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ORIGINAL ARTICLE

# A sensitive colorimetric assay for identification of *Acinetobacter baumannii* using unmodified gold nanoparticles

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## Abstract

**Aims:** *Acinetobacter baumannii* is a global health problem, which threatens many healthcare settings. The current study aims to develop a detection assay for *Ac. baumannii* using unmodified gold nanoparticles (AuNPs).
**Methods and Results:** Fifty-three *Ac. baumannii* clinical isolates were collected from Egyptian hospitals. Bacterial isolation and biochemical identification of isolates were carried out followed by DNA extraction using boiling method and PCR amplification of the 23S–16S rRNA intergenic spacer sequences (ITS). AuNPs were synthesized using citrate reduction method. Detection and optimization of *Ac. baumannii* amplicons using unmodified spherical AuNPs were performed using species-specific DNA oligonucleotide. The nano-gold assay was able to colorimetrically detect and distinguish *Ac. baumannii* from other Gram-negative bacteria. The turnaround time of the assay is about 2 h including sample treatment and amplification. The assay detection limit is 0·8125 ng of DNA.
**Conclusions:** The developed colorimetric assay is sensitive, fast and reliable and can be used for identification of *Ac. baumannii*.
**Significance and Impact of the Study:** There is a need to develop robust, rapid, and specific methods for detection of *Ac. baumannii* isolated from clinical specimens. The developed nanogold assay prototype allows sensitive, specific and rapid detection of amplified DNA of *A*. baumannii and represents a reliable diagnostic tool to aid routine laboratory identification of this pathogen.

## Introduction

*Acinetobacter baumannii* has become global health threat and burden especially in hospitals. *Acinetobacter* spp. was found to be the reason behind a wide range of hospital-acquired infections (HAIs) such as nosocomial pneumonia, urinary tract infections, meningitis and superinfections in burn patients (Bergogne-Berezin and Towner 1996). In the period from 2006 to 2009, the worldwide prevalence rate has been reported to be about 6·8% in North America, 7% in Europe, 18·6% in Latin America and 17·5% in Asia (Gales *et al.* 2011). In addition, a terrifying increase in HAIs caused by multidrug-resistant *Ac. baumannii* has been lately recognized in many Egyptian hospitals (Al-Hassan *et al.* 2013; Fouad *et al.* 2013). *Acinetobacter baumannii* is characterized by its extraordinary ability to persist in the environment. It has magnificent ability to acquire resistance mechanisms against several antibiotic classes including third-generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides (Bergogne-Berezin and Towner 1996).

*Acinetobacter* is a Gram-negative, oxidase-negative, catalase-positive and nonmotile coccobacilli. There are more than 20 genomic *Acinetobacter* species, but only few of them are considered potentially pathogenic. *Acinetobacter baumannii* was found to be the most clinically relevant

species (Carr *et al.* 2002; McGowan 2006). *Acinetobacter* spp. can be easily misidentified. It sometimes resists decolourization step during Gram stain, thus may be identified incorrectly as a Gram-positive, which adversely affects drug choice. In addition, no unique metabolic reaction was able to differentiate *Acinetobacter* from other nonlactose fermenter Gram-negative bacteria. Due to these microbial criteria, phenotypic identification of *Acinetobacter* is particularly challenging (Howard *et al.* 2012).

Traditional biochemical and other identifications systems as the API 20NE system and the Vitek GNI card encountered difficulties to identify *Ac. baumannii* species (Dijkshoorn *et al.* 2007). A breakthrough in the identification of *Acinetobacter* species was achieved with the development of advanced molecular methods such as amplified 16S rRNA gene restriction analysis (ARDRA) (Vaneechoutte *et al.* 1995), amplified fragment length polymorphism (AFLP) (Janssen *et al.* 1997), ribotyping (Gerner-Smidt 1992), tRNA spacer fingerprinting (Ehrenstein *et al.* 1996), restriction analysis of the 16S–23S rRNA intergenic spacer sequences (ITS) (Dolzani *et al.* 1995) and sequence analysis of the 16S–23S rRNA gene spacer region (Chang *et al.* 2005). Although molecular methods have been proven as reliable and sensitive techniques for species identification, but the obstacles associated with these techniques (e.g., high cost, being laborious, need for complicated instrument and well-trained personal) restricted their dissemination in routine laboratory identification of *Acinetobacter*.

