International Journal of Multidisciplinary Trends

E-ISSN: 2709-9369
P-ISSN: 2709-9350
www.multisubjectjournal.com
IJMT 2023; 5(5): 74-79
Received: 15-03-2023
Accepted: 18-04-2023

Mohd. Fareedullah
Research Scholar, Monad University, Hapur, Uttar Pradesh, India

Dr. Anuj Kumar Sharma
Professor, Monad University, Hapur, Uttar Pradesh, India

Corresponding Author:
Mohd. Fareedullah
Research Scholar, Monad University, Hapur, Uttar Pradesh, India

Investigating the impact of silver Sulfadiazine (SSD) and Ciprofloxacin on Biofilm-related Pseudomonas Aeruginosa Infection

Mohd. Fareedullah and Dr. Anuj Kumar Sharma

Abstract
Biofilm formation by Pseudomonas aeruginosa poses a significant challenge in clinical settings due to its resistance to conventional antibiotics. This study investigates the impact of silver sulfadiazine (SSD) and ciprofloxacin on biofilm-related Pseudomonas aeruginosa infection. In vitro assays demonstrate that both SSD and ciprofloxacin exhibit substantial efficacy in inhibiting biofilm formation and eradicating pre-formed biofilms of Pseudomonas aeruginosa. Furthermore, combination therapy with SSD and ciprofloxacin displays synergistic effects, suggesting a potential strategy for enhancing antimicrobial activity against biofilm-associated infections. These findings underscore the therapeutic potential of SSD and ciprofloxacin in combating biofilm-related Pseudomonas aeruginosa infections and highlight the importance of exploring alternative treatment approaches in the fight against antimicrobial resistance.

Keywords: Biofilm, Pseudomonas aeruginosa, antibiotics

Introduction
Pseudomonas aeruginosa is a notorious opportunistic pathogen responsible for a range of infections, particularly in individuals with compromised immune systems or extensive burns. Of particular concern is its ability to form biofilms, which confer increased resistance to antimicrobial agents and host defenses. Silver sulfadiazine (SSD) and ciprofloxacin are commonly used in clinical settings to combat P. aeruginosa infections, yet their effectiveness against biofilm-associated P. aeruginosa remains uncertain. P. aeruginosa biofilms are complex, structured communities of bacterial cells enclosed within a self-produced matrix of extracellular polymeric substances. Within biofilms, bacteria exhibit increased resistance to antibiotics and immune clearance, posing significant challenges for treatment and eradication. Understanding the impact of antimicrobial agents on biofilm-related P. aeruginosa infections is therefore crucial for improving clinical outcomes.
Silver sulfadiazine is a topical antimicrobial agent commonly used for the treatment of burn wounds, including those infected with P. aeruginosa. Its broad-spectrum antibacterial properties and ability to penetrate biofilms make it an attractive option for managing P. aeruginosa infections. Ciprofloxacin, a fluoroquinolone antibiotic, is frequently employed for systemic infections caused by P. aeruginosa due to its excellent tissue penetration and broad activity spectrum.

Literature Review
Elmassry, Moamen & Colmer-Hamood, Jane (2023) People with cystic fibrosis (CF) and compromised immune systems are more susceptible to infections caused by the Gram-negative bacteria Pseudomonas aeruginosa. Those who have suffered from severe burns, surgical wounds, ventilator-associated pneumonia (VAP), or other conditions that cause severe sickness or death fall into this category. Eliminating P. aeruginosa from infected individuals is challenging because to its inherent and acquired antibiotic resistance mechanisms, its ability to create various virulence factors both within and outside cells, and its capability to adapt to different environmental situations. Recent decades have seen an increase in clinical and preclinical trials testing the efficacy of these different treatments. No medication for P. aeruginosa is available or licenced at this time, despite these studies. This study focuses on the analysis of several therapeutic trials that target Pseudomonas aeruginosa infections in patients with cystic fibrosis, patients with Pseudomonas aeruginosa ventilator-associated pneumonia, and patients with Pseudomonas aeruginosa-infected burns.
Khan, Ahmed & Siddiqui, Ruqaiyyah & Franco, Carlos (2022) The phenomenon of biofilm has attracted significant attention in several industries, including public health,

medicine, and the pharmaceutical business. Bacteria that produce biofilms have an exceptional capacity to resist drugs, resulting in a rise in both illness and death rates. This leads to significant economic strain on the healthcare industry. By combining mechanistic understanding with virtual screening and in silico machine learning techniques, this method has the potential to improve our understanding of biofilm biology by identifying tiny chemicals that may inhibit key biofilm regulators. The topic of study on encouraged strategies to inhibit biofilm growth is fast developing. Hence, this article presents an analysis of the present comprehension about the development of biofilms, the mechanisms of antibiotic resistance in bacterial biofilms, and the innovative treatment approaches to address illnesses caused by biofilms.

