ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Effect of Gold Nanoparticles on Gram negative bacteria *Aeromonas hydrophila*

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## *Manuscript Info*

***Manuscript History:***

Received: 15 May 2014
Final Accepted: 19 June 2014
Published Online: July 2014

***Key words:***

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## *Abstract*

In order to know the effect of the gold nanoparticles on Aeromonas hydrophila antigens on the white mice , ( 18 ) mice ( from both sex ) was used and divided onto 5 groups each group immunized with 0.2 ml of the extracted antigens except the control group which injected with 0.2 ml of phosphate buffer saline . serum collected after ( 20 ) days of immunization.

Several immunological methods was used to determine the presence of antibodies that formed as a result of the mouse immune response . The results showed that most of the used antigens in the study gives positive results in SAT test while the LPS antigens with 10 mg/ml and Pili antigen gives negative gives negative results to the presence of antibodies on slide agglutination test .

While all antigens understudy gives appositive results to the presence of Antibodies in ELISA Test , and This results was confirmed by the using of counterimmunoelectrophoresis technique which give the same result with ELISA test .The immunization with gold nanoparticles showed higher immune responses for the antibodies . it was noticed from the immunological test results that the antigens with the gold nanoparticles gives high percentage for the presence of antibodies which indicate that these antigens made strong immune response in comparison with the non-gold nanoparticles antigens. Also the LPS antigens with 200 mg/ml and the whole cell antigens of A. hydrophila gives the highest percentage for the presence of antibodies in comparison with the other extracted antigens used understudy.

This results was compared with control samples which give negative result to the present of antibodies



## Introduction

Aeromonashydrophila is gram negative bacteria , coccobacilli , non-spore forming , facultative anaerobic , motile , positive to Oxidase and Catalase ( Alperi et al.,2010 ) .

A.hydrophila was identified as acausative agent of human disease such as septicemia , meningitis , wound infection and diarrhea due to exposed to contaminated water ( EvangelilaBarrebet al.,2010 ; Messi.,2003 ) .

The Aeromonads have wide variety of virulence factors include Hemolysin ,cytotonic and cytotoxic enterotoxins , s-layer , Leucocidins and endotoxin which work together to cause disease , Also in the host . Aeromonas species produced awide range of exoenzymes several of them believed to contribute in pathogenesis( G.Y. Trouer et al.,2000 ).

Discovery of virulence factors is important to understand bacterial pathogenicity and its interactions with the host that can be serve as excellent targets in treatment and vaccine developing( Hsing-Juwu et al.,2008 ) .

For the majority of disease caused by bacterial pathogens , pathogenesis is multifactorial ,so it is difficult to determine precisely the role of any given factor . however , there are correlations between strains isolated from particular diseases and expression of particular virulence determinants ,which suggests their role in particular

diseases ( Shokrollah et al.,2009 ).A better understanding of the relationship between immune responses to specific pathogens and protection is needed( Lowy,1998 ).

Nanotechnology has dynamically developed as an important field of modern research withpotential effects in electronic and medicine (Glomm 2005, Chan 2006, Boisselier and Astruc2009). Nanotechnology can be defined as a research for the design, synthesis, andmanipulation of structure of particles with dimension smaller than 100nm. A new branch ofnanotechnology is nanobiotechnology. Nanobiotechnology combines biological principleswith physical and chemical procedures to generate nano-sized particles with specificfunctions. Nanobiotechnology represents an economic alternative for chemical and physicalmethods of nanoparticles formation. These methods of synthesis can be divided on intracellular and extracellular (Ahmad et al. 2003).

The nanoparticles can act as antigen carriers to deliver and release antigens to specific targets, and enhance the immune response against a variety of antigens as adjuvants( Hong Ren et al.,2013 ) .

## Material and Methods

**Bacterial isolate :**

Clinical isolates was collected from wound , burns and pus samples in Al-Jamhoree Teaching hospital in Mosul City/Iraq , (from both sex ) by using sterile cotton swabs and cultured on MacConkey agar and Blood agar , plates was incubated at $37^{o}$c for 24 hour .gram stain ,biochemical test and Antibiotic sensitivity were done for identified isolates .

**Extraction and preparation of A. hydrophila antigens**

Several methods were used for extraction according to the type of extracted antigens .

Preparation of whole cell antigen of A.hydrophila was employed according to ( Shimada & Arakawa.,1999 ; Shimada & Arakawa.,1994 ), extraction of Lipopolysaccharide was done according to (A.Mirzaei etal.,2011 ; S.chibberetal.,2004 ) and the extraction of Pili by ( Helaine . S.,2005 ) .LPS and Pili were detected using TLC(Elizabeth A etal.,1983 , Caroff M& Karibian D.1990, Nanette S. etal.,2004, KapustinaNV etal.,2004) .

