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Synergistic Effect of Selenium Nanoparticles (SeNPs) With Various Antibiotics as an Antimicrobial Activity

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***Abstract*-- Nanoparticles (NPs) are advantageous in treating bacterial infections. Scientists found that Selenium Nanoparticles (SeNPs), owing to their unique structure and properties, may be more effective than antibiotics as they have a larger surface area and therefore can be more in contact with the external environment. The antibacterial effect of selenium may be due to the fact that at a particular concentration nano-selenium interacts with the bacterial cell surface and penetrates into the cell, thus causing damage. Some studies in recent years have suggested the use of combination of antibiotics+SeNPs, the synergistic effect of which often surpasses their individual's inhibitory activity. In our work we performed synergetic effect of SeNPs and 3 commercial antibiotics. We found that the Nanoparticles enhanced the reaction rates of antibiotics in a synergistic mode as well as in its own way on different kinds of pathogens.**

I. INTRODUCTION

Over the last decade, there has been a remarkable global focus on conventional as well as biogenic metallic Nanoparticles as innovative tools for combating the high rates of antimicrobial resistance [1], [2]. Resurgent interest in NPs has been stimulated by the appearance of drug-resistant bacteria and the increasing rate of hospital infection outbreaks.

NPs in particular have demonstrated broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria. The NPs have a direct contact with the bacterial cell wall, without penetrating the cell, which would be less prone to promoting resistance in bacteria than antibiotics [3]. Due to their excellent antimicrobial resistance properties, NPs have been widely used in many fields. In fact, the application of NPs in fighting bacteria has decreased bacterial infection [4].

Several NPs have been documented for antimicrobial activity [5], as the bacteria are less likely to develop resistance to nanoparticles, the nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics [6], [7], [8].

More recent option from the perspective of nanotechnologies and bacterial infections are Selenium Nanoparticles (SeNPs). SeNPs were investigated for various medical applications and as a potential material for orthopedic implants [9], [10], as anti cancer agent, Selenium has been investigated for various medical applications such as anticancer applications. Selenium as a dietary supplement has been demonstrated to reduce the risks of various types of cancers including prostate cancer [9], [10] lung cancer [11]and esophageal and gastric-cardiac cancers.

Currently, studies indicating the ability of the selenium compounds to inhibit bacterial growth and formation of bacterial biofilms are also available [12]. Scientists found that SeNPs, owing to their unique structure and properties, may be more effective than antibiotics as they have a larger surface area and therefore can be more in contact with the external environment [13]. The antibacterial effect of selenium may be due to the fact that at a particular concentration nano-selenium interacts with the bacterial cell surface and penetrates into the cell, thus causing damage. Selenium compounds are effectively used as anti-fungal agents in shampoos for the treatment of the dry scalp [13].

Many vast range of microbes are used to study the antimicrobial effect of SeNPs few of such microbes we used are E.coli, Pseudomonas, Staphylococcus aureus, Salmonella, Bacillus subtilis, Candida, zygomycota etc. The pathogenic microorganisms used for the present study were E.coli, Pseudomonas, Staphylococcus, Bacillus & Salmonella.

Some studies in recent years have suggested the use of combination of antibiotics, the synergistic effect of which often surpasses their individual's inhibitory activity [14], [15]. In our work we performed the inhibitory activity of SeNPs on 5 different pathogens, inhibitory activity of antibiotics on 5 different pathogens. Then the zone of inhibition of SeNPs was compared to that of Antibiotics

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inhibitory Zone. In the final stage we performed the synergetic effect of SeNPs+commercial antibiotics the pathogenic microbes and measured their synergistic activity.

II. MATERIALS AND METHODS

Synthesis of Selenium Nanoparticles

Selenium nanoparticles (SeNPs) was synthesised according to Sheng-YiZhang et al; protocol [16] with little modifications.

Collection of Pathogenic Sample

The samples were collected from various places and organizations. The pus sample was collected Hospitals, Hyderabad. The water sample was collected from Musi River, Moosrambagh, Hyderbad. The soil sample was collected from Purana Pul bridge, Hyderabad.

Inoculation and Isolation Pathogenic Samples

The samples were isolated and inoculated based on the protocols of Gopal Reddy et al., [17].