Several nanoparticles have been used to develop versatile testing strategies which offer technical advantages over traditional methods. Strategies based on use of gold nanoparticles (AuNPs) have particularly stood out in this arena (Azzazy *et al.* 2012). The distinguished physicochemical properties of AuNPs have been utilized for many diagnostic applications. The unique optical phenomenon called surface plasmon resonance (SPR) is responsible for characteristic colour of colloidal AuNPs (Shen *et al.* 2006). AuNPs colour in solution depends on the dispersion and interparticle distance between AuNPs. Dispersed AuNPs appear red in colour while aggregated ones appear blue (Jin *et al.* 2003). Interactions between single-stranded DNA (ss-DNA) and double-stranded DNA (ds-DNA) with AuNPs have been investigated (Li and Rothberg 2004). ss-DNA can adsorb onto the negatively charged surface of unmodified AuNPs via the attraction with the exposed positively charged nitrogenous bases of ss-DNA. On the other hand, ds-DNA cannot adsorb onto the AuNPs surface due to the repulsion with the negatively charged phosphate backbone. The adsorption of ss-DNA on the surface of AuNPs increases the overall negative charge and electrostatic repulsion between AuNPs and thus allows them to resist salt-induced aggregation (Li and Rothberg 2004). This concept has been successfully employed for detection of hepatitis C virus in serum (Shawky *et al.* 2010) and detection of *Mycobacterium tuberculosis* isolated from clinical specimens (Hussain *et al.* 2013). In this study, a nanoparticle-based assay prototype was developed for detection of clinically isolated *Ac. baumannii* strains.

## Materials and methods

### Bacterial strains

A total of 62 strains were used in this study to assess the performance of the developed assay. Nine reference strains were used; *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumonia* ATCC 23495, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Streptococcus pneumoniae,* ATCC 33400, *Ac. baumannii* ATCC 17978 and 53 *Ac. baumannii* clinical isolates were recovered from intensive care units of three hospitals. *Acinetobacter baumannii* isolates were biochemically identified, in addition to identification by analysis of the $bla_{\mathrm{Oxa\text{-}51}}$-like genes (Prashanth and Badrinath 2000; Turton *et al.* 2006).

### Bacterial DNA extraction and amplification of ITS region

Bacterial DNA was extracted by the boiling method (Millar *et al.* 2000). Bacterium-specific universal forward (5′-TTGTACACACCGCCCGTC-3′) and reverse primers (5′-TTCGCCTTTCCCTCACGGTA-3′) (Gurtler and Stanisich 1996) were synthesized (Metabion, Steinkirchen, Germany) and used to amplify a DNA fragment of 23S-16S rRNA intergenic gene spacer (ITS). PCR was performed with 5 $\mu$l (50 ng) of template DNA in a reaction mixture (50 $\mu$l) containing 75 mmol $l^{-1}$ Tris-HCl (pH 8·8), 20 mmol $l^{-1}$ ammonium sulphate, 1·5 mmol $l^{-1}$ $MgCl_2$, 0·8 mmol $l^{-1}$ deoxyribonucleoside triphosphates (0·2 mmol $l^{-1}$ each), 1 $\mu$mol $l^{-1}$ primer (each) and 1 U of *Taq* DNA polymerase (Fermentas, Ottawa, Canada). The PCR started with a denaturation step (94°C for 3 min), followed by 35 cycles of denaturation (94°C for 1 min), annealing (55°C for 1 min) and extension (72°C for 1·5 min), and a final extension step at 72°C for 7 min. Amplicons were visualized on 1·5% wt/vol agarose gel stained with ethidium bromide. PCR product was purified using Wizard SV gel and PCR clean-up system Promega, USA, Cat no. A9282. DNA concentration was quantified using a UV-Vis spectrophotometer (model 7315; Jenway, Worcestershire, UK).

### Preparation and characterization of AuNPs

Preparation of spherical AuNPs were performed by citrate reduction method (Sato *et al.* 2003). Briefly, a solution of $HAuCl_4$ (50 ml $H_2O$, 0·167 ml 10% $HAuCl_4$) was refluxed with stirring. Then, a solution of sodium citrate (0·1 mol $l^{-1}$, 1·94 ml) was rapidly added. The solution remained boiling for 15 min and, then, cooled at room temperature. The resulting wine-red coloured solution was filtered using a 0·45-$\mu$m syringe filter and transferred into a clean glass bottle. The shape and size of the synthesized AuNPs were characterized by scanning electron microscopy (SEM; Model: Leo Supra 55). The size distribution of the formulated AuNPs was studied by dynamic light scattering measurement using Malvern Zeta sizer (Model3000HSA). Spectrophotometric analysis of the AuNPs was performed by scanning in the range of 400–700 nm using a UV- Vis spectrophotometer (model 7315; Jenway).

