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Full Length Research Paper

Antibacterial efficacy of colloidal silver alone and in combination with other antibiotics on isolates from wound Infections

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A total of ten clinical bacterial isolates comprising five isolates of *Escherichia coli* isolated from surgical wound patients at the Federal Medical Centre, Abakaliki and five isolates of *Staphylococcus aureus* isolated from the wound sites of burnt patients at the University of Nigeria Teaching Hospital, Enugu were evaluated for their susceptibility to colloidal silver at 5 and 20 ppm using agar dilution method and the killing rate technique. Also, interaction studies between colloidal silver and some conventional antibiotics were carried out against the clinical isolates of *Escherichia coli* and *S. aureus* using disc diffusion technique. The result of the study shows that the test organisms were sensitive to the colloidal silver at both concentrations and also to some of the antibiotics in the paper disc. The killing rate studies revealed that the colloidal silver is highly bactericidal against the test isolates at both concentrations. The drug interaction study showed no antagonism, indicating that concomitant use of colloidal silver with these antibiotics may not affect the therapeutic efficacy of either of these agents.

Key words: Antibacterial, colloidal silver, clinical bacteria isolates, antibiotic discs, killing rate, wound infections.

INTRODUCTION

The upsurge of bacteria resistance to most conventional antibiotics has become a point of worry to the medical institution and these problems have led to the continual search for possible alternative antimicrobial agents that can destroy these resistant microorganisms without any side effect and at a lower cost. Colloidal silver was one alternative identified to be very useful in treating septic wounds with low toxicity and little or no microbial resistance. This agent is non-allergic, possesses very high broad spectrum antimicrobial activity and could boost healing process and the immune system;

(1). Colloidal silver is a suspension of submicroscopic metallic silver particles in a colloidal base which does not attack bacteria directly but rather causes deactivation of enzymes responsible for their respiration, multiplication and metabolism of the organism (2). One side effect of colloidal silver is a permanent blue-gray discolorations of the skin and deep tissue known as Argyria.
(3). In the present study, we attempted to evaluate the potential use of colloidal silver in septic wound infections caused by microorganisms and to check if combinations with conventional antibiotics will increase or decrease the antimicrobial activity of the antibiotic and/or the colloidal silver.

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MATERIALS AND METHODS

Culture media: The culture media used include nutrient broth and nutrient agar (Lab M, United Kingdom). All media were prepared according to manufactures' instructions.

Microorganisms: The clinical isolates used are five isolates of *E. coli* isolated from surgical wound patients at the Federal Medical Centre, Abakaliki and five different strains of *S. aureus* isolated from wound site of a burnt patient from University of Nigeria Teaching Hospital (UNTH) Enugu, Enugu State Nigeria. These isolates were

Table 1. Result of sensitivity studies of colloidal silver against *E. coli* and *S. aureus* at 5 ppm and 20 ppm concentrations

***E. coli* isolate nos**	**5 ppm**	**20 ppm**
1	+	+
11	+	+
111	+	+
1V	+	+
V	-	+
S. aureus isolate nos		
1	+	+
11	+	+
111	+	+
1V	+	+
V	+	+

(-)= Growth of organism was not inhibited by 5 ppm and 20 ppm of colloidal silver (+) = Growth of organism was inhibited by 5 ppm and 20 ppm of colloidal silver

identified using standard procedure for isolating and identifying bacteria (Chessbrough, 2001).

Drugs: Colloidal silver concentration (formor International USA) and commercial antibiotic disc (Maxicare medical laboratory Enugu) were obtained from Enugu state, Nigeria.

SENSITIVITY STUDIES

Agar dilution method: A 19 ml sterile molten nutrient agar was poured aseptically into each of the twenty Petri dishes with 1 ml of 2-folds serially diluted colloidal silver. This was allowed to gel and 0.5 MacFarland equivalent standards of the test organisms (*E. coli* and *S. aureus*) were streaked on the surface of the Petri dishes containing the molten agar and colloidal silver. These were incubated at 37°C for 24 h after which the plates were observed for microbial growth.

Interaction studies: Interaction between the colloidal silver and various antibiotics discs was determined by the disc diffusion technique. Exactly 19 ml each of sterile molten nutrient agar was poured aseptically into ten different Petri dishes and were divided into two groups (A and B). Group A consists of five plates each containing 19 ml of nutrient agar and 1 ml 5 ppm of colloidal silver seeded with 0.5 MacFarland equivalent of *E. coli* while group B also consists of five plates each containing 19 ml of nutrient agar and 1 ml 5 ppm of colloidal silver seeded with *S. aureus*. A Gram positive antibiotic disc was aseptically placed on the surface of plates seeded with *S. aureus* (Group A) while Gram negative antibiotic discs were also aseptically placed on the surface of plates seeded with *E. coli* (Group B) using sterile forceps. Another ten Petri dishes each containing 20 ml of sterile nutrient agar designated group C were prepared. Five were seeded with *E. coli* and five with *S. aureus*. Gram negative antibiotic discs were placed on the surface of plates seeded with *E. coli* while Gram positive antibiotic discs were placed on plates containing *S. aureus* The overall set up was done in duplicate and was incubated at 37°C for 24 h after which the inhibition zone diameter of plates containing colloidal silver, test organism and antibiotic disc and those containing only the antibiotic disc and the test organisms were measured.

