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Evaluation of Antibacterial Potential of Silver Nitrate – An *In vitro* Approach

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Abstract: The exploration of antibiotic and antiseptic resistance in the hospitals are happened by various reasons including continuous usage of same antiseptics and disinfectants for surface cleaning, inappropriate and misuse of antimicrobials, No follow up of the patients and discontinuation of the course of drugs. This may create a demand for new and long term effective drugs against antibiotic resistance. Comparing to the chemicaly fabricated drugs, metals are considered as the better choice of replacement. From the ancient times, the basic understanding of antimicrobial properties of metal particles like silver, mercury, copper and arsenic are quite available in practice; but their scientific evidences are lacking. Thus this study may provide some information related to the determination of *in vitro* antibacterial activity of various concentrations of silver solution against several bacterial pathogens isolated from wounds. The evident record of this study highlighted that 10^{-2} mM silver solution showed maximum bactericidal effect on *Staphylococcus aureus*, *Providencia rettigeri* and *Pseudomonas aeruginosa* with 17mm, 16mm and 16mm inhibitory zone respectively. Other test bacterial species also inhibited but not upto the mark. We may recommend the high concentration of silver solution in the form of antiseptic spray as surface bactericidal mask for wound management.
Keywords: Antibiotic resistance, silver, bactericidal nature.

INTRODUCTION

Microbes are ubiquitous and be the source of various human infections that cause mild (treatable) to severe illnesses (complicated). The emergence of superbugs due to the increase in misusing the antibiotics may lead to the advent, further treatment failure and antibiotic disproportionate action observed. To overcome such situations, identification of effective antimicrobial agents are important. The major actions of the antibacterial agents are either bactericidal or bacteriostatic along with that effective for a longer time period and prevent the recolonization of microbes [1-3]. In the 20th century, silver is first approved to use as an antimicrobial agent, but the usage of silver is limited in early cases due to its acute to chronic systemic damage (hepatic, spleenic and renal dysfunction) but no records are found regarding systemic failure. But recently, silver is widely used in the medical field due to its high potent ability to act as antimicrobial activity [4].

Currently, the development of medicine at global standards are observed that enhance the quality life, but still facing various struggles because of the threat of emerging and re-emerging microbial infections. The following are the major reasons for the emergence of habitants as superbugs in various environments that are observed as high risk

- Misuse of antimicrobials by the clinicians
- Abuse of the antibiotics in the health care environment including pharmacy and primary health care clinics, and animal husbandaries
- Overuse of antibiotics during immunosuppressive state, pre, during and post surgical interventions
- Self medication or usage of secondary prescriptions

To overcome the above high risk situations, an alternative should be evoked. By traditional understanding of the biomaterials usage by the historic peoples, it was found interestingly that the infection control was done with self limited hand remedy where herbs and metals in solution or in dissolved form. The adaptation of understanding the interactions of metal ions with the microbes are mainly due to three reasons.

- Creating a reversible mechanism to the resistant microbes,

- Developing a new drug target for the pathogen and
- Reducing the toxicity of the metal ions in the complex form [5, 6].

Traditionally, silver ions are largely used as a potent antimicrobial agent either in simple direct form (silver sprays) or complex indirect form (silver pastes – bashpam) [7, 8]. Chemically defined that the purest form of metallic silver is inert and thus does not react with human tissues or kill the microorganisms. The chemoreduction from metallic to ionic form in the presence of aqueous media, the silver molecules activated leads to cytolysis. High level Ag^+ ion concentration inhibits the pathogens by the simpler mechanisms like arresting the action of respiratory paths, destroying cell wall, inhibiting protein synthesis, impairing the essential enzymes and disturbing the metabolic activity of the microbes including genomic alterations [9, 10]. Depends upon the concentration of the silver solution, the mechanism of silver ions on microbes may change. The condensed silver prevent the DNA replication that leads to apoptosis [11, 12]. This evidences motivated us to investigate the *in vitro* antibacterial activity of the silver nitrate solution against wound infection causing bacterial pathogens.

MATERIALS AND METHODS

Sample collection and culturing

The pus/ wound samples of the patients were collected and preliminary dispensed in the nutrient broth without contamination for pre-culturing. Futher, these were inoculated into the Nutrient agar, MacConkey agar, Blood agar and incubated at 37^{o}C for 24 hours. The specific physical, chemical and biochemical characterization for confirming the presence of bacterial pathogens in the samples were done.

*In vitro a*ntimicrobial activity of silver solution

Various concentrations (10^{-1} to 10^{-4} mM) of silver solution were prepared. Each bacterials isolates were swabbed on Muller Hinton Agar plates. The standard and known sized wells were prepared on agar plates and 100µl of various concentrations of silver solution were loaded in the appropriate wells. All the processed plates were inocubated at 37^{o}C for 24 hours and the zone of minimum and maximum inhibition were recorded.

RESULTS AND DISCUSSION

It was possible to isolate *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* sp., *Klebsiella pneumoniae*, *E. coli*, *Enterococcus* sp and *Flavobacter* sp. The antibacterial result, showed that the *S. aureus*, *K pneumoniae* and *P. aeruginosa* showed maximum zone of inhibition about 20mm each; moderate level of inhibition was observed among *Proteus* sp, *E. coli* and *Flavobacter* sp about 18mm, 16mm and 16mm respectively. *Enterococcus* sp only showed a very least level of inhibition (Table-1).