### Colorimetric detection of *Acinetobacter baumannii* isolates amplified ITS region DNA using AuNPs

Colorimetric AuNPs-based detection of isolates: the nano-gold assay was carried out using amplicons of ITS region. For sequence detection, 1 $\mu$l of amplification product was added to 9 $\mu$l hybridization buffer, which contained two oligotargeters (target-specific oligonucleotide sequences; one of which is genus-specific and the other species-specific). The reaction mixture contained 0·166 $\mu$mol $l^{-1}$ oligotargeters, 11 mmol $l^{-1}$ phosphate buffer (pH 7·4) and 210 mmol $l^{-1}$ NaCl. After 3 min of denaturation at 95°C and 1 min of annealing at 50°C, 30 $\mu$l of AuNPs were added and the colour change was observed and photographed within 10 . The oligotargeter sequences used were (ACB 5′-GACTGGTTGAAGTT ATAGATAAAAGAT-3′) and (ACI 5′-AATTCATATACCA AAACGCTCGATTC-3′) (Ko *et al.* 2008). Each sample was scanned using a UV–Vis spectrophotometer in the range from 400 to 700 nm.

### Optimization of *Acinetobacter baumannii* AuNPs assay prototype

One microlitre of different DNA target consternations (0·4–6·5 ng $\mu l^{-1}$) was mixed with 9 $\mu$l hybridization buffer containing 11 mmol $l^{-1}$ phosphate buffer (pH 7·4), 210 mmol $l^{-1}$ NaCl and different oligotargeter concentrations (0·11–0·22 $\mu$mol $l^{-1}$). The mixture was denatured at 95°C for 3 min and then annealed at 50°C for 1 min and 30 $\mu$l AuNPs were added. The absorbance maxima of the reaction mixtures were determined by scanning between 400 and 700 nm. In negative control, 1 $\mu$l of *E. coli* DNA was mixed with hybridization buffer.

### Sequencing of ITS region and 16S rRNA genes

Six randomly selected isolates of *Acinetobacter* isolates were sequenced for ITS and 16S rRNA genes regions for species confirmation. Sequencing was performed for amplicons of 16S rRNA gene regions that were amplified by bacterium-specific universal primers 8FPL (5 -AGA-GTTTGATCCTGGCTCAG-3 ) and 1492RPL (5 -GGTT ACCTTGTTACGACTT-3 ) (Gurtler and Stanisich 1996). The PCR mixtures and cycling conditions were the same as described above, and the amplicons were purified with PCR clean-up system (Promega, Madison, WI) and sequenced in both directions using the two primers listed above. Comparison of ITS region and 16S rRNA gene sequences were performed to reference sequences using the BLASTn tool in NCBI database.

### Ethics statement

Approval to perform all experiments in the present study was validated by the research ethics committee of the Faculty of Pharmacy, Cairo University.

## Results

In the present study, *Ac. baumannii* ATCC 17978 reference strain was used as a positive control while other reference strains were used as negative controls. Fifty-three clinically isolated *Ac. baumannii* were recovered from intensive care units of three hospitals. The isolated *Acinetobacter* strains were identified biochemically as an oxidase-negative, nonfermenting, nonmotile, and Gram-negative coccobacillli. Detection of $bla_{Oxa-51}$-like carbapenemase gene was spotted among all the tested 53 clinical isolates, which asseverate the biochemical results. The isolated *Acinetobacter* strains exhibited high level of resistance to different antibiotic classes including carbapenems, quinolones and aminoglycosides as determined by the disc diffusion method (data not shown). ITS region amplification was performed using universal primers and gel electrophoresis of PCR product showed a DNA amplicons about 1200 bp.

Characterization of prepared spherical AuNPs was confirmed by electron microscope scanning as illustrated in (Fig. 1a). The mean diameter of the prepared AuNPs was found to be 13 ± 2 nm (Fig. 1b). UV/Visible scanning of AuNPs displayed a maximum absorption peak $\lambda_{max}$ at 520 nm.