Determination of rate of kills using colloidal silver

All the ten test bacteria isolates were grown in nutrient broth at 37°C for 24 h after which each of the test organism were re-inoculated into 3 ml of fresh nutrient broth and was incubated for 1 h at 37°C to activate the organism. A 0.5 ml MacFarland equivalence of the test organisms were added into 3 ml of 5 ppm of colloidal silver solution in ten different test tubes and samples taken from each tube at different time intervals ranging from 5 to 20 min. An appropriate arithmetic serial dilution of the test organism suspended in sterile normal saline was made and spread plated in an over-dried nutrient agar plates. These were incubated for 24 h at 37°C after which the viable cell count was estimated. The viable count of tubes containing only the test organisms without colloidal silver served as control.

RESULTS AND DISCUSSIONS

The result in Table 1 shows that the test organisms were completely susceptible to the therapeutic effect of colloidal silver at both concentrations. This proves the works of other authors that colloidal silver is a wonder antibiotic that has the ability to destroy over 650 microorganisms at a very low concentration without having any deleterious effect in the body tissues (Becker, 1978). Tables 2 shows that the combination interaction of colloidal silver with some gram positive Antibiotics such as (novobiocin, ciproval, ampicillin/cloxacillin, cephalexin, gentamycin, erythromycin and cloxacillin) did not increase the therapeutic efficacy of the drug showing that combining colloidal silver with some antibiotics will not always increase therapeutic action. Although it was observed in Table 3 that the combination interaction of colloidal silver with some gram negative antibiotics (ampicillin, nalidixic acid, nitrofurantoin, tetracycline, ciproval, chloromphenicol) increased the therapeutic effect of the drugs but these does not suggest that colloidal silver should be combined with other drugs for therapeutic purposes. It may be suggested that one should not combine colloidal silver with other drugs as it has been established that it does not really have a synergistic interaction with other drugs. This authenticates the works of some authors that states that colloidal silver does not show any increased activity when combined with other drugs cacy of drugs when combined with them, but that it is more effective when used alone (Lloyd and Zane, 1996). Tables 4 and 5 also show that the killing potency of colloidal silver towards the test organisms was very high at both concentrations. The broad spectrum antimicrobial activity of colloidal silver observed in this study suggests that it may be effectively used as an alternative antimicrobial agent for treatment of septic wounds / burns.

Colloidal silver contains minute silver particles of the ranges of 0.001 microns which results in its overall increased surface area. It has been proposed that this increased surface area is responsible for its quick and fast penetration into the cell wall of bacteria. Colloidal silver works by inhibiting and disabling the oxygen metabolism enzyme bacteria which finally kills off the microbes in an incredibly short period of time (Duncan, 1993). The isolates of *S. aureus* showed zero sensitivity to ampicillin/cloxacilin combination, erythromycin and cloxacillin and this

Table 2. Combined effect of colloidal silver and various antibiotics against *S. aureus*.

Inhibition zone diameter (IZD) (mm)					
Antibiotics/antibiotic + colloidal silver	**Isolate I**	**Isolate II**	**Isolate III**	**Isolate IV**	**Isolate V**
NB	8	10	18	17	12
NB + CS	13	14	16	12	16
% change in IZD	62.5 [i]	40 [i]	11.1 [d]	29.4 [d]	33.3 [i]
AC	0	0	0	0	0
AC + CS	0	0	0	0	0
% change in IZD	0 [ndi]	0 [ndi]	0 [ndi]	0 [ndi]	0 [ndi]
GN	16	19	20	18	20
GN + CS	24	18	20	20	22
% change in IZD	50 [i]	5.3 [d]	0 [ndi]	11.1 [i]	10 [i]
E	0	0	0	0	0
E + CS	2	0	0	0	0
% change in IZD	200 [i]	0 [ndi]	0 [ndi]	0 [ndi]	0 [ndi]
CX	9	6	0	10	12
CX + CS	10	8	0	11	15
% change in IZD	11.11 [i]	33 [i]	0 [ndi]	10 [i]	25 [i]
CVL	19	22	20	18	22
CVL + CS	19	20	16	18	22
% change in IZD	0 [ndi]	9.1 [d]	20 [d]	0 [ndi]	0 [ndi]
TFX	22	24	21	23	20
TFX + CS	26	30	28	28	26
% change in IZD	18.2 [i]	25 [i]	33 [i]	21.7 [i]	30 [i]
CD	19	20	20	22	18
CD + CS	30	26	26	25	28
% change in IZD	57.9 [i]	30 [i]	30 [i]	13.6 [i]	55.6 [i]
CL	0	0	0	0	0
CL + CS	0	0	0	0	0
% change in IZD	0 [ndi]	0 [ndi]	0 [ndi]	0 [ndi]	0 [ndi]
DX	23	20	22	18	16
DX + CS	19	22	18	20	18
% change in IZD	21.1 [d]	10 [i]	18 [d]	11 [i]	12.5 [i]