**Exposition of extracted antigens understudy to precipitated Gold Nanoparticles**

The extracted antigens understudy was exposited to Gold Nanoparticles precipitated by plasma using ( supply from Quorumtech Q 150RES England company ) in physics department /mosul university,, by using 10 mM current , 1nM thickness and $1*10^{-1}$mBar pressure .

**Immunization**

Eighteenwhite mice BALB/c , age (6-8)weeks , was used in our experiment . the mice were adapted in animals house for 10 days before immunization .

Mice injected with antigens subcutaneous between the shoulders blades after sterilized it with 70% ethanol .mice were divided into 5 groups :

1st group : immunized with whole cell antigen of A.hydrophila with and without Gold Nanoparticles (GNP).
2nd group : immunized with LPS 10 □g/ml of A.hydrophila with and without Gold Nanoparticles (GNP).
3rd group : immunized with LPS 200 □g/ml of A.hydrophila with and without Gold Nanoparticles (GNP).
4th group : immunized with Pili antigens of A.hydrophila with and without Gold Nanoparticles (GNP).
5th group : ( Negative control ) injected with 0.2 ml of phosphate Buffer Saline ( $P^{H}$ 7.2 ) .as in table ( 1 ) :

**Serum preparation**

The blood wasdrawn from mouse s heart during dissection , mouse was anesthetized using ether , and after dissection process the needle was introduced into ventricle of the heart to draw sufficient volume of blood . The collected blood left one hour in room temperature for clotting , then centrifuged at 2500 rpm for 10 min . the serum stored in refrigerator till use .( HemA, Smith A .,1998)

**Immunological methods**

Different immunological methods were used to determine the presence of the antibodies formed against the previous prepared antigens , the used immunological methods are :

- Slide agglutination test ( SAT ) ( Nakazawaetal.,1983 )
- Enzyme Linked Immunosorbentassay ( ELISA )
- Counter Immunoelectrophoresis ( CIEP ) ( Edwards etal.,1982 )

## Results and discussion

**Table ( 1 ) : Injection Mice by different Antigens .**

| Type of antigens | | Concen. of Ag g/ml | Volume of Ag solution ml | Volume Of Ag boost immunization | Injection site | days |
|---|---|---|---|---|---|---|
| **Antigens with GNP** | **Antigens without GNP** | | | | | |
| WCA of A.hydrophila | WCA of A.hydrophila | 70 | 0.2 | 0.1 | s.c | 20 |
| LPS | LPS | 10 | 0.2 | - | s.c | 20 |
| LPS | LPS | 200 | 0.2 | - | s.c | 20 |
| Pili | Pili | 70 | 0.2 | 0.1 | s.c | 20 |

**Bacterial isolate :**

Clinical isolates of A.hydrophila were diagnosed by Grams stain andbiochemical tests( Abbott et al.,2003 ; Carnahan and Joseph.,2005 ). Antibiotic susceptibility test was undertaken by the desk method on muller-hinton agar plates , isolates were classified as Sensitive ( S ) , Intermediate ( I ) or Resistant ( R ) according to the guideline of the Clinical and Laboratory Standard Institute (CLSI..,2013).

**Immunological methods**

The serum samples were collected from the immunized mice with the extracted antigens and the formed antibodies were determined by using three different immunological methods . The result from these methods were summarized in table ( 2 ) .

The results showed that most of the antigens used in the study gives positive results in SAT test in percentage ( 50% ), while LPS 10 mg/ml and Pili antigen give negative results to SAT test . SAT consider as fast and simple test , and give the result less than three minute , also it s require only a little amount of serum or antigen , so we can use drop from both serum and antigen to show the result .

ELISA test was used also to determine the presence of IgG antibodies , and give positive results to the presence of antibodies that formed by the immune response,This results was confirmed by using CIEP method which gives the same results of ELISA test .Counter immunoelectrophoresis used in antigen-antibody system in which the antigen migrates towards the anode during electrophoresis , forming a precipitation band with the homologous antiserum which is back-migrating due to electroendosmosis and the precipitation band formed only between the antigens and antibody wells ( Randall A.Smithet al.,1981 )the figure ( 1 ) show the precipitate lines of antigen and antibody in CIEP test .