Among the laboratory isolated bacterial pathogens, *Serratia* sp alone showed maximum inhibition about 20mm; moderate level of inhibition observed in *Providentia rettigeri* (18mm), *Salmonella typhi* (16mm) and *K. oxytoca* (15mm) and *S. paratyphi* B showed low level of zone about 10mm (Figure-1). According to this study result, it was confirmed preliminarily that the silver ions have a potential to act against bacterial pathogens.

Table-1: *In vitro* antibacterial activity of silver solutions

S. No	Bacterial pathogens	Concentration of Ag solution verses zone of inhibition (mm)			
		1%	**2%**	**3%**	**4%**
1	*Proteus* sp	15	16	18	18
2	*Staphylococcus aureus*	10	14	16	20
3	*Klebsiella pneumoniae*	10	13	17	20
4	*Escherichia coli*	10	12	14	16
5	*Pseudomonas aeruginosa*	17	20	20	20
6	*Enterococcus* sp	-	5	8	11
7	*Flavobacter* sp	10	12	14	16

A study reported that the effective inhibition was a silver solution in the gram negative *E. coli* than the gram positive *S. aureus*, due to the bacterial cell wall thickness, that may prevent the action of silver on bacterial cells; thus the concentration of the peptidoglycan play vital role [8]. The lowest inhibition showed in *E. coli* and maximum in *Enterococcus* sp were also reported in previous studies [6], but in this study reversely revealed that the maximum level inhibition in *E. coli* compares with *Enterococcus* sp. This may be happening because of the evolution of the strain. The antimicrobial activity of microbial residues leads to the lysis of *P. aeruginosa* was also recorded [3].

In the wound dressing management, silver ions releasing antiseptics were used, further if the concentration is sub-lethal, they may have a chance to produce resistant bacterial pathogens, so the clinicians must aware about the antiseptics used for wound dressing. When using higher concentrations of silver ion that are acting as antiseptic leads to the maximum inhibition of the wound infectious pathogens [4, 10, 13]. Silver ions incorporation with bacterial cells inhibit the

respiratory system, thus consequently leads to cytolysis [14]. Low level of silver ions concentrations doesn't affect the mechanism and function of the bacterial cells, in this stage there are living susceptibility continued in cells normally, whereas using higher concentrations disrupts the bacterial pathogens [15].

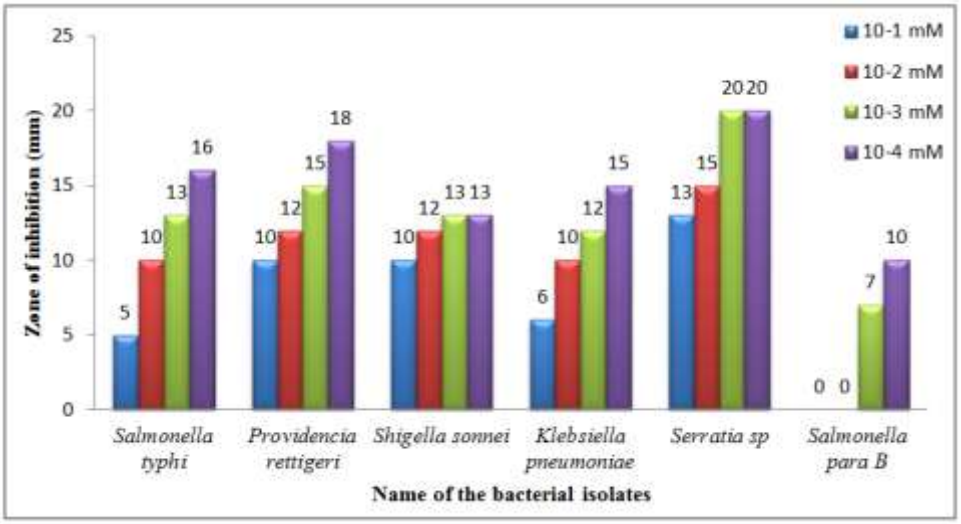

Fig-1: *In vitro* antibacterial activity in laboratory isolated bacteria

The mechanisms of the silver ions on microbial cells required the uptake and utilization of the substrates that affecting cell division and further morphological changes, and detachment of cytoplasm observed [8,16]. The study highlighted the effect of media also play important role in the activation of the silver ions that kills bacterial cells with possible chemical interference [17]. The bioapplications of the silver ions that interact with the proteins of the bacterial pathogens provide a better significant [18].

The usage of silver as antibacterial molecule is dose dependent that binding to the cell wall and modulate the cellular signaling [19], binding with thiols and DNA [20], damage the cell membrane with multilocus principle leads to disturbances in permeability [21, 22], alterations in the genetic make up that encodes envelop proteins [23], destabilization of outer membrane leads to dysfunction of proton motive force [23], disturbing oxygenic environment leads to cellular damage [24]. The synergistic effect of silver ions with antibiotics also provided better results [20].

CONCLUSION

By this study, we presented the *in vitro* ability of the various concentrations of silver ions against the clinical bacterial isolates from wounds and laboratory strains. We further noticed the gap in understanding the mode of action of the silver on various bacterial cells. The possibilities of overcoming the antibacterial resistance may be possible by these types of scientific investigations and further the pharmaceutical companies have to come forward to take these types of result oriented projects for large scale production for human welfare.

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