

Short Communication

***In vitro* evaluation of the activity of colloidal silver concentrate against *Pseudomonas aeruginosa* isolated from postoperative wound infection**

Iroha I. R.^{1*}, Amadi E. S.¹, Orji J.O.³ and Esimone C. O.²

¹Department of Applied Microbiology, Ebonyi State University, PMB 053 Abakaliki, Nigeria.

²Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria.

³Department of Applied Microbiology, Ebonyi State University, P.M.B. 053 Abakaliki Ebonyi State Nigeria.

Accepted 15 May, 2008

The high rate of hospital acquired infections caused by pathogenic *Pseudomonas aeruginosa* as an opportunistic infection is currently a serious global health problem. The problem is not only with the diseases caused by this organism but with the rate at which this organism develops resistance to some vital antimicrobial agents. This increase in resistance of *P. aeruginosa* in recent times underscores the need to search for an alternative antimicrobial agent. This study was therefore, designed to evaluate the antimicrobial activity of colloidal silver concentrate against some strains of pathogenic *P. aeruginosa* isolated from post operation eye wound infection, using agar- well diffusion, broth dilution and the killing rate kinetics methods. The results showed that colloidal silver concentrate have an antibacterial activity against pathogenic *P. aeruginosa* and the killing rate kinetics studies reveal that the test organisms were completely killed within 90 min. Further exploitation of colloidal silver for the treatment of multi-drug resistant *P. aeruginosa* infections is hereby suggested.

Key words: Colloidal silver concentrate, pathogenic *Pseudomonas aeruginosa*, killing rate kinetics, post operative wound infection.

INTRODUCTION

Pseudomonas aeruginosa is a virulent opportunistic pathogen that is mostly implicated in hospital-acquired infections especially in the intensive care unit (ICU) (Goossens, 2003). It is responsible for the outbreak of nosocomial infections in different parts of the world and contributes to the morbidity and mortality in hospitalized patients. Despite efforts made to introduce a wide variety of antimicrobial agents with anti- pseudomonal activity, *P.aeruginosa* is still resistant to a variety of antimicrobial agents (Karlowsky et al., 2003).

P. aeruginosa incidence in post wound infection has increased compared to a decade back. Post operative wound infection is a wound infection that occurs after surgical operation and the rate of this infection varies from one hospital to another (Oguntibeju and Nwobu, 2004). The use of silver to treat wound infection after surgery has been a regular practice from the ancient time.

The term 'colloidal silver' refers to substances that consist of ultrafine particles that do not dissolve but remain suspended in a medium of different matter. These ultra-fine particles are larger than most molecules but so small that they cannot be seen with the naked eyes. The particles are electrically charged to activate the germicidal quality of the silver and allow the particles to remain suspended in the solution of deionized water (Lloyd and Zane, 1996). While most antibiotics inhibit about half dozen or so of pathogenic microorganisms, silver kills over 650 pathogenic microorganisms (Wickless and Schwader, 2004). Most importantly, unlike conventional antibiotics, microorganisms cannot build resistance to the action of silver (Warren, 2003). Many forms of bacteria, viruses and fungi utilize special enzymes for their metabolism and silver acts as a catalyst effectively disabling these enzymes (Hall et al., 1999).

The effectiveness of colloidal silver is as a result of its small particle size, which also makes it less toxic. Silver's strong antibacterial properties have been found extens-

*Corresponding author. E-mail: ifynero@yahoo.com.

Table 1. Susceptibility of *Pseudomonas aeruginosa* to colloidal silver.

Test Organism	Inhibition zone diameter (IZD mm)
<i>Pseudomonas aeruginosa</i> (01)	29.00
<i>Pseudomonas aeruginosa</i> (02)	27.00
<i>Pseudomonas aeruginosa</i> (03)	31.00
<i>Pseudomonas aeruginosa</i> (04)	33.00
<i>Pseudomonas aeruginosa</i> (05)	30.00

sively useful in treating some serious infections caused by pathogenic microorganism (Wickless and Schwader, 2004). The present study investigated the *in vitro* activity of colloidal silver against *P. aeruginosa* implicated in post operation eye wound infections in Enugu, using agar well diffusion, broth dilution techniques and killing rate kinetics methods.