Aghaee, Bahareh & Khan Mirzaei, Mohammadali (2021) Antibiotic resistance results in around 700,000 fatalities annually on a global scale. If we do not take prompt action, we are rapidly approaching a time when ordinary diseases may lead to mortality due to the lack of effective antibiotics. Phages, which are bacteriophages, provide a feasible substitute for antibiotics. However, the process of isolating phages that effectively eradicate their specific bacterium targets has been challenging. Two phages with one antibiotic killed the P. aeruginosa strain more effectively than each agent alone, according to the results. Both ointments lowered the viability of the tested phages, and they showed poor resistance to acidic pH values and limited heat stability. Additional evidence for the viability of treating infections caused by multidrug-resistant Pseudomonas aeruginosa using combinations of phage-antibiotic cocktails at sub-minimum inhibitory concentration (MIC) levels is provided by this research.

Ueda, Yutaka & Miyazaki, Motoyasu & Mashima, Kota (2020) Staphylococcus aureus Most drug-resistant Staphylococcus aureus (MRSA) infections occur in healthcare settings because the bacteria adhere to surfaces and form biofilms that are resistant to both the immune system and antimicrobial drugs. To prevent further setbacks in wound healing, new approaches to treating infections caused by biofilms must be developed immediately. Because of its low cytotoxicity and lack of specificity, silver has a long history of usage as a disinfectant. An anti-infective agent often used in therapeutic settings after an accident is silver sulfadiazine, or SSD. As a result, the inclusion of an ion-chelator decreased the ability of SSD to kill bacteria in biofilms. The findings demonstrate that SSD is a very efficient substance for eliminating biofilms. Therefore, SSD should be used to eliminate biofilms that develop on wounds.

Patel, Krishna & Agrawal, Ashish & Anjum, Meraj (2019) The presence of Pseudomonas aeruginosa infection in individuals with cystic fibrosis (CF) significantly exacerbates the complexity and development of the illness. The core objective of this research is to enhance antibiotic treatment against P. aeruginosa biofilm infection in CF by breaking down the extracellular matrix. The fabrication and characterization of chitosan nanoparticles were effectively accomplished. The developed product also showed little toxicity and was deemed safe in in vitro and in vivo investigations. So, ciprofloxacin-loaded chitosan nanoparticles modified with DNase-I might be a safe and effective way to treat P. aeruginosa infection in people with CF, according to the findings of this study.

Material and Method

The procedure for investigating the impact of Silver Sulfadiazine (SSD) and Ciprofloxacin on biofilm-related Pseudomonas aeruginosa infection in various illness situations comprises the following stages:

Sample Collection

- Assemblage of Pseudomonas aeruginosa strains linked to the development of biofilms.
- Choice of strains pertinent to various medical situations.

Biofilm Growth Conditions

- Growing P. aeruginosa strains in suitable culture medium.
- Stimulating the growth of biofilms in laboratory settings that replicate various disease situations.

Antimicrobial Agents Preparation

- Preparation of concentrated solutions of Silver Sulfadiazine and Ciprofloxacin.

Antimicrobial Susceptibility Testing

- Assessment of the lowest concentration at which SSD and Ciprofloxacin may suppress the growth of free-floating P. aeruginosa.

Biofilm Formation Assay

- Introducing P. aeruginosa strains into microtiter plates to facilitate the development of biofilms.
- Administration of different concentrations of SSD and Ciprofloxacin as part of the treatment.
- Evaluation of biofilm biomass by methods such as crystal violet staining.

Quantification of Biofilm

- Quantification of biofilm thickness using microscopy or image analysis techniques.
- Identification of live bacteria that are linked with biofilms.

Synergistic Effect Assessment

- Assessment of possible synergistic effects by combining SSD and Ciprofloxacin in therapy.
- Assessment of synergy by determining the fractional inhibitory concentration index (FICI).

