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Colloidal Silver as a New Antimicrobial Agent

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Abstract: Silver has the advantage of having broad antimicrobial activities against Gram-negative and Gram-positive bacteria. This research was the result of bioassay experimentation on the effects of colloidal silver on multidrug resistant bacteria. The initial idea was to determine the antimicrobial activity of colloidal silver. So it could be used as a powerful *in-vitro* antimicrobial agent. Antimicrobial activity was determined by means of agar diffusion. Resistant clinical isolates of *Staphylococcus aureus, Escherichia coli, pseudomonas aregnosa* and *Salmonella typhi* were used as the test organisms. It was concluded this study showed successful formation of colloidal silver and their antibacterial activity against all tested pathogens.

Key words: Antimicrobial activity · Silver · Bacteria

INTRODUCTION

The emergence and spread of antibiotic resistance is an alarming concern in clinical practice. Increasingly, agents with ‘antimicrobial’ effects are being coated on materials and medical devices [1] as a prophylaxis to prevent bacteria from growing or for therapeutic use. The new technology of impregnation of silver nanoparticles [2] is enabling a wider range of these medical products to be available to clinicians. The use of metallic silver as an antimicrobial agent has long been recognized [3, 4]. Dilute solutions of silver nitrate had been used since the 19^{th} century to treat infections and burns before the introduction of silver sulphadiazine cream [5].

Silver has the advantage of having broad antimicrobial activities against Gram-negative and Gram-positive bacteria and there is also minimal development of bacterial resistance [6].

The antimicrobial activity of silver has been recognized by clinicians for over 100 years [4]. In addition, many reports suggested that hygienic benefits have been associated with the use of silver for considerably longer time. In the same time, records showed that Hippocrates recognized the role of silver in the prevention of disease and the Romans stored wine in silver vessels to prevent spoilage. However, only in the last few decades the mode of action of silver as an antimicrobial agent has been studied without any rigour [7]. Silver nano-particles have also been demonstrated to exhibit antimicrobial properties both against bacteria [8] and viruses [9] with close attachment of the nano-particles themselves with the microbial cells / virus particles being demonstrated with activity being size dependent [9]. Despite this, the principle activity of silver is as results of the production of silver ions within an aqueous matrix [10]. This therefore implies that for silver to have an antimicrobial effect, free water must be present. Interruption of cell wall synthesis resulting in loss of essential nutrients has been shown to occur in yeasts [11] and may well occur in other fungi. Antiviral activity of silver ions has been recorded and interaction with –SH groups has been implicated in the mode of action. The association of silver nano-particles with the envelope of certain viruses has been suggested to prevent them from being infective [9].

The current work was designed to throw lights on the *in vitro* antibacterial activity of colloidal silver with special emphasis on the comparison between silver solutions, sulphonamides and trimethoprime alone or in combination.

MATERIALS AND METHODS

Preparation of the Colloidal Silver Suspension in Pure Water: Colloidal silver solution was electrically prepared. Pure silver wires were used as both the positive and negative electrodes. The pure silver wires were etched by the DC pulse arc-discharge in pure water [12].

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Determination of Antibiotics Susceptibility (By Disc Diffusion Method): The tested isolates were sub cultured on nutrient agar plates for 24 hours at 37°C. 3-4 similar colonies were selected and aseptically transferred into 5ml of Muller Hinton broth and incubated at 37°C for 4-5 hours. One ml of the previous suspension was transferred to Muller Hinton agar tube and homogeneously suspend using vortex then the mixture was poured in a Petri dish and kept to dry for 10 minutes. Antibiotic discs were applied using aseptic technique with 1cm apart with gentle pressure to allow complete diffusion.

The Antibiotic Discs Were Supplied from Oxoide Company:

- Chloroamphinicol © 30mcg
- Cefaclor (Cj) 30mcg
- Cefadroxil (Cfr)
- Ciprofloxacin (Cf) 5mcg
- Erythromycin (E) 15mcg
- Levofloxacin (Le) 5mcg
- Norfloxacin (Nx) 10mcg
- Sulphonamides&Trimethoprime (Stx) 23.75/1.25mcg
- Tobramycin (Tb) 10mcg
- Incubate plates 24 hours at 37°C.

The diameter of the inhibition zones were measured and compared to a reference table to differentiate the isolates into sensitive, intermediate or resistant [13]. As shown in Table 1.

RESULTS

Antimicrobial susceptibility test for different antibiotic groups and sliver solution against four isolates were determined using diffusion method (Table 1).

Table 2 shows that all tested bacteria were resistant to more than one antibiotic. The important observation was the antibacterial activity of colloidal silver against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* which exhibit superior effect compared with other antibiotics.

Figure 1 Showed the antimicrobial susceptibility test for the different used antibiotic groups and sliver against four isolates.