NDI = No difference in interaction; I = overall % increment in activity when antibiotic is administered concomitantly with colloidal silver; D = overall % decrease in activity when antibiotic is administered concomitantly with colloidal silver; C.S. Collodial Silver (5ppm) Gram positive antibiotic Disc; NB = Novomycin AC = Ampicilln/Cloxacillin; CD = Clindamycin GN = Gentamicin; TFX = Traflox; E = Erythromycin CX = Cephalexin CL = Cloxacillin; DX = Doxycycline; CVL=Ciproval

Table 3. Combined effect of colloidal silver and various antibiotics against *E. coli*. IZD (mm)

Percentage IZD E. coli and Colloidal silver	**Isolate I**	**Isolate II**	**Isolate III**	**Isolate IV**	**Isolate V**
N	0	0	0	0	0
N + CS	13	15	16	10	13
% change in IZD	1300 [i]	1500 [i]	1600 [i]	1100 [i]	1300 [i]
TFX	26	24	28	26	26
TFX + CS	26	22	28	24	25
% change in IZD	0 [ndi]	8.3 [d]	0 [ndi]	7.7 [d]	3.8 [d]
Nor	13	12	14	13	13
Nor + CS	11	10	12	11	11
% change in IZD	15.4 [d]	16.7 [d]	14.3 [d]	15.4 [d]	15.4 [d]
COT	14	13	16	14	15
COT + CS	14	12	15	9	13
% change in IZD	0 [ndi]	7.7 [d]	6.25 [d]	35.7 [d]	13 [d]

Table 3. contd.

G	17	18	19	17	17
G + CS	17	18	20	18	18
% change in IZD	0 [ndi]	0 [ndi]	5.3 [i]	5.9 [i]	5.9 [i]
T E	11	14	15	10	13
T E + CS	14	20	35	14	23
% change in IZD	27.3 [i]	30 [i]	133 [i]	40 [i]	77 [i]
GP	25	24	27	25	25
GP + CS	26	28	29	27	28
% change in IZD	4 [i]	16.7 [i]	7.4 [i]	8 [i]	12 [i]
Nal	17	18	20	17	18
Nal + CS	24	22	25	21	23
% change in IZD	41.2 [i]	22.2 [i]	25 [i]	23.5 [i]	27.8 [i]
ChL	7	4	5	4	5
ChL + CS	9	10	14	12	13
% change in IZD	28.6 [i]	150 [i]	180 [i]	200 [i]	140 [i]
Amp	0	0	0	0	0
Amp + CS	10	11	12	10	11
% change in IZD	1000 [i]	1100 [i]	1200 [i]	1000 [i]	1100 [i]

I = overall % increment in activity when antibiotic is administered concomitantly with colloidal silver
D = overall % increment in activity when antibiotic is administered concomitantly with colloidal silver
CS = Colloidal Silver; GN = Gram Negative antibiotic Disc; N = Nitrofurantoin; TFX = Traflox; Nor = Norfloxacin; Cot = Cotrimazole; G = Gentamycin; TE =Tetracycline; Cip = Ciproval; Nal = Nalidixic acidChl = Chloramphenicol; A = Ampicillin

Table 4. Killing rate constant of colloidal silver concentrate (5 and 20 ppm) against *S. aureus.*

Isolates Nos	5ppm K(min^{-1})	20ppm K(Min^{-1})
01	-0.6218	-0.00322
02	-0.175	-0.0046
03	-0.237	-0.00368
04	-0.22	-0.01405
05	-0.488	-0.0115

Table 5. Killing rate constants of colloidal silver concentration (5 and 20 ppm) against *E. coli.*

Isolate Nos	5ppm K(min^{-1})	20ppm K(min^{-1})
06	-0.0109	-0.00398
07	-0.00875	-0.00046
08	-0.01036	-0.00014
09	-0.00944	-0.00069
10	-0.0069	-0.00046

this effect did not improve when combined with colloidal silver. Generally, the results of the interaction studies showed that colloidal silver can potentate the effect of some antibiotics against pathogenic organisms. Given the diverse cellular mechanism of action of colloidal silver, such potentiation effects may not be completely unexpected (Marshall and Killoh, 1915).

Also, the rapid killing potential of colloidal agent is because minute silver was able to attach to cell receptors and inhibit microbial cellular reproduction and respiration by attacking the important prokaryotic enzymes involved in microbial cell metabolism and the result also revealed that the killing rate was not concentration – dependent (Lloyd and Zane, 1996). We therefore conclude that due to the low toxicity associated with colloidal silver and its high therapeutic activity against pathogenic microorganisms, it can be suggested as an alternative to antibiotics for chemotherapy.

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