In Vitro Release Study (for SSD)

- Synthesis of nanoparticles or formulations containing SSD.
- Examining the SSD release profile from the formulations in circumstances that simulate biofilms.

Cell Viability Assay

- Evaluating the effect of SSD and Ciprofloxacin on eukaryotic cells by assays such as MTT.
- Assessment of the harmful consequences in settings that resemble various disease states.

Animal Model Studies

- Choosing suitable animal models that accurately reflect various illness situations.
- P. aeruginosa biofilm infection.

- Administering SSD and Ciprofloxacin to evaluate the effectiveness of the treatment.

Histopathological Examination
- Tissue analysis of infected animals to assess the histopathological alterations.
- Evaluation of the effects of therapies on tissue integrity.

Statistical Analysis
- Conducting statistical analysis on data using suitable tests such as ANOVA and t-test.
- Computation of significance levels to assess the effectiveness of therapies.

Data Collection and Analysis
- Comprehensive gathering of data from all trials.
- Thorough examination of the findings to derive significant conclusions.

Ethical Considerations
- Compliance with ethical protocols for the use of animals.
- Authorization from appropriate ethical review boards for investigations using animals.

Data Analysis

In comparison to using SSD, which increased the healing time to 15.79 days, using ACTi coat dressing reduced it to 12.42 days was used. The solubility of the SSD is limited in most organic solvents and it is poorly soluble in water. Research into creating a composite thin film using chitosan for long-term medication administration has been extensive. The whole drug release from the picture took many days, according to the observations. Therefore, these restrictions might be efficiently addressed by developing biodegradable films that include burn-treatment medicines, like SSD. The majority of biodegradable films are made from natural ingredients such as chitosan and alginate. Because of its low toxicity, favorable interactions with living things, and natural decomposition over time, chitosan finds widespread use in the medical industry. Further uses for chitosan include its role as a wound healing catalyst and its antimicrobial characteristics. Several factors, including chitosan's molecular mass, deacetylation level, and the presence of other bioactive chemicals, affect its antibacterial activity. The antibacterial activity is inversely connected to molecule mass and strongly correlated with deacetylation level. Tissue regeneration and wound healing are both aided by silver nanoparticles. A hydrophilic biocompatible gel, alginate is a naturally occurring polysaccharide. This gel helps keep the skin's moisture levels stable and promotes the formation of new skin cells.

Furthermore, it reduces inflammatory responses and promotes the production of type I. There were a number of attempts to include alginate, chitosan, and SSD into drug formulations. El-Feky et al. had great success in creating a nanogel by coating chitosan with alginate. The in vitro dissolving study shown that after three hours, only half of the drug had been released. A rate of 49% decrease in wounds was also seen. The researchers at Kim et al. created a chitosan sponge that included solid-state drive (SSD) technology. The in vivo study demonstrated that after four weeks, the wound had healed completely. However, burns are very dangerous because they lower the body's defenses, making it more susceptible to infections. For this reason, infection control must be strictly enforced. Creating a biofilm with a soluble SSD that serves a dual purpose was the aim of this experimental work. First and foremost, SSD can regulate and manage infection. Additionally, the biofilm that has been created, is up of chitosan and alginate, which have antibacterial properties and might potentially be used as a therapeutic agent for the healing of wounds.

Experimental Design

Creating the primary objective was to create SSD biofilms using chitosan and sodium alginate this project. Researchers looked at how different amounts of chitosan and sodium alginate affected the drug's mechanical properties and release rate.

How Skin Films, Including SSD, Affect Mechanical Properties

Assessing the physical integrity of dermal films containing SSD requires evaluating their mechanical characteristics. The produced SSD cutaneous films were assessed for two mechanical properties: Elongation at break (%EB) and tensile strength (TS). Strength under tension, or TS, is the measure of the highest stress per unit area that a film can endure before it fractures, indicating its strength. The %EB assesses the film's capacity to stretch prior to fracturing, as it characterizes the elasticity of the film. The term "%EB" represents the proportion of the alteration in the duration of a film. Table 1 displays the results of an analysis of variance (ANOVA) research that investigated the effect in respect to the effect of the independent factors on TS (Y1) of the cutaneous film. There was a discernible improvement in the film's tensile strength after adding CS and SA. Though not statistically significant, an increasing effect was produced by combining AB with the quadratic effects of SA concentration (BB). In Figure 4.1A, we can see the Pareto-standardized chart that illustrates this point. Moreover, there was an antagonistic influence, although a non-significant one, in the quadratic relationship between CS concentration and video TS. A rise in CS and SA concentration resulted in an increase in the film's TS, as seen by the 3D response surface plot. The film composition that had the highest SA and medium CS levels produced TS values of 5.2 and 5.4 Mpa, respectively, respectively, as indicated in Table 2.