Colorimetric testing of the clinical isolates of *Ac. baumannii* and the reference strains using AuNPs was performed in parallel. Species-specific oligotargeter was used to target the ITS region. In the nano-gold assay, positive samples appeared as blue to purple colour, while negative

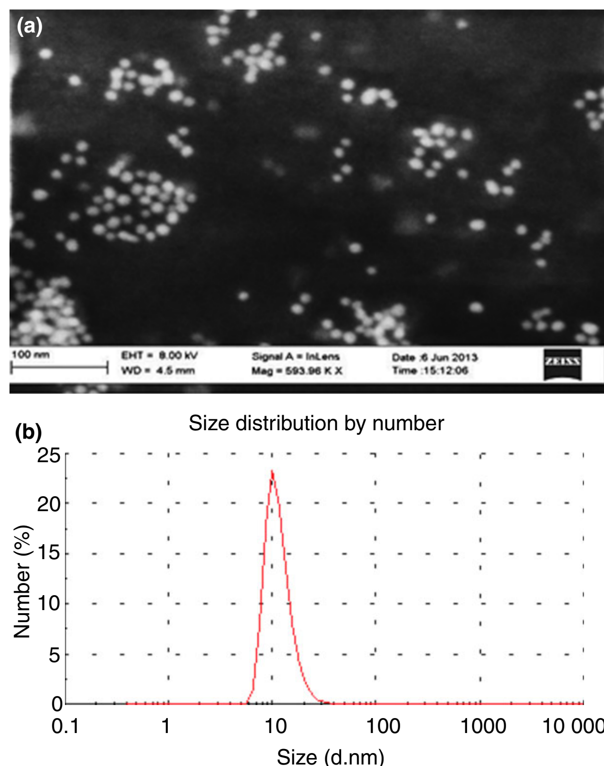


**Figure 1** (a) Scanning electron monographs of the prepared AuNPs. One drop of AuNPs was placed on silicon slide and left to dry, then examined using field emission scanning electron microscopy (Model: Leo Supra 55). (b). Analysis of the size distribution of the prepared AuNPs measured by dynamic light scattering (DLS, Malvern Zeta sizer 3000HSA; Malvern Instruments, Malvern, UK).

ones had a red colour. Characterization of the changes in UV–visible absorption spectra for both negative and positive samples is illustrated in (Fig. 2); positive sample showed shift in the absorption peak to 610 nm while the negative controls had a maximum absorption peak at 520 nm. The oligotargeter concentration was adjusted to prevent aggregation of AuNPs in absence of target yet allows the particles to aggregate in presence of low target concentration. Too much oligotargeter would decrease the sensitivity of the assay for low target concentration and *vice versa*. In the absence of DNA target, low oligotargeter consternations (0·11 $\mu$mol l$^{-1}$) were unable to stabilize the AuNPs particles, causing AuNPs to aggregate and a peak shift to 530 nm (false positive). Doubling the oligotargeter concentration to 0·22 $\mu$mol l$^{-1}$ reduced the ability of the assay to detect lower target concentration (0·8125 ng $\mu$l$^{-1}$). The oligotargeter concentration of 0·166 $\mu$mol l$^{-1}$ was found optimal to stabilize AuNPs and allow the detection of low target concentration. The negative control appeared red in colour with absorption peak at 520 nm, while the positive control appeared blue in colour with absorption peak of 610 nm.

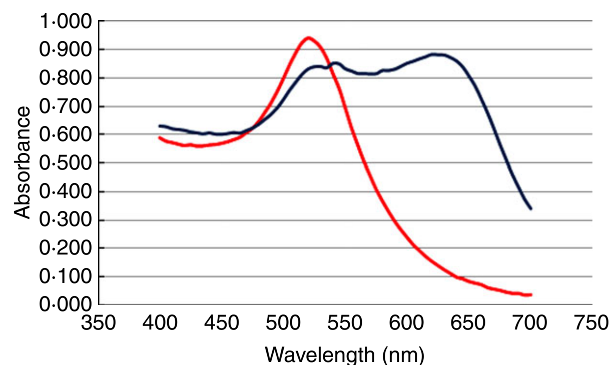


**Figure 2** Spectrophotometeric analysis showing the absorption peak of negative sample (non aggregated AuNPs; red) at 520 nm and positive sample (aggregated AuNPs; blue) at 610 nm. *Escherichia coli* ATCC 25922 was used as a negative control and *Acinetobacter baumannii* (ATCC 17978) as a positive control. (——) Negative and (——) Positive.