MATERIALS AND METHODS

Isolation of test organisms

The test organisms used for this study were five clinical isolates of *P. aeruginosa* isolated from surgical ward of Enugu State University Teaching Hospital (ESUTH), Nigeria, from patients that have undergone operation in the eye. The test organisms were identified and characterized by using standard laboratory technique (Chessbrough, 2002).

Culture media

The culture media used were nutrient agar, Mueller Hinton agar, MacConkey agar, Cetrimide agar, Simmon citrate agar and peptone water (Biotech Laboratories United Kingdom) and the media were prepared according to the manufacturers' specifications in 1000 ml flask and sterilized by autoclaving at 121°C for 15 min.

Preparation of colloidal silver

The colloidal silver solution (Formor products, USA) was prepared using the manufacturer's specifications by adding 1 ml of colloidal silver concentrate to 3 parts (3 ml) of distilled water.

Agar well diffusion technique

An inoculum of the test organism equivalent to 0.5 Macfarland standard was seeded on a Mueller Hinton agar plate. These were allowed to stand for 1 h to gel. An 8 mm diameter well with a depth of about 4 mm was aseptically bored on the Petri dish containing the agar using a sterile cork borer. Prepared colloidal silver solution was used to fill the hole and was incubated at 37°C for 18-24 h.

Broth dilution technique

Colloidal silver concentrate was prepared in a decreasing two-fold serial dilution for each of the test organism in five different test

Table 2. Minimum inhibitory concentration (MIC µg/ml) of colloidal silver against *Pseudomonas aeruginosa*.

Test organism	MIC µg/ml
<i>Pseudomonas aeruginosa</i> (01)	0.078
<i>Pseudomonas aeruginosa</i> (02)	0.078
<i>Pseudomonas aeruginosa</i> (03)	0.078
<i>Pseudomonas aeruginosa</i> (04)	0.313
<i>Pseudomonas aeruginosa</i> (05)	0.313

tubes. Exactly 1 ml each of the serially diluted colloidal silver solution was added into each of the test tubes containing 3 ml of double strength of Mueller Hinton broth. An inoculum of the test organism equivalent to 0.5 Macfarland Standard was added to the test tubes containing the serially diluted colloidal silver. The sixth tube containing the test organism and the Mueller Hinton agar, without colloidal silver served as a control. The tubes were incubated at 37°C for 18-24 h after which the tubes were observed for turbidity or no turbidity.

Killing rate studies

Exactly 5 ml of double strength Mueller Hinton broth was aseptically prepared in four different test tubes and was inoculated with the test organisms. This was incubated at 37°C for 18-24 h. A 0.5 Macfarland equivalent standard of the test organism was re-inoculated into five different test tubes of a double strength Mueller Hinton agar and were incubated for 1 h to activate the organisms. Samples were taken from each of the test tube at different time intervals ranging from 20- 90 min. These samples were spread plated on the surface of an over-dried Mueller Hinton agar plate and incubated at 37°C for 24 h, after which the viable cell counts were taken. The viable cell count of the tube containing only the test organisms without colloidal silver served as a control.

RESULTS AND DISCUSSION

The result of sensitivity studies (Table 1) shows that *P. aeruginosa* was susceptible to antimicrobial action of colloidal silver concentrate. The MIC results (Table 2) of colloidal silver reveals that the test organisms were susceptible within the ranges of 0.078 to 0.313 µg/ml. The killing rate studies showed that the population of *P. aeruginosa* was drastically reduced to non- existing level at the 90th min (Figure 1). The first order killing rate constant was estimated to be 0.084 min⁻¹. This demonstrates that colloidal silver has an antibacterial activity against *P. aeruginosa*.

The rate at which microorganism develops resistance to conventional antimicrobial agents poses a serious public health problem globally. *P. aeruginosa* is known to be resistant to many antimicrobial agents and has been implicated in post operative wound infections (Oguntibeju and Nwobu, 2004). Faced with the challenge of increasing incidence of infections caused by *P. aeruginosa* and its resistance problem, an attempt was made to establish the *in vitro* antibacterial activity of colloidal silver concentrate in our environment.