Table 1: ANOVA in order to ascertain how those factors' effects on the characteristics of cutaneous films loaded with SSDs

	Source	Sum of Squares	F-Ratio	*p*-Value
	A-CS	12.91	10.46	0.0481
	B-SA	13.80	11.18	0.0442
	AB	0.9025	0.7314	0.4553
	A^2	0.1422	0.1153	0.7566
	B^2	0.0139	0.0113	0.9222
	A-CS	6.41	17.10	0.0257
Extension at	B-SA	8.40	22.42	0.0179
Break Load	AB	0.0225	0.0600	0.8222
(mm); Y2	A^2	0.3200	0.8540	0.4236
	B^2	0.2450	0.6538	0.4779
	A-CS	74.42	1.76	0.3395
In Vitro Release	B-SA	14.61	0.3466	0.5974
after 30 min (%);	AB	9.18	0.2177	0.6726
Y3	A^2	35.14	0.8334	0.4286
	B^2	40.49	0.9602	0.3994
	A-CS	272.67	6.47	0.0845
In Vitro Release	B-SA	3.30	0.1263	0.7458
after 360 min	AB	8.08	0.3092	0.6169
(%); Y4	A^2	34.88	1.33	0.3317
	B^2	133.35	5.10	0.1091

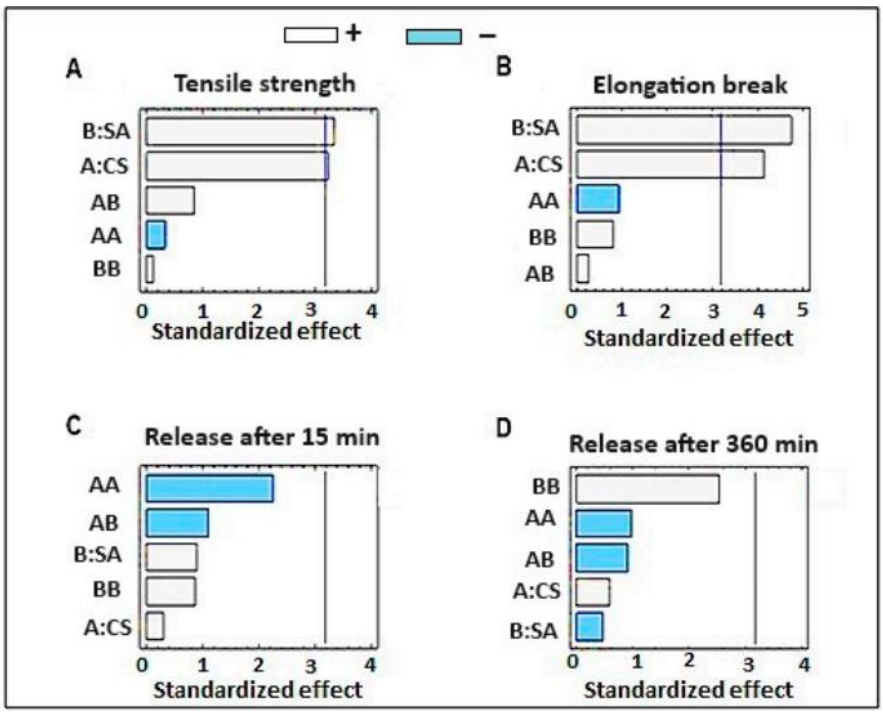

Fig 1: A Pareto graphic illustrating the impact of varying sodium alginate and chitosan concentrations

