Article Citation:** Khurshheed L, Yasmin R, Riaz N, Shabbir R, Imdad K, Iqbal S. Combating antibiotic resistance in gram-negative bacteria through synergistic effect of silica nanoparticles and antibiotics. Professional Med J 2021; 28(7):1282-1287. <https://doi.org/10.29309/TPMJ/2021.28.09.6199>

controllable particle size and shape and biocompatibility, have made them perfect therapeutic nano-carriers.<sup>10-13</sup> Nanoparticles owing to their greater surface area can disrupt microbial membrane and move in into the cells without difficulty (Xiu et al., 2012). Some nanoparticles can produce antimicrobial effects through free radicals which they generate.<sup>14</sup> These particles can revive the bactericidal activity of the antibiotics by making a complex with  $\beta$ -lactam antibiotics, such as penicillin.<sup>15</sup>

This article focuses on the innovative synthesis of completely mono-dispersed SiNPs and evaluation of their efficiency against gram-negative pathogens when combined with antibiotics.

## MATERIAL & METHODS

This cross sectional study was conducted at Institute of Biogenetic Engineering, Islamabad from 2015 to 2017.

### **Antibacterial activity against MDR bacteria**

MDR strains of E.Coli and P.auginosae were obtained from clinical isolates of patients affected from UTIs and ear infection respectively from Microbiology Lab of the tertiary care hospitals in Islamabad.

### **Antibiotics Sensitivity Profiling**

The antibiotics susceptibility patterns of the ESBLs producing E.coli and P.auginosae were determined by the disc diffusion method on Muller Hinton Agar (MHA) plates according to the CLSI guidelines 2013. The isolates were cultured on MHA plates by using a sterilized loop. Antibiotics discs of different concentrations were placed on the surface of these plates that have been inoculated by ESBLs producing E.coli and P.auginosae. The plates were incubated overnight at 37°C.

### **Synthesis of Silica Nanoparticles**

Silica nanoparticles were prepared by three different modifications using general Stober Method and the synthesized SiNPs were named as S1, S2 and S3.

Tetraethyl orthosilicate (TEOS 98%), Cetyltrimethyl-ammonium bromide (CTAB 99%), Ammonium hydroxide solution (NH<sub>3</sub> 32%), Hydrochloric acid solution (HCL 38%), Absolute Ethanol 99%.

**S1 synthesis:** In this method, a small amount of CTAB(0.3gm) was added in 100ml of absolute ethanol and 8ml TEOS solution followed by NH<sub>3</sub>(10 $\mu$ l). To ensure complete mixing the solution was kept for 15mins stirring at high speed. The further reaction proceeded in a drying oven at 100°C temp for 24hours.

**S2 synthesis:** TEOS added in ethanol by (8:100) ratio. Then few drops of NH<sub>3</sub> were added in solution to maintain pH by 7.5 and reaction was proceeded at 60°C and was kept stirring for two hours. The solution further proceeded to centrifugation for 15mins at maximum speed followed by 3times washing with HCL(0.5ml) and absolute ethanol solution. The obtained mixture was then added in ethanol and distilled water

solution, initially stirred for 2hours at 60°C, and then dried at 100°C by keeping it in drying oven for 24 hours.

**S3 synthesis:** TEOS was added to double solvent of Ethanol (8:200 ratios) and distilled water solution. NH<sub>3</sub> was added to maintain its pH and kept stirring for 30mins at 60°C followed by drying in an oven at 100°C for 24hours. All three dried samples were carefully collected, stored and characterized.

### **Drug-Nanoparticles Synergistic Antibacterial Assay**

Colonies of different Pathogens including E.coli and P.auginosa were cultured in LB to check the biological activity of SiNPs, antibiotics and combination of both against these pathogenic ESBLs producing strains. Two quinolone antibiotic Ciprofloxacin (CIP) and beta-lactam antibiotics Piperacillin-Tazobactam (TZP) combined with SiNPs to check the synergistic effect in LB. The bacteria showed sensitivity and resistance towards these antibiotics respectively.

### **Preparation of Culture Media**

This includes Bactotryptone (3gm), Yeast (1.5gm), NaCl (3gm) (OXOID, UK). All the ingredients were mixed in 200ml distilled water and kept for one-hour continuous stirring then NaOH was added to maintain its pH at 7.5 and after that 300ml volume was adjusted followed by autoclave.