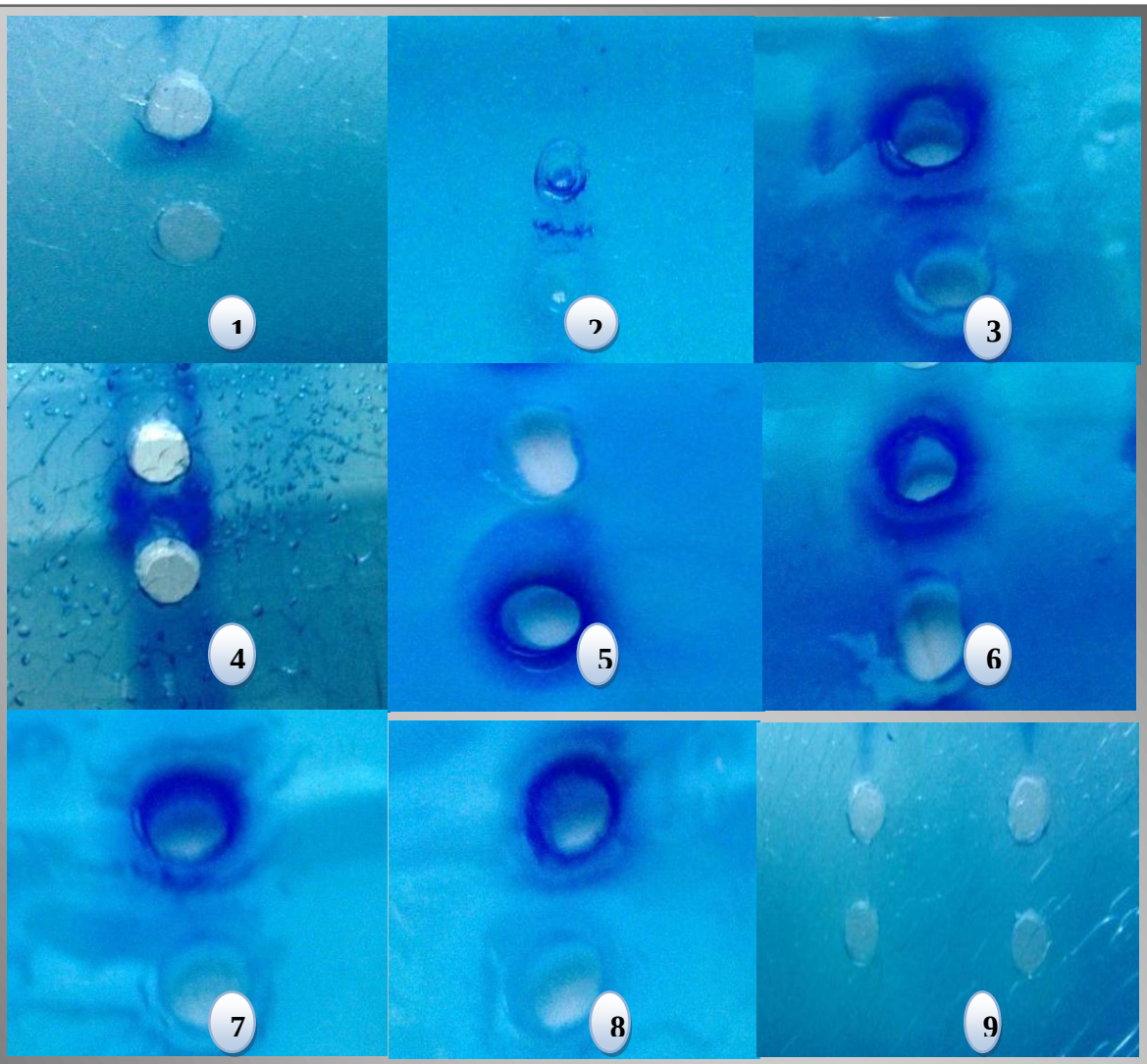


FIG ( 1 ) : counter immunoelectrophoresis of sera :

1=WCA
2=WCA+GNP
3=LPS 200mg/ml
4=LPS 200mg/ml +GNP
5=LPS 10mg/ml
6=LPS 10mg/ml +GNP
7=Pili antigen
8=Pili antigen +GNP
9= Negative control

CIEP is a good adjunct to diagnosis of infectious disease but it should not be the only laboratory method used to rule out or confirm an infection . use of the CIEP test to identify bacteria is acceptable , provided that the conditions needed to detect soluble antigens in culture supernant are met and that specific and high titer antisera are used ( Jone G. Fuung&Konard W.,1981 ) .

