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Effect of Silver nanoParticles on Carbapenem Resistant Gram-negative Bacteria

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ABSTRACT

Background: Gram-negative bacteria are common causes of both community-acquired and hospital-acquired infections. β-Lactam drugs are often the primary therapeutic option for serious infections, and carbapenems in particular are often considered agents of last resort. Thus, the emergence and spread of carbapenem-resistant (CR) bacteria are a significant public health concern. Infections with these resistant bacteria are associated with higher mortality rates than those for infections caused by carbapenem susceptible organisms. There have been considerable efforts to develop new antimicrobials drug against this type of resistant bacteria. Silver Nanoparticles (AgNPs) have emerged as promising weapons in the anti-microbial arsenal. Their anti-microbial efficacy is attributable to multiple mechanism of action.

Objective: The aim of this study was to evaluate effect of AgNPs on CR Gram-negative bacteria.

Materials and Methods: Effect of AgNPs on CR Gram-negative bacteria alone and in combination with carbapenems. 50 CR Gram-negative bacterial samples were included in this study. They were divided into 4 types; Acinetobacter, *E. coli*, klebseilla and *P. aeruginosa*. Each type was divided into (subtypes) metalo and non metalo carbapenemase producers.

Results: There was statistically significant difference between all types of CR Gram-negative bacteria with AgNPs alone and some of them with AgNPs plus antibiotic (P-value < 0.05). A comparison of the effect of AgNPs alone between different types of detected CR Gram-negative bacteria showed different levels of response. Lowest response was observed with klebsiella (metalo), while highest was remarked with *P. aeruginosa* (metalo). Highest synergy with highly statistically significant difference (P-value < 0.001) occurred with Meropenem in *P. aeruginosa*

Conclusion: AgNPs showed anti-microbial effect against CR Gram-negative bacteria alone and synergistic effect against some of detected CR Gram-negative bacteria with certain antibiotics.

Keywords: AgNPs, CR Gram-negative bacteria, synergistic effect

INTRODUCTION

Gram-negative bacteria are common causes of both community-acquired and hospital-acquired infections, including urinary tract, bloodstream, and lower respiratory tract infections [(1)].

β-Lactam drugs are often the primary therapeutic option for serious infections, and carbapenems in particular are often considered agents of last resort. Thus, the emergence and spread of carbapenem resistant bacteria (CRB) are of significant clinical and public health concern [(2)].

Infections with these resistant bacteria are associated with higher mortality rates than those for infections caused by carbapenem susceptible organisms [(3)].

There have been considerable efforts to develop new antimicrobials by screening natural products, modifying existing antibiotics and synthesizing antimicrobial peptides. Synthetic macro molecules such as polymers that mimic host defense peptides are frequently used as biomedical agents [(4)].

Nanoparticles (NPs) have emerged as promising weapons in the anti-microbial agents. Their anti-microbial efficacy is attributable to multiple mechanism of action. For example, their large surface area enabling high synergy arising from multivalent interactions. Also, NPs functionalized with small molecule ligands exhibit broad-spectrum activity against bacteria [(5)].

However, an alternative strategy is to use NPs in combination with existing antibiotics to combat MDR bacterial infections [(6)].

This study was performed to evaluate effect of AgNPs on CR Gram-negative bacteria alone and in combination with carbapenems.

MATERIALS AND METHODS

Materials:

1. 50 bacterial isolates; They were divided into 4 types; Acinetobacter, *E. coli*, klebseilla and *P. aeruginosa*. Each type was divided into (subtypes) metalo and non metalo carbapenemase producers. This study was conducted in collaboration between the Clinical Pathology department and other clinical departments Al-Houssien University Hospital, Faculty of Medicine, Al-Azhar University. **The study was approved by the Ethics Board of Al-Azhar University.**

All isolates were collected from Al-Houssien University Hospital over the period from 22 th December 2017 to 15th May 2018. Appropriate consent was obtained from the patient participating in this study after explaining the sampling procedure as well as the aim of our work to them.

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Inclusion criteria: 50 bacterial isolates (from: sputum, urine & wound) were collected from in & outpatients of Al-Houssien university hospital. All bacterial isolates were CR Gram-negative. Patients of all ages were included.

Exclusion criteria: Other types of organisms (Gram-positive, fungi,..). Bacteria showed intermediate resistance.

2. Silver nanoparticles; Properties: Appearance: Yellow &Liquid, Concentration: 1000 ppm., Avg. Size: 10 ± 3 nm, spherical shape. They were applied alone by two methods (Complete cut & sterile disc) and in combination with different carbapenems (mentioned below).

3. Sterile discs (SD067); sterile discs was used for testing AgNPs alone, Appearance: Sterile filter paper discs of 6mm diameter.

4. Carbapenems antibiotic discs (Meropenem, Imipenem and Ertapenem); antibiotic concentration in disc: 10 µ.

5. Culture media: MHA plates, blood agar, macconky plates, brain heart infusion broth,….ect.

Statistical analysis:

All results were analyzed using Statistical package for social science (SPSS V.15, IBM Corp. U.S.A).

Descriptive statistics used for quantitative data were; Mean ±SD while categorized data were represented as numbers and percentages. Chi square test was used for comparison of sex.

Friedman One way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were used to compare means of parametric data of different groups.

Pearson correlation coefficient was used to check for correlation between two quantitative parametric data.

For all analysis, a two-tailed test was used and p <0.05 was considered statistically significant.

RESULTS

As regard AgNPs, they showed synergistic effect with Meropenem in *E. coli* (metalo and non metalo) and *P. aeruginosa* (metalo). Also synergism happened with both Imipenem and ertapenem in klebsiella (non metalo) and in *P. aeruginosa*. Highest synergy with highly statistically significant difference (P-value < 0.001) occurred with Meropenem in *P. aeruginosa* (metalo) as shown in table (1). Other types of bacteria and carbapenems showed non statistically significant difference (P-value > 0.05). A comparison of the effect silver nanoparticles alone between different types of detected CR Gram-negative bacteria showed different levels of response with statistically significant difference (P-value < 0.05). Lowest response was noted with klebsiella (metalo), while highest response was observed with *P. aeruginosa* (metalo) as shown in table (2).

Table (1): Comparison between MEM and MEM + Ag as regard to *P. aeruginosa* (metalo) sensitivity.

Variables \ Groups		MEM (N = 7)	MEM + Ag (N = 7)	T-test p-value
P. aeruginosa (metalo)	Mean	0	13.8	0.001
	±SD	0	3.8	

Table (2): Comparison between different types of CR Gram-negative bacterial isolates detected in Al-Houssien university hospital as regard to effect of silver nanoparticles by Complete cut (punch out) method.

Groups / Variables		Acineto non-metalo	Acineto Metalo	*E. Coli* non-metalo	*E. Coli* metalo	Kleb non-metalo	Kleb metalo	Pseudo. Metalo	T-test p-value
Complete Cut	Mean	12	11.7	13	12.8	11.5	9.5	18.1	0.024
	±SD	4.6	8.09	1.6	3.1	1.9	4.7	5.5	
	N	4	4	11	14	4	6	7	

DISCUSSION

Data collection from Al-Houssien Hospital showed that Gram-negative bacteria are common causes of community-acquired and hospital-acquired infections, including urinary tract, wound, and lower respiratory tract infections as recorded by ***Agarwal et al.*** [(1)]. Also, results of several studies showed that CR is common in Gram-negative bacteria than Gram-positive bacteria. Only three samples were CR Gram-positive to fifty CR Gram-negative bacterial samples was found in Al-Houssien University Hospital.

In this study, patient's data showed that CR is mostly associated with immunosupperesed patient especially in patients with chest infection and UTI. These patients were susceptible to risk factors such as; hypertension, diabetes, treatment with chemotherapy and ICU admission which play important role in developing CR Gram-negative bacteria. These risk factors show less association with developing CR Gram-negative bacteria in wound.

Infections with these resistant bacteria are associated with higher mortality rates than those for infections caused by carbapenem susceptible

organisms [3]. Most cases are multidrug resistant (MDR) infections and require prolonged antibiotic treatment which are associated with extensive health care costs and further contribute to an increase in antibiotic tolerance in surviving bacterial cells. Recent data suggests that annual global deaths caused by MDR infections will reach 10 million by 2050 [7].

There have been considerable efforts to develop new antimicrobials. Nanoparticles (NPs) have emerged as promising weapons in the anti-microbial agents. Their anti-microbial efficacy is attributable to multiple mechanism of action [5]. In this study we used silver nanoparticles for many reasons such as; low coast in comparison to other metal NPs, high bactericidal effect and many antibacterial applications were done commercially.

This study showed that silver nanoparticles have anti-microbial effect on detected samples by both methods (complete cut and sterile disc methods). Complete cut was used by ***Abd El Fattah et al.***, while no published study use the sterile disc method. They help carbapenems to increase the inhibitory zone but do not reach the level of sensitivity by 20 micron of 1000 ppm silver nanoparticles, this also is going with ***Abd El Fattah et al.*** [8].

CONCLUSION

Regarding effect of silver nanoparticles alone on carbapenem resistant Gram-negative bacteria; Silver nanoparticles have anti-microbial effect on detected samples by both methods (complete cut and sterile disc methods). Maximum effect happens with *P. aeruginosa*, while least happens with klebsiella (metalo). AgNPs combination has good inhibitoray activity, but not in all detected types. They helped carbapenems to increace the inhibitory zone but do not reach the level of sensitivity at 20 micron of 1000 ppm AgNPs. Dose adjustment of AgNPs may help to reach level of sensitivity.

RECOMMENDATION

Continuous support and frequent observation are required for such a proGram to be successful. Concern has been raised on the toxicity of chemical agents used in AgNPs synthesis. Thus, it is essential to develop a green approach for AgNPs production without using hazardous substances to the human health and environment. Using nanosilver as a component of building materials (aspiration tube & endotracheal tube) may be of value. Thus, our results should provide a valuable reference for the future treatment of CR Gram-negative bacteria.

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