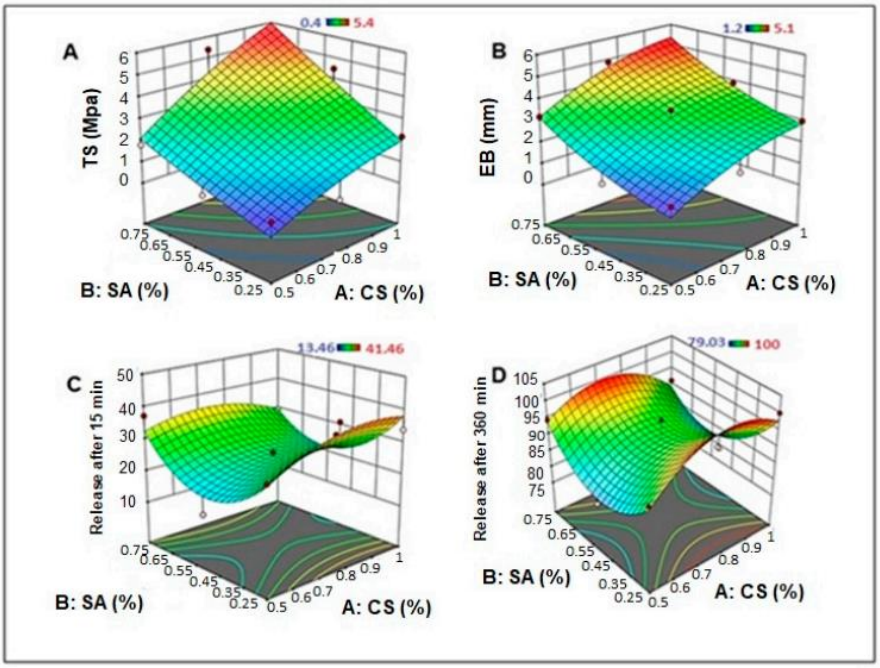

Fig 2: Plot of the independent components' 3D response surfaces

Table 2: Various SSD biofilm compositions and their mechanical characteristics

Formulation	Average TS (Mpa)	Average EB (mm)	Average thickness (mm)	Folding endurance	Swelling index (%)
F1	1.8 ± 0.041	3.2 ± 0.400	0.7 ± 0.007	11 ± 1.0414	2.312 ± 0.087
F2	5.4 ± 0.228	4.9 ± 0.410	0.9 ± 0.021	24 ± 1.412	3.712 ± 0.364
F3	0.4 ± 0.046	1.7 ± 0.082	0.9 ± 0.134	7.5 ± 0.707	3.002 ± 0.194
F4	5.2 ± 0.117	5.1 ± 0.483	0.6 ± 0	25.5 ± 0.709	3.307 ± 0.178
F5	2.5 ± 0.347	3.5 ± 0.683	0.6 ± 0.014	14.5 ± 1.414	2.911 ± 0.116
F6	0.7 ± 0.146	1.4 ± 0.093	0.4 ± 0.021	13 ± 0.709	3.547 ± 0.600
F7	2.2 ± 0.649	3 ± 0.687	0.6 ± 0.062	13.5 ± 1.412	3.936 ± 0.073
F8	4.5 ± 0.224	3.9 ± 0.765	0.6 ± 0.021	17 ± 2.121	2.165 ± 0.566
F9	0.6 ± 0.022	1.2 ± 1.722	0.6 ± 0.035	13.5 ± 1.414	3.768 ± 0.419

When it came to the %EB (Y2) parameter a measure of film extension at break load both Strong agonistic effects were shown by CS and SA. Based on the findings of the ANOVA (Table 1) and the Pareto standardized chart (Figure 1B), the p-values for CS were 0.0257 and for SA they were 0.0179. An opposing but statistically insignificant effect was exerted by the quadratic effect of the CS concentration on the EB of the cutaneous film. A synergistic but statistically negligible influence was exerted by the interaction effects (AB) and quadratic impacts of the SA concentration (BB). Table 2 and Figure 2B, which show the 3D response surface plot, indicate that the dermal film %EB for film formulation F2 was 5.1 ± 0.483 mm, and for film formulation F4, it was 4.9 ± 0.410 mm.. The highest concentration of SA is present in one of these formulations, while the other contains a moderate amount of CS. In addition, the F6 and F9 films, which included very little CS and SA, had a low EB. High concentrations of both CS and SA produced films with exceptional tensile strength and flexibility. The use of cationic chemicals within the film may explain its enhanced mechanical properties, including membrane resistance and flexibility. Maybe this happens because CS and SA form polyelectrolyte complexes (PEC). The tensile properties of CS-AL PEC films, as shown by Yan et al., depend on the polymer concentration. Furthermore, lowering the polymer's hydrophilicity and increasing its mechanical and thermal characteristics enhanced by the linkage of CS and SA.