### **Bacterial Growth Assessment in L.B**

The L.B culture of the strain was detected for its resistance towards the drug and nanoparticles combination. A serial dilution of 20 mg per ml SiNPs stock was used with 2 mg drug per ml against 100 $\mu$ l of strain in 5ml L.B culture. The synergism of beta-lactam antibiotics with a combination of silica nanoparticles was evaluated to see the growth kinetics of the ESBL strain. The strains of ESBL E.coli. and P. aeruginosae was cultured in the L.B media with the test compound and incubated at 37 °C. The O.Ds at 600nm was recorded for each sample of silica nanoparticles conjugate with the drug at an interval of two hours using Nanodrop (Thermo 2000C). The readings were recorded at 2, 4, 6 and 24 hrs.

**RESULTS**

**Antibiotics sensitivity profiling against MDR bacteria**

25, 000 Col/ml of *Escherichia coli* was isolated from clinical samples of Urine infection and heavy growth of *Pseudomonas aeruginosa* was isolated from ear swab. Susceptibility and resistance of both strains were determined against different antibiotics.

**Bacterial growth assessment in LB**

The synergism of fluoroquinolones ciprofloxacin beta-lactam antibiotics Piperacillin-Tazobactam with a combination of silica nanoparticles (S1, S2 and S3) was evaluated in LB grown culture to determine the sensitivity of *Escherichia coli* and *pseudomonas aeruginosa*. A serial dilution of 20 mg per ml SiNPs stock was used with 2 mg drug per ml against 100 µl of strain in 5 ml L.B culture.

**Ciprofloxacin and SiNPs Synergistic activity**

S1, S2 and S3 alone did not show any potential activity to inhibit bacterial growth of both the strains but the combined therapy of Ciprofloxacin and S1, S2 and S3 respectively retarded the growth of *P. aeruginosa* almost absolutely whereas *E. coli* showed minimal growth inhibition. The growth pattern for both strains is shown in Figure-1 and Figure-2 respectively.

**Piperacillin-Tazobactam and SiNPs Synergistic activity**

S1, S2 and S3 alone did not show any potential activity to inhibit the bacterial growth but combined therapy of Piperacillin-Tazobactam with S1, S2 and S3 inhibit the normal growth pattern of *E. coli* as compared to the control. (Figure-3)

Similarly, growth of *Paeruginosae* was retarded with this combination therapy as described in Figure-4.

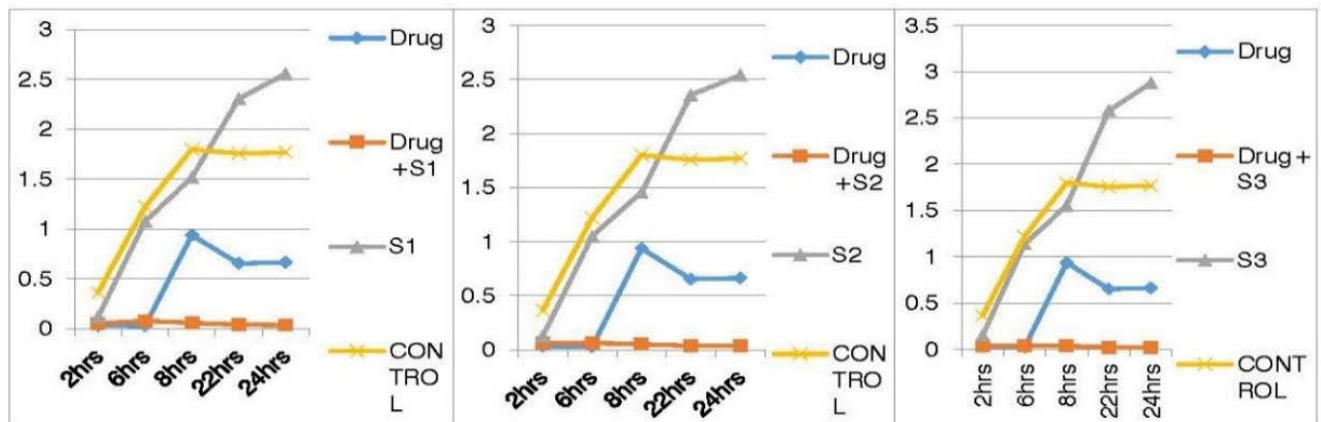


Figure-1. Growth curve of MDR *P.aeruginosae* against the combination of S1, S2, S3 and Ciprofloxacin respectively in 24 hours.