The identification of Microorganisms were done by staining techniques and by biochemical tests. After the isolation and identification of various organisms, the antibiotic sensitivity test and selenium nanopartical inhibitory tests by diffusion methods were performed. And also the synergistic effect of SeNPs of different concentration with various antibiotics was done.The plates were incubated at 37oC for 24 hrs. The inhibition zone diameters of them were measured and the Synergistic effect was calculated by the following equation.

$$\text{Synergistic effect} = (B\text{-}A)/A \times 100$$

Where,

'A' is Zone of inhibition for antibiotics

'B' is Zone of inhibition for the antibioitic + SeNPs

III. RESULTS

The pathogenic organisms which were collected from various sources were identified as Pseudomonas, Bacillus, E.coli, Staphylococcus based on morphological and biochemical tests. Their morphological characters, showed brown greenish colour, slightly yellow colour, pink colour colonies colonies, Golden yellow colour, pale yellow colour colonies on agar medium. In gram staining they were observed as rod shaped pink colour, rod shaped purple colour, Cocci shaped pink colour colonies, Cocci shaped purple colour, rod shaped pink colour confirming Gram-ve, Gram+ve, Gram-ve,, Gram+ve Gram –ve for Pseudomonas, Bacilli, E.coli, Staphylococcus, Salmonella respectively.

The result of various Biochemical test infer that the sugar fermentation was observed by gas bubble formation in durhams tubes in all the pathogens, giving +ve result. While indole test the Bacillus, E.coli formation of dark red colour layer indicates +ve results and light pink colour layer indicates –ve results in Pseudomonas, Staphylococcus, Salmonella. The Methyl Red test indicates bright red colour in all the pathogens, giving +ve results. While Voges-Proskauer test the Bacillus and Staphylococcus showing pink colour layer that indicates +ve results, while Pseudomonas, E.coli, Salmonella indicates –ve results. While Hydrogen sulphide test all pathogens are showing –ve results. While Citrate utilization tests Pseudomonas, Bacillus subtilis, Staphylococcus, Salmonella are showing +ve results, E.coli shows –ve result. While Gelatin Hydrolysis Pseudomonas, Bacillus subtilis, Staphylococcus, Salmonella are showing +ve results, E.coli shows –ve result.

Table:1-Diameter of Inhibition Zone (mm) of difeerent concentartions of SeNps against various pathogens.

S. No	Pathogen	SeNPs			Antibiotics		
		50µl (5µg)	100µl (10µg)	150µl (15µg)	Penicillin	Streptomycin	Tetracyclin
1.	Pseudomonas	25 mm	31 mm	39 Mm	10 mm	30 mm	_
2.	B. Subtilis	40 mm	46 mm	50 Mm	10 mm	25 mm	_
3.	E. coli	30 mm	40 mm	43 Mm	10 mm	30 mm	30 mm
4.	Staphylococcus	35 mm	35 mm	40 Mm	7 mm	25 mm	30 mm
5.	Salmonella	30 mm	35 mm	50 Mm	10 mm	26 mm	30 mm

Table: 1, Interprets the result of diameter of the inhibition zone in mm of all pathogens. The highest antimicrobial activity of 150µl SeNPs was seen in Bacillus subtilis, salmonella followed by E.coli, Staphylococcus, pseudomonas. The increasing level of SeNPs concentration lead to inncrease in diameter of inhibition zone, i.e., when SeNPs concentation were increased from(5µg to 15µg, the diameter of inhibition zones were also increased (Fig1,2).

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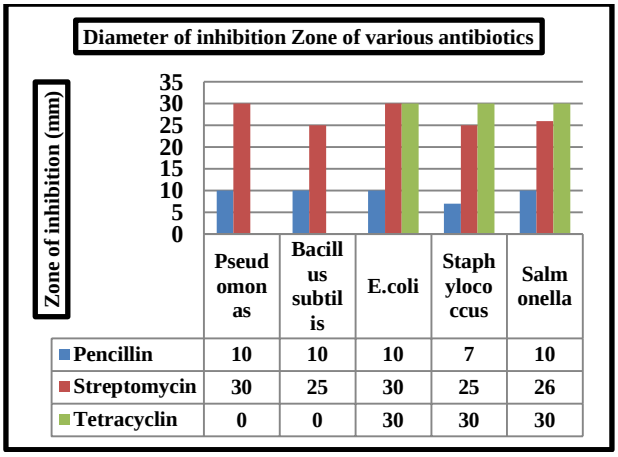