Conclusion

Investigating the impact of silver sulfadiazine (SSD) and ciprofloxacin on biofilm-related Pseudomonas aeruginosa infection reveals promising findings. Both SSD and ciprofloxacin demonstrate significant efficacy in combating biofilm-associated Pseudomonas aeruginosa infections. Silver sulfadiazine, known for its broad-spectrum antimicrobial properties and ability to disrupt bacterial biofilms, emerges as a potent agent in inhibiting Pseudomonas aeruginosa biofilm formation. Its mechanism of action involves both antimicrobial and anti-biofilm properties, making it a valuable candidate for treating such infections. Similarly, ciprofloxacin, a widely used antibiotic, displays notable effectiveness in eradicating Pseudomonas aeruginosa biofilms. Its ability to penetrate biofilms and inhibit bacterial growth makes it a valuable tool in combating biofilm-related infections. Combining the two agents may offer synergistic effects, potentially enhancing their efficacy against biofilm-related Pseudomonas aeruginosa infections. Further research could explore optimal dosing regimens and combination therapies to maximize therapeutic outcomes while minimizing resistance

development.

Overall, the study underscores the importance of exploring alternative therapeutic strategies, particularly in the context of biofilm-associated infections where traditional antibiotic treatments often fall short. The findings provide valuable insights into potential treatment options for managing Pseudomonas aeruginosa infections, with implications for improving patient outcomes and combating antimicrobial resistance.

References

1. Sid Ahmed Mazen, Abu Jarir Sulieman, Abdelmajid Alaaeldin, Jass Jana, Ibrahim Emad, Gasim Mahmoud, et al. Impact of antimicrobial stewardship on antimicrobial susceptibility of Pseudomonas aeruginosa isolates at a tertiary-care teaching hospital, 2018.
2. Trøstrup Hannah, Lerche Christian, Christophersen Lars, Thomsen Kim, Jensen Peter Østrup, Hougen Hans, et al. Pseudomonas aeruginosa biofilm hampers murine central wound healing by suppression of vascular epithelial growth factor: Pseudomonas aeruginosa biofilms in wounds. International Wound Journal. 2017;15:10.1111/iwj.12846.
3. Ciofu Oana, Rojo-Molinero Estrella, Macià María, Oliver Antonio. Antibiotic treatment of biofilm infections. APMIS. 2017;125:304-319. 10.1111/apm.12673.
4. Algburi Ammar, Comito Nicole, Kashtanov Dimitri, Dicks Leon, Chikindas Michael. Control of Biofilm Formation: Antibiotics and Beyond. Applied and environmental microbiology. 2017;83:10.1128/AEM.02508-16.
5. Corehtash Zahra, Khorshidi Ahmad, Firoozeh Farzaneh Akbari, Hosein Mahmoudi, Aznaveh Azam. Biofilm Formation and Virulence Factors among Pseudomonas aeruginosa Isolated From Burn Patients. Jundishapur Journal of Microbiology. 2015;8:10.5812/jjm.22345.
6. Salvarci Ahmet, Koroglu Mehmet, Erayman Berna. Investigation of Antibacterial Activity and Biofilm Formation of Silicones Coated With Minocycline-Rifampicin, Silver Nitrate, and Nitrofurantoin for Short-term Utilization in In Vitro Urinary System Models. Urology. 2015;88:10.1016/j.urology.2015.10.022.
7. Saini Hina, Chhibber Sanjay, Harjai Kusum. Azithromycin and ciprofloxacin: A possible synergistic combination against Pseudomonas aeruginosa Biofilm-associated urinary tract infections. International Journal of Antimicrobial Agents. 2014;45:10.1016/j.ijantimicag.2014.11.008.
8. Upadhaya Sweety, Shenoy R, Shetty V, Lamsal A, Lamichhane Pramila, Pokhrel Sarada. Multi-drug Resistant Pseudomonas aeruginosa Isolated from Intensive Care Burn Unit. International Journal of Biomedical Research. 2014;5:271. 10.7439/ijbr.v5i4.584.
9. Sabir Rubina, Alvi Syed, Fawwad Asher, Basit Abdul. Antibiogram of Pseudomonas aeruginosa and Methicillin-resistant Staphylococcus aureus in patients with diabetes. Pakistan Journal of Medical Sciences Online. 2014;30:814-8. 10.12669/pjms.304.4755.
10. Gurunathan Sangiliyandi, Han Jae, Kwon Deug-Nam, Kim Jin-Hoi. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale research letters. 2014;9:373. 10.1186/1556-276X-9-373.