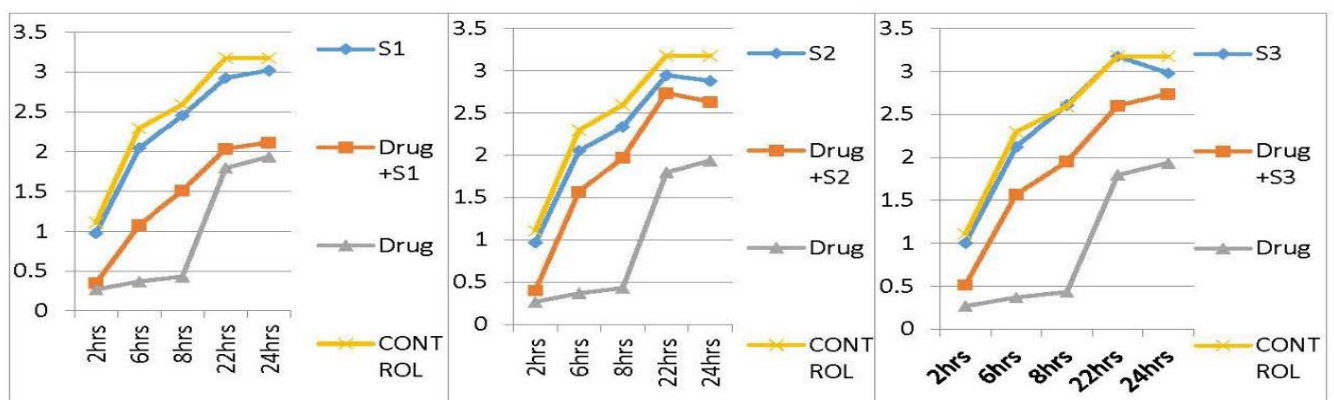


Figure-2. Growth curve of ESBL *E.coli* against the combination of S1, S2, S3 and Ciprofloxacin respectively in 24 hours.