Determination of the nano-gold assay detection limit was carried out by serial dilution of target DNA. The assay was able to detect minute concentration of DNA target down to 0·8125 ng $\mu$l$^{-1}$, as shown in (Fig. 3).

Sequences of ITS and 16srRNA gene obtained from six randomly selected *Ac. baumannii* strains were analysed using the NCBI Blastn tool. Results of sequence analysis revealed 97–98% homology with *Ac. baumannii* species. Moreover, analysis of the used ssDNA oligotargeters showed complete specificity to ITS sequences.

## Discussion

Identification of the isolated *Acinetobacter* strains was performed biochemically as well as by identifying *bla*$_{\text{Oxa-51}}$-like genes. The *bla*$_{\text{Oxa-51}}$-like genes assumed to be intrinsic to *Acinetobacter* species and its detection among all clinical *Acinetobacter* strains proves that isolated strains belong to *Ac. baumannii* species (Turton *et al.* 2006). ITS region is considered an ideal region to characterize different bacterial species; thereby, genus- and species-specific oligotargeters were used to recognize ITS region of *Acinetobacter* (Ko *et al.* 2008). Reference strains were first used to optimize the assay conditions before to testing clinical *Acinetobacter* isolates.

AuNP-based assay was developed for identification of *Ac. baumannii*, which includes extraction of DNA from colonies followed by PCR amplification and detection amplicons using oligotargeters and unmodified spherical AuNPs. Reference strains were first used to optimize the assay conditions before to testing clinical *Acinetobacter* isolates.

The nano-gold assay is affected by different factors, which must be optimized to have the functional assay.

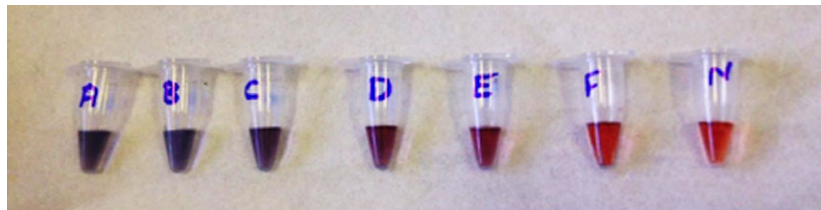


**Figure 3** Nano-gold assay detection limit of *Acinetobacter baumannii* PCR amplicon. A: 13 ng $\mu l^{-1}$; B: 6·5 ng $\mu l^{-1}$; C: 3·25 ng $\mu l^{-1}$; D: 1·625 ng $\mu l^{-1}$; E: 0·8125 ng $\mu l^{-1}$; F: 0·406 ng $\mu l^{-1}$; and N: negative (*Escherichia coli* ATCC 25922).

These factors comprise temperature, size and shape of AuNPs in addition to oligotargeters and salt concentrations. High temperature can result in AuNPs aggregation. However, in this assay, AuNPs is added after the annealing step, which enabled the use of optimal temperature for annealing without interference with AuNPs solution stability. Control of the particles size, shape and charge was achieved by using the appropriate concentration of the sodium citrate, which acts as reducing and capping agent. Different oligotargeters ratios were used to stabilize the AuNPs colloidal in presence of appropriate NaCl concentration

Principle of the AuNPs assay is based on the ability of ssDNA oligotargeters to stabilize the colloidal AuNPs preventing their salt-induced aggregation. In the positive sample (target present), oligotargeters will hybridize with their complementary DNA sequence in the target; therefore, addition of AuNPs will lead to its aggregation by the salt available in hybridization buffer, resulting in blue colour formation. On the other hand, if target is absent, ssDNA oligotargeters will remain free in the solution, stabilizing colloidal AuNPs, preventing their salt-induced aggregation and maintaining the red colour of the solution.

Detection limit of the assay was carried out using two-fold serial dilutions of amplicons. The assay prototype had a limit of detection 0·8125 ng $\mu l^{-1}$ (Fig. 3). The turnaround time of the assay was only 2 h, which is much shorter than conventional methods for biochemical detection (Prashanth and Badrinath 2000) and is comparable to other molecular assays but at a significantly lower cost. The results of the assay prototype declared complete concordance with other identification results.

Because of challenging phenotypic detection criteria of *Ac. baumannii*, conventional biochemical methods and routinely used commercial kits, such as the API 20NE system were not affirmed as cogent approaches for species identification. On the other hand, DNA molecular techniques such as amplified ribosomal DNