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Antibacterial activity of nano goldparticles synthesized by *Bacillus Sps*.

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Abstract
The synthesis and bioactivity of gold nanoparticles has been extensively studied. However, the antibacterial activity of gold nano particles individually or in combination with different antibiotics has not been demonstrated. In the present study the effect of gold nano particles on the antibacterial activity of different antibiotics was evaluated against *Staphylococcus aureus, k.pnemonea, P.aureginosa, E.coli.* Disk diffusion method was used to determine the antibacterial activity of various antibiotics in the absence and presence of sub inhibitory concentration of gold nano particles. A clinical isolates of *S. aureus, k.pnemonea, p.aureginosa,E.coli.* were used as the test strains. In the presence of sub-inhibitory concentration of gold nano particles the antibacterial activities of all antibiotics have. Increased from minimum 2 mm to a maximum of 8 mm. The highest increase was observed for ceftazidime followed by genthamycin. These results signify that the gold nano particles potentiate the antimicrobial action of cephalosporins and amino glycosides, suggesting a possible utilization of nano compounds in combination therapy against *S. aureus, k.pnemonea, p.aureginosa,E.coli*.

Keywords: Gold nano particles, bacillus, synergestic effect.

INTRODUCTION

The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology today. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological micro-organisms such as bacteria.

Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [1]. Biological methods are regarded as safe, cost-effective, sustainable and environment friendly processes for the synthesis of nanoparticles.

Gold nanoparticles have been successfully synthesized using various Bacteria (2), Fungi 3.

Over the recent decade, gold nanoparticles (NPs) [4] have attracted significant interest as a novel platform for various applications such as nanobiotechnology and biomedicine [5–7] because of convenient surface bioconjugation [8] with molecular probes and remarkable plasmon- resonant optical properties [9]. Recently published examples include applications of NPs to biosensorics [10], genomics [11,12], clinical chemistry [13], immunoassays [14], immune response enhancement [15], detection and control of microorganisms [16], and targeted delivery of drugs or genetic and immunological substances [17].Conjugates of gold NPs with antibiotics and antibodies also have been used for selective photothermal killing of protozoa and bacteria [18]. The development of new resistant strains of bacteria to current antibiotics (19) has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [20].

The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs [21, 22]. Consequently there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy [23-24]. Investigations have been carried out on the biological activities of gold nano particles; however, the effects of nano-particles on the activities of antibiotics have not been demonstrated.

In the present study, gold nanoparticles are synthesized using *Baccillus sp*. The study deals with the effect of biologically synthesized gold nanoparticles and effect on gold nano particles on the antibacterial activity of different antibiotics was evaluated against *Staphylococcus aureus, k.pnemonea, P.aureginosa, E.coli* .

MATERAILS AND METHODS

Preparation of Gold Nano Particles

Gold nano particles were synthesized by usingt bacillus sp. These gold nano particles were used to test the antibacterial activity as well as synergistic effect on antibiotis action against pathogenic bacteria *Staphylococcus aureus, k.pnemonea, P.aureginosa, E.coli* .

Microbial Assay Gold Nano Particles

Antibacterial activity of gold nano particles were studied by the agar-well-diffusion method, wherein a bacterial suspension was added to sterile nutrient agar at 45 _C and the mixture was solidified on a Petri dish. A 20-mL volume of the medium was poured into a Petri dish (diameter, 90 mm) on a horizontally leveled surface. After the medium had solidified, 6-mm-diameter wells were made in the agars (at six wells per dish) that were equidistant from one another and from the dish edge. The wells received either 0,25, 35, 45 55 and 100µl of gold nano particles and equal volumes of the free antibiotic solution and NP mixture. The Petri dishes were incubated in a thermostat at 37^{o}C for 24 h. After incubation, the diameter of the

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zone of bacterial-growth inhibition was measured with an accuracy of ±0.1 mm. The mean inhibition-zone diameter were determined. All experiments were repeated thrice.

Antibacterial assay

A clinical isolates of *S. aureus k.pnemonea, p.aureginosa, E.coli.* were obtained from the MTCC . The disk diffusion method was used to assay the activity of 4 different antibiotics against test strains on Mueller-Hinton agar (Hi Media, INDIA) plates according to the CLSI [25]. The standard antibiotics disks were procured from Hi Media. To determine combined effects, each standard paper disc was further impregnated with sub-inhibitory concentration of gold nano particles. A single colony of test strains was grown overnight in Mueller -Hinton broth on a rotary shaker (200 rpm) at 35°C. The inoculums were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard. A lawn of the test organism was made on the agar plate using a sterile cotton swab and the antibiotic discs and gold nanoparticles separately (with and without gold nano particles) were placed on the bacterial lawn. After incubation at 37°C for 24 hrs, the zones of inhibition were measured. The assays were performed in triplicate.

RESULTS AND DISCUTION

Therapeutic roles for gold in different diseases have been established in recent years. Gold oxide has a very good potential to move into the clinic [26]. In this investigation the effect of gold nano particles on the antibacterial properties of different antibiotics was investigated against *S. aureus, K. pnemonea, P. aureginosa, E.coli* .using the disk diffusion method.

The synergetic effect of the Gold nano-particles as observed by the increase in diameter of inhibition zones (mm) around the different antibiotic disks with gold nano-particles have been recorded in respect of certain antibiotics [Table-1]. The activities of all the antibiotics have increased in the presence of nanosize gold particles against the test strains.

The effect of gold nano particles were investigated against *S. aureus , k.pnemonea, P.aureginosa, E.coli* . Table 2 shows The inhibitory activity were more aginest *S.aureus* compared to *S. aureus , K. pnemonea, P.aureginosa, E.coli.*

Table 1. Synergistic effect of gold nano particles on Antibacterial activity of antibiotics against bacteria

S.No	Antibiotic	*S.aureus*		*K.pnemoniae*		*P.aureginosa*		*E.coli*	
		Ab only (mm)	With nano gold(mm)	Ab only (mm)	With nano gold(mm)	Ab only (mm)	With nano gold(mm)	Ab only (mm)	With nano gold(mm)
1	Genthamycin	28	31	24	28	31	38	18	22
2	Ceftazidime	28	30	32	37	21	25	20	28
3	Vancomycin	13		00		00		09	
4	Methicillin	00		00		00		06	

Table 2. Antibacterial activity of gold nano particles

S.No	*Concentration (µl)*	*S.aureus (mm)*	*K.pnemoniae (mm)*	*P.auriginosa (mm)*	*E.coli (mm)*
1	Controle	00	00mm	00mm	00mm
2	25	10	09	10	08
3	35	14	12	11	09
4	45	16	13	14	12
5	55	19	15	15	15
6	100	23	19	20	20

Anti bacterial study indicates that antibiotic + gold nano particles Sythesized by Baccillus sp exhibit more zone of inhibition compared to standard antibiotics used (Table 1). The zone of inhibition on *E.coli* , *k.pnemonea, P.aureginosa were* more when compared with the zone of inhibition exhibited on *S.aureus.* The biosynthesized gold nanoparticles with the antibiotic ceftazidime [30mm, 37mm, 25mm and 28mm] exhibit high zone of inhibition as compared with genthamycin [31mm, 28mm, 38mm and 22mm]. the synergistic activity of biosynthesized gold nanoparticles were more against gram negative bacteria compaired to gram positive (table no 1). The highest increases in the inhibition zones were observed for ceftazidime (4-8 mm) than the genthamycine (3-7mm)

Gold nano particle showed no synergetic effect on the antibacterial activity against the test strain. It should be pointed out that the gold nano particles content of 100 µg/disc was chosen to guarantee that the effect produced was due to the combination and not to the effect of the gold nano particles itself (18-22). So the effect observed in this condition could be due to the antibiotic-gold nano particles combination. At the concentration tested, gold nano particle significantly Improved antibiotic efficacy against *S. aureus, k.pnemonea, P.aureginosa, E.coli* when combined with antibiotics . At this time the reason of these enchantments and the reason for these differences are not known and merits investigation. Efflux transporter mediated bacterial resistance to different antibiotics and gold nano particles may inhibit this efflux pump system [27].

Comparison of these data with the findings in the literature [28, 29, 30], showing enhancement of antibacterial activity in the presence of NPs, suggests that two conditions at minimum are necessary (but insufficient) for such effects to be observed. First, antibiotic–NP conjugates should be stabilized, and their spectrum and color should correspond to those of single-particle non aggregated colloids. Second, the amount of the antibiotic covering the particle surface should be large enough to ensure an increase in the local antibiotic concentration at the site of bacterium–particle contact.

CONCLUSION

The biosynthesis of nano size gold particles was carried out

successfully using bacillus sp. The small nanometer scale gold particles which enhanced activity of several antibiotics. Further work is needed to find out the exact reasons for enhancement of activity of antibiotics in presence of gold nano particles.

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