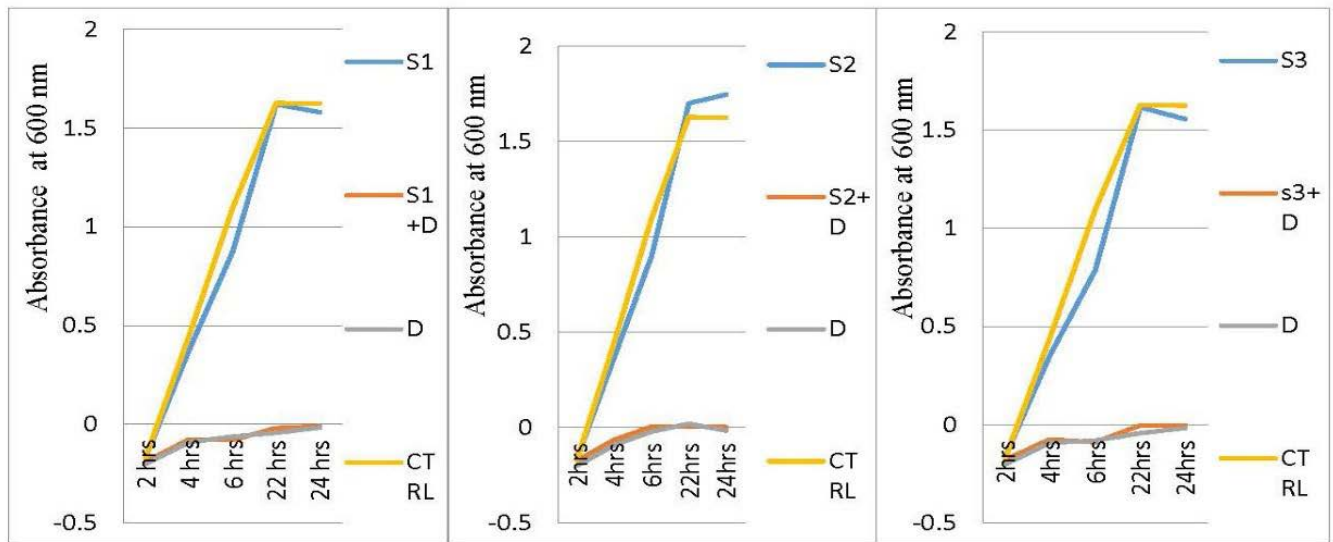


Figure-3. Growth curve of ESBL *E. coli* against the combination of S1, S2, S3 and Piperacillin-Tazobactam respectively in 24 hours.

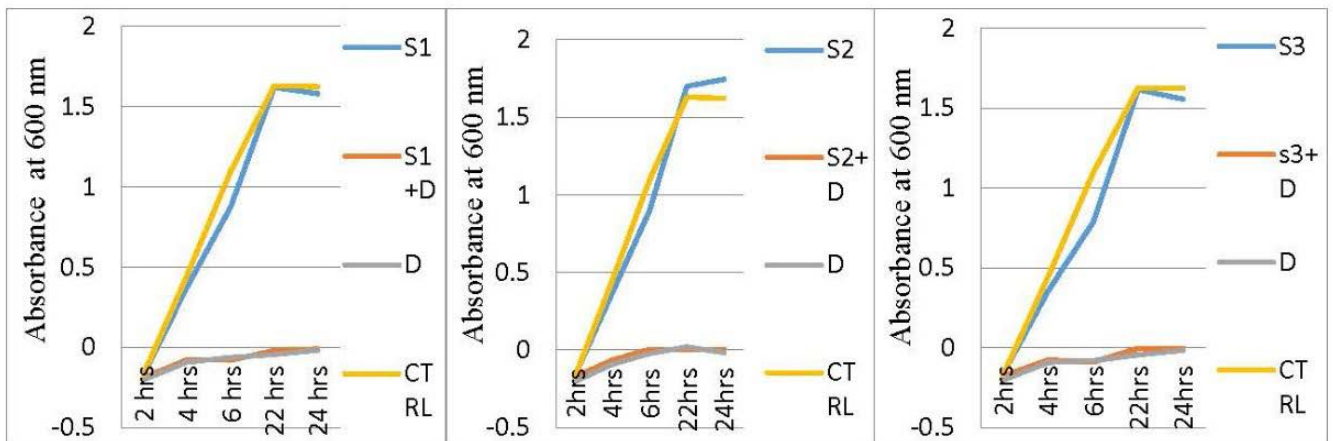


Figure-4. Growth curve of MDR *Paeruginosae* against the combination of S1, S2, S3 and Piperacillin-Tazobactam respectively in 24 hours.

## DISCUSSION

Antibiotic resistance has evolved as a serious threat against treating severe infections like ear and urinary tract infections. MDR and ESBLs producing *Paeruginosae* and *E.coli* are the pathogens with different sensitivity profile for commercially available drugs. Production of these uro-pathogens causes inactivation of a large number of antibiotics and present major therapeutic dilemma since the choice of antibiotics is limited.<sup>16</sup>

The combined use of prevalent antibiotics (eg, amphotericin B, oxacillin, cloxacillin, amoxicillin, cephalexin, cefotaxime, ceftazidime,

vancomycin, streptomycin, and erythromycin) and nanoparticles can treat bacterial diseases.<sup>8</sup> Existing studies have established that Gram-positive and Gram-negative bacterial infections can be managed by the inexpensive and effective antibacterial activity of silica-coated silicon nanostructures.<sup>17</sup>

In this study, we checked the synergistic effect of two drugs with SiNPs for two different strains. It was of great interest to find that S1, S2, S3 and ciprofloxacin, which was affecting the growth of *P. aeruginosae* alone, showed bactericidal activity in combination at 24 hours but synergism of SiNPs and drug was not observed for absolute growth

inhibition of *E. coli*. However, combined therapy of Piperacillin -Tazobactam with S1, S2 and S3 inhibited the normal growth pattern of both *E. coli* and *P. aeruginosa* as compared to the control.

Nanomaterials have great potential to inhibit bacterial infection, but many challenges persist for clinical trials. Some of these include assessing the interactions of Nano antibiotics with cells, tissues and organs, for dose adjustments and identification of suitable routes of administration.<sup>18</sup>

## CONCLUSION






Combined application of silica nanoparticles and antibiotics inhibited the growth of MDR gram-negative bacteria in vitro.

Copyright© 18 Nov, 2020.

## REFERENCES

- Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou GJFiph. **The “old” and the “new” antibiotics for MDR Gram-negative pathogens: For whom, when and how.** 2019; 7:151.
- Taneja N, Rao P, Arora J, Dogra AJJoMR. **The occurrence of ESBL & Amp-C [beta]-lactamases & susceptibility to newer antimicrobial agents in complicated UTI.** 2008; 127(1):85.
- Stamm WE, Norrby SRJTJoid. **Urinary tract infections: Disease panorama and challenges.** 2001; 183(Supplement\_1): S1-S4.
- Miyoshi-Akiyama T, Tada T, Ohmagari N, Viet Hung N, Tharavichitkul P, Pokhrel BM, et al. **Emergence and spread of epidemic multidrug-resistant *Pseudomonas aeruginosa*.** 2017; 9(12):3238-45.
- Hassan SA, Jamal SA, Kamal MJJoB, Sciences A. **Occurrence of multidrug resistant and ESBL producing *E. coli* causing urinary tract infections.** 2011; 7(1).
- Parhi P, Mohanty C, Sahoo SKJDdt. **Nanotechnology-based combinational drug delivery: An emerging approach for cancer therapy.** 2012; 17(17-18):1044-52.
- Jafari S, Soleimani M, Salehi RJJoPM, Biomaterials P. **Nanotechnology-based combinational drug delivery systems for breast cancer treatment.** 2019; 68(14):859-69.
- Rai M, Kon K, Gade A, Ingle A, Nagaonkar D, Paralikar P, da Silva SS. **Antibiotic resistance: can nanoparticles tackle the problem.** *Antibiot. Resist.* 2016.
- Knežević NŽ, Durand J-OJN. **Large pore mesoporous silica nanomaterials for application in delivery of biomolecules.** 2015; 7(6):2199-209.
- Manzano M, Vallet-Regí MJAFM. **Mesoporous silica nanoparticles for drug delivery.** 2020; 30(2):1902634.
- Möller K, Bein TJCoM. **Talented mesoporous silica nanoparticles.** 2017; 29(1):371-88.
- Croissant JJAM. *Adv. Healthcare Mater.* 2018, 7, 1700831; b) JG Croissant, Y. Fatieiev, NM Khashab. 2017; 29:1604634.
- Zhang Q, Liu F, Nguyen KT, Ma X, Wang X, Xing B, et al. **Multifunctional mesoporous silica nanoparticles for cancer-targeted and controlled drug delivery.** 2012; 22(24):5144-56.
- Samal S, Das PKJP. **Microbial biofilms: Pathogenicity and treatment strategies.** 2018; 6(1):16-22.
- Pageni P, Yang P, Chen YP, Huang Y, Bam M, Zhu T, et al. **Charged metallopolymer-grafted silica nanoparticles for antimicrobial applications.** 2018; 19(2):417-25.
- Ramazanzadeh RJEJoM. **Etiologic agents and extended-spectrum beta-lactamase production in urinary tract infections in Sanandaj, Iran.** 2010; 15(2):57.
- Tank C, Raman S, Karan S, Gosavi S, Lalla NP, Sathe V, et al. **Antimicrobial activity of silica coated silicon nano-tubes (SCSNT) and silica coated silicon nano-particles (SCSNP) synthesized by gas phase condensation.** 2013; 24(6):1483-90.
- Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, et al. **Nano-strategies to fight multidrug resistant bacteria—“A Battle of the Titans”.** 2018; 9:1441.

### AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Lubna Khursheed	MS student Performing experiment, compiling reslts.	
2	Raheela Yasmin	Article writing, Proof reading, compiling data.	
3	Naveeda Riaz	Supervisor, technical support guidance.	
4	Rabbia Shabbir	Proof reading.	
5	Kaleem Imdad	Proof reading and rechnical support.	
6	Seemab Iqbal	Research support.	