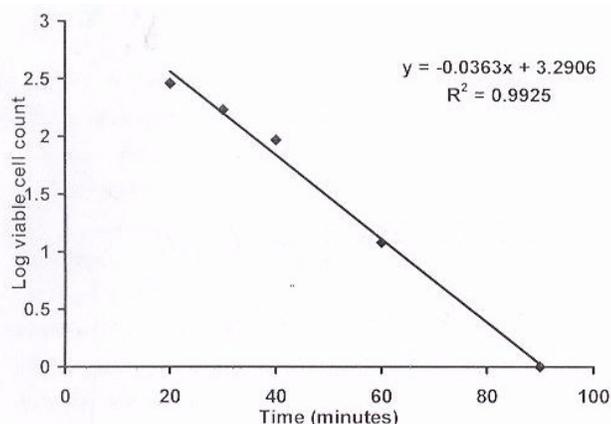


Figure 1. Effect of colloidal silver on the killing rate of *Pseudomonas aeruginosa*.

Colloidal silver is one agent that has a tremendous antimicrobial effect and has been used successfully in the treatment of many infectious diseases without visible side effect (Wickless and Schwader, 2004). Our study has further confirmed the potent antibacterial activity of colloidal silver concentrate against *P. aeruginosa*. This presents a clear indication for the potential applicability of colloidal silver in the treatment of some post operative wound infections caused by this organism. Presently, the use of colloidal silver in the treatment of various infectious diseases is growing exponentially. It has been shown that its presence near a virus, fungi, bacteria or any other single cell pathogen disables its oxygen metabolizing enzymes, and within a few minutes the pathogen suffocates, dies and is cleared out of the body by the immune lymphatic and elimination system (Hall et al., 1999). Unlike pharmaceutical antibiotics, which destroy beneficial enzymes, colloidal silver leaves these beneficial enzymes intact (Hall et al., 1999). An added advantage to the use of silver is that it has variously been shown to have high safety profile *in vivo* and cannot interact or interfere with other medicines being taken (Iroha et al., 2007). Furthermore, it is known not to destroy the normal microbial flora of the gut thereby avoiding the negative effects of traditional antibiotics that kill good bacteria in the lower digestive tract (Beaker and Spadaro, 1981).

The result from Figure 1 showed that the killing potency of colloidal silver is very high, killing a given population of *P. aeruginosa* within 90 min. This could be attributed to its small particle size that increases its surface area, which enables it to penetrate the organism's cell membrane. Also, the ionic (positive charge) nature is considered the primary benefiting feature of colloidal silver being able to aggressively attach to microbial cells and kill them (Hall et al., 1999). In the present study, colloidal silver was shown to demonstrate very potent antibacterial activity against *P. aeruginosa* isolated from post operative eye infection. This activity should be urgently explo-

red in the development of highly active antibacterial therapeutic agents to combat the rapidly increasing infectious diseases that currently challenge the present arsenal of antibacterial drugs.

REFERENCES

- Beaker RO, Spadaro JA (1981). Treatment of orthopedic infection with electrically generated silver ions J. bone surg. 60A: 871-881.
- Chessbrough M (2002). Morphology and characterization of *P. aeruginosa*. District Laboratory practice in tropical countries part 1 and 11. pp. 193-194.
- Goossens H (2003). Susceptibility of multiple *P. aeruginosa* in intensive care units: Results from the European MYSTIC study group. Clin. Microbiol. Infect. 9: 988-993.
- Hall RE, Bender G, Marquis RE (1999). Inhibitory and bactericidal antimicrobial action of electrically generated silver ions. J. Oral. Maxillofac. Surg. 45: 783-787.
- Iroha IR, Esimone CO, Orji JO, Imomoh OO (2007). Antibacterial efficacy of colloidal silver alone and in combination with other antibiotics on isolates from wound infections. Sci. Res. Essay 2(8): 338-341.
- Karlowsky JA, Draghi DC, Jones ME, Thornsherry C, Friedland IR, Shaham DF (2003). Surveillance for antimicrobial susceptibility among clinical isolates of *P. aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United states, 1998-2001. Antimicrob. Agents Chemother. 47:1681-1688.
- Lloyd MC, Zane BCN (1996). Colloidal Silver, the antibiotic alternative. The Association of advanced colloidal silver research USA. pp. 503-604.
- Oguntibeju OO, Nwobu RA (2004). Occurrence of *P. aeruginosa* isolated in post operative wound infection. Pak. J. Med. Sc. 20: 187-91.
- Warren J (2003). Colloidal silver today, the all natural will spectrum germ killer. Health living production Summertown USA.
- Wickless SC, Schwader TA (2004). Medical mystery- the answer. New Engl. J. Med. 352: 2349- 2350.