ELISA is an immunological technique used todiagnose various infectious diseases of human, animals aswell as plants which is more than 99% sensitive and specificthan any other serological test (Kemeny&Challacombe,1989).

Inspite ofDifferent serological tests have been used for antigendetection such as rapid slide agglutination test, agar gelprecipitation test but ELISA is best method for detection ofantigen (Rehmanet al., 2002).TheseELISAs are readily adaptable to clinical laboratoriessince they avoid the use of radiolabels, arerelatively inexpensive, and require very smallamounts of patient serum.(KATHLEEN SNELL JAGGER.,1982)
From the results of immunological tests were noticed that the antigens with Gold nanoparticles gives higher percentage for the presence of antibodies which indicate that these antibodies enhanced immune responsestronger than antigens without Gold nanoparticles also the Lipopolysaccharide 200 mg/ml and whole cell antigens gives high percentage for the presence of of antibodies compared with other extracted antigens understudy .
This results was compared with control samples which give negative result to the presence of antibodies .

The immunization with Nanoparticles showed high response of antibodies , whereas nanoparticles could work as antigen carrier to transport and release antigens to the targets and enhanced immunological response against different antigens the table ( 2 ) show the percentage of presence of antibodies formed against antigen with and without nanoparticles ..

**Table ( 2 ) : the percentage of presence of antibodies formed against antigen with and without nanoparticles by using different immunological methods understudy**

| Antigens understudy | SAT | | | | | | ELISA | | | | | | CIEP | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Un diluted serum | 1:2 | 1:4 | 1:8 | 1:16 | % | Un diluted serum | 1:2 | 1:4 | 1:8 | 1:16 | % | Un diluted serum | 1:2 | 1:4 | 1:8 | 1:16 | % |
| **WCA** | + | + | + | - | - | 60 | + | + | + | - | - | 60 | + | + | + | - | - | 60 |
| **WCA with GNP** | + | + | + | + | - | 80 | + | + | + | + | - | 80 | + | + | + | + | - | 80 |
| **LPS 10 mg/ml** | - | - | - | - | - | 0 | + | - | - | - | - | 20 | + | - | - | - | - | 20 |
| **LPS 10 mg/ml with GNP** | - | - | - | - | - | 0 | + | - | - | - | - | 20 | + | - | - | - | - | 20 |
| **LPS 200 mg/ml** | + | + | + | - | - | 60 | + | + | + | - | - | 60 | + | + | + | - | - | 60 |
| **LPS 200 mg/ml with GNP** | + | + | + | + | - | 80 | + | + | + | + | + | 100 | + | + | + | + | + | 100 |
| **Pili antigen** | - | - | - | - | - | 0 | + | - | - | - | - | 20 | + | - | - | - | - | 20 |
| **Pili antigen with GNP** | - | - | - | - | - | 0 | + | - | - | - | - | 20 | + | - | - | - | - | 20 |

(+)=positive ( - ) = negative

Generally, gold nanoparticles provide non-toxic routes to drug and gene deliveryapplication. Gold nanoparticles are capable of delivering large biomolecules (peptides,proteins, or nucleic acids like DNA or RNA) (Ghosh at al. 2008) .
Particle size is a critical factor that affects the immune response. Generally,small nanoparticles are known to be more effective targeted-delivery systemthan large particles because small nanoparticles can easily penetrate

thebiological barriers. So one would expect stronger immune response from smallnanoparticle-antigen conjugates( Gutierro,I et al.,2002 ; Vila, A et al.,2004 ).

The long term toxicity of GNP is not well known. It is suggested that GNP have, in general, low acute toxicity both in vitro and in vivo, and its toxicity depends on particle size, concentration, and surfaces properties.[42] Specifically in the eye there is some data on GNP toxicity. In different animals models the intravenous, intravitreal, or corneal administration of GNP seem to have no evident retinal and/or corneal toxicity. It is suggested that GNP have, in general, low acute toxicity both in vitro and in vivo, and its toxicity depends on particle size, concentration, and surfaces properties( Bakrisl. Pulido IS.et al.,2008 ;KhlebtsovN, DykmanL.,2011 ;SharmaA et al.,2011 ).
Nanoparticles can easily enter cellsalthough the mechanism(s) involved are not wellunderstood.The nanoparticle influx occurs by endocytosis The particles are inserted and diffused through the lipid bilayer of the cell membrane Furthermore, these nanoparticles were shown to be able to enter the cells even after linkage to proteins such as antibodies(Dai .,Y.Tanet al.,2002;Shikman,N.W&Oconnel,et al.,2005 ) .

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