Figure:1 Diameter of Inhibition Zone of various antibiotics

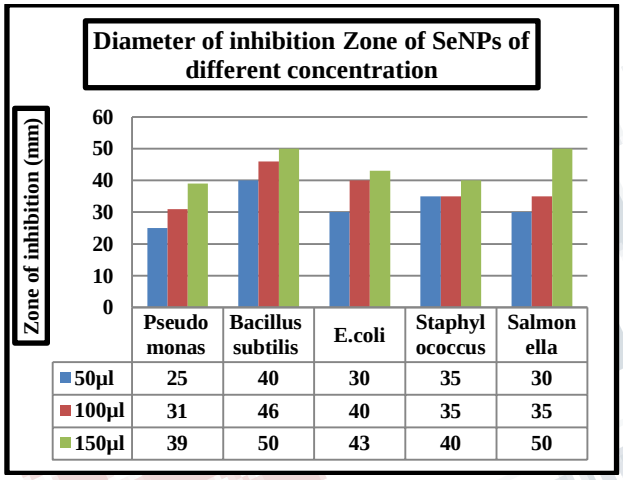
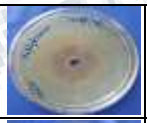
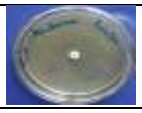
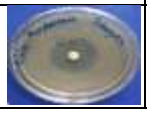


Figure:2 Diameter of Inhibition Zone Of SeNPs of different concentration

In the present study three antibiotics (Pencillin, Streptomycin, Tetracyclin) were used as control gainst SeNPs to compare their inhibitory activity against pathogenic bacteria. Penicillin showed 10mm zone of inhibition for pseudomonas, e.coli, Bacillus subtilis, Salmonella and 7mm for Staphylococcus. while streptomycin showed highest zone of Inhibition 30mm for Pseudomonas and E.coli, Salmonella showed 26mm, Bacillus, Staphylococcus showed 25mm zone of inhibition. Tetracyclin showed highest zone of Inhibition (30mm) for E.coli, Staphylococcus, Salmonella while Psedomonas, Bascillus Zone of Inhibition was not observed. over all the highest zone of inhibiton of antibiotics was less compared to that of SeNPs (Fig 3).

Figure 3: Minimum inhibitory Zone of Intiation of SeNPs and Antibiotics on Pathogens

S. No	Antimicobial Agent	Zone of Inhibition (ug)		
		5	10	15
Bacillus				
1	SeNPs			
2	Antibiotics			
E.coli				
1	SeNPs			
2	Antibiotics			
Streptococcus				
1	SeNPs			
2	Antibiotics			
pseudomonas				
1	SeNPs			
2	Antibiotics			
Salmonella				
1	SeNPs			
2	Antibiotics			

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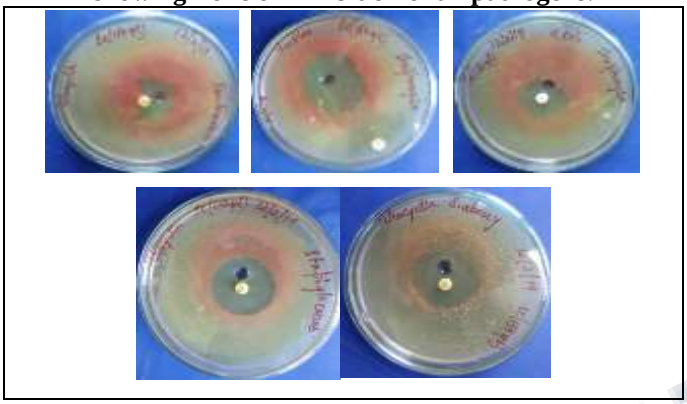
After the individual sensitivity tests of antibiotics and SeNPs on pathogens, the further experment was done on combined sinsitivity of both antibiotics+SeNPs on pathogens (fig 4).

Figure:4 Synergistic effect of SeNPs+Antibiotics showing Zone of Inhibition of all pathogens.



The combination of SeNPs with different antibiotics was investigated against five pathogenic bacteria using the disc diffusion method. The diameter of the inhibition zone in mm around the different antibiotic disks with SeNPs was determined as shown in (Table:2). The highest increased fold area was found for Tetracycline in presence of SeNPs 150µl against Pseudomonas (83%). The highest fold area was observed against Bacillus subtilius (167%), followed by E.coli (140%), Staphylococcus (66%), Salmonella (66%). It was found that the Nanoparticles enhanced the reaction rates of antibiotics in a synergistic mode as well as in its own way on different kinds of pathogens (Fig 5, 6).

Table:2 Diameter of Inhibition Zone (mm) of synergistic effect of SeNPs with various antibiotics

Pathogens	Antibiotics	Diameter of Inhibition Zone			
		SeNPs (mm)	Antibiotics (mm)	Se + Ab (mm)	Fold area increasing %
Pseudomonas	Tetracyclin	43	30	55	83
Bacillus subtilis	Streptomycin	39	30	80	167
E.coli	Streptomycin	50	25	60	140
Staphylococcus	Tetracyclin	50	30	50	66
Salmonella	Tetracyclin	40	30	50	66%

Figure:5 Diameter of Inhibition Zone (mm) of synergistic effect of SeNPs with various antibiotics

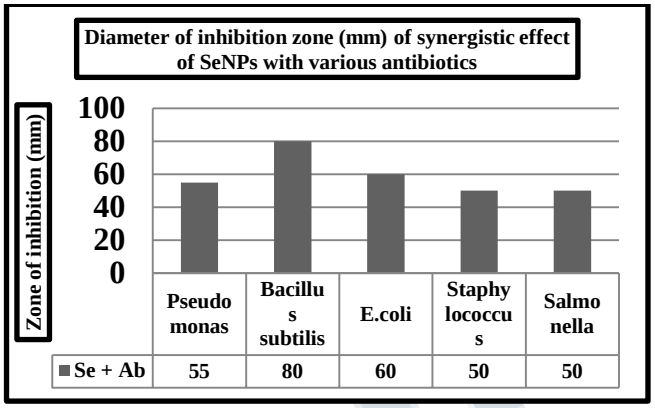
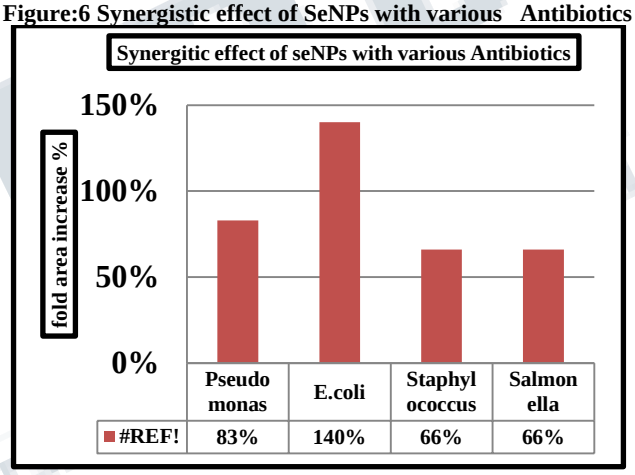


Figure:6 Synergistic effect of SeNPs with various Antibiotics



IV. DISCUSSION

Determination of the inhibition zone of SeNPs at different concentration:-According to Bahig El-Deeb et al., [14], the highest antimicrobial activity of 100 µl SeNPs was seen in the order of S. aureus and B. cereus (29mm) followed by MRSA (27 mm), S. agalactiae (25 mm) and E. coli (13 mm). However, apart from E. coli, SeNPs did not show a significant effect on all bacterial growth of Gram negative bacteria. The increases of SeNPs concentration lead to increase of diameter of inhibition zone, when the SeNPs concentrations were increased from 100µl (10µg) to 150µl (15µg), the diameter of inhibition zones were increased. Similar results were observed in our study.

According to our study, the highest antimicrobial activity of 150µl SeNPs was seen in Bacillus subtilis, salmonella followed by E.coli, Staphylococcus, and pseudomonas. The increase of SeNPs concentration lead to increase in diameter

of inhibition zone, when the SeNPs concentration were increased from 50µl (5µg) to 100µl (10µg) to 150µl (15µg), the diameter of inhibition zones were increased.

In the study [14], they used six antibiotics to compare their inhibitory activity when combined with SeNPs against pathogenic bacteria. They found that the SeNPs have greater Zone of Inhibition when compared to individual antibiotic the similar results were observed in our study.

Overall it was found that our results were similar to [14], where the synergistic effect of antibiotic+SeNPs showed highest Inhibitory Zone when compared to individual Minimum Zone of Inhibition (MIZ) of antibiotic and SeNPs respectively.

V. CONCLUSION

In the present study, all tested combination of SeNPs + Antibiotics showed higher synergistic inhibition against the growth of the pathogenic bacteria, compared to individual inhibition. This combinatorial approach may serve as adjunct to the existing therapies and may help to retrain the escalating nosocomial threats.

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