DISCUSSION

The emergence and spread of antibiotic resistance is an alarming concern in clinical practice, Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize,

Table 1: Zone diameter interpretation charts for the antibiotics used according to NCCL2007

				Diameter of zone of inhibition in mm		
Antibiotic Class	Antibiotic Name	Symbol	Disc content	Resistant mm or less ®	Intermediate mm (I)	Sensitive mm or more (S)
Amphenicols	Chloroamphenicol	C	30 mcg	12	13-17	18
Aminoglycosides	Tobramycin	Tb	10 mcg	12	13-14	15
Betalactams	Cefaclor	Cj	30 mcg	14	15-17	18
cephalosporins	cefadroxil	Cfr	30 mcg	14	15-17	18
Sulphonamides & Trimethoprime	Sulphonamides & Trimethoprime	Stx	23.75/1.25 mcg	10	11-15	16
Macrolides	Erythromycin	E	15 mcg	13	14-22	23
Quinolones	Cibrofloxacin	Cf	5 mcg	15	16-20	21
	Levofloxacin	Le	5 mcg	15	16-18	19
	Norfloxacin	Nx	10 mcg	12	13-16	17

Table 2: Antimicrobial susceptibility test for different antibiotic group and sliver against four isolates

	Diameter of zone of inhibition in mm of resistant Bacterial isolates			
Antibiotic Name	*Staphylococcus aureus*	*Pseudomonus aregnosa*	*Salmonella typhi*	*Echerishia coli*
Chloroamphenicol	I	R	R	I
Tobramycin	R	S	I	R
Cefaclor	R	R	R	R
cefadroxil	R	R	R	R
Sulphonamides & Trimethoprime	I	R	R	R
Erythromycin	R	R	R	R
Cibrofloxacin	R	S	R	R
Levofloxacin	R	S	R	R
Norfloxacin	R	S	R	R
Silver	S	S	S	S

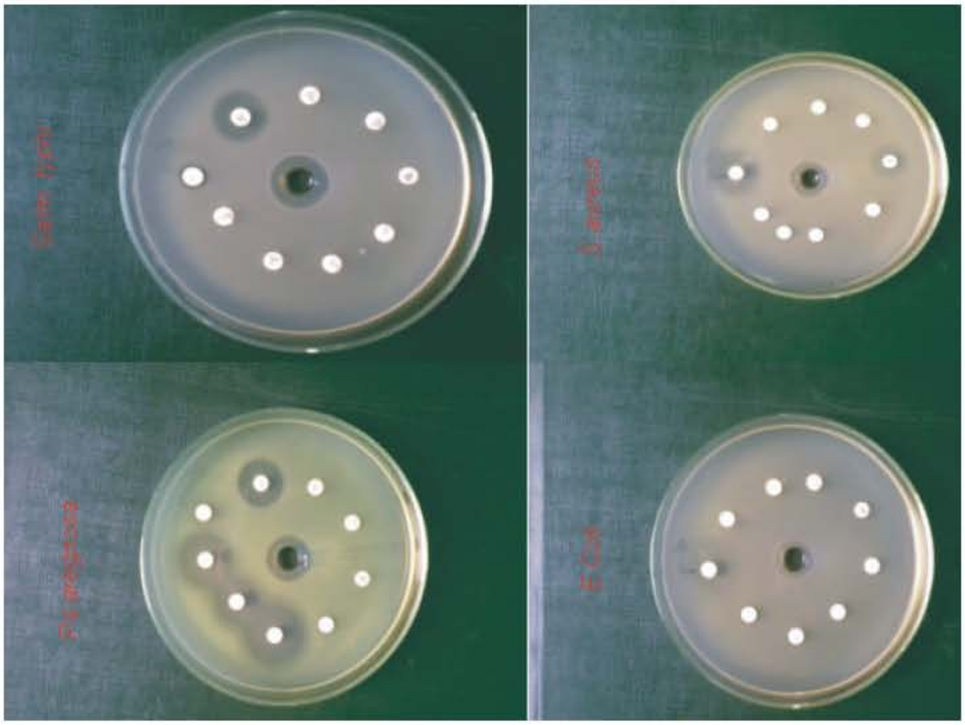

Fig. 1: Antimicrobial susceptibility test for the different used antibiotic groups and sliver against four isolates

which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent.

The present study showed successful formation of colloidal silver and their antibacterial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi (S. typhi)* and *Pseudomonas aeruginosa* (*P. aeruginosa*). Colloidal silver showed a strong bactericidal effect against *E. coli*, *S. aureus* and *P. aeruginosa*, these resuls were in agreement with those reported by Bryaskova *et al*. [14].

In the present experiment, colloidal silver showed highly potent antibacterial activity toward both Gram-positive and Gram-negative bacteria. This condition may be due to its accumulation in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell. Meanwhile, studies have demonstrated that silver ions interact with sulfhydryl (-SH) groups of proteins as well as the bases of DNA leading either to the inhibition of respiratory processes [15] or DNA unwinding [16]. Inhibition of cell division and damage to bacterial cell envelopes was also recorded [17] and interaction with hydrogen bonding processes has been also, demonstrated to occur [18]. However, the mechanism depends on both the concentration of silver ions present and the sensitivity of the microbial species to silver. In the same time, it was reported that contact time, temperature, pH and the presence of free water have clear impact on both the rate and extent of antimicrobial activity [19]. However, in the present study the spectrum of activity was very wide and the development of resistance was not recorded. Silver ions clearly do not possess a single mode of action. They interact with a wide range of molecular processes within microorganisms resulting in a range of effects from inhibition of growth, loss of infectivity to cell death. Another theory explained that colloidal silver works as a catalyst which can disable the enzymes that all unicellular bacteria, fungi and viruses use for their oxygen metabolism. Unlike with antibiotics, resistant strains have never been known to develop tell now.

These nontoxic nanomaterials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials and may be solve the problem of the emergence and spread of *in vitro* antibiotic resistance.

Nanosilver could be electrically prepared with low cost, to be applied for external uses only, not for systemic application and further investigations still needed.

In conclusion, nanosilver solution has a powerful antimicrobial activity against multi drug resistant clinical Ggram positive and Gram negative bacteria and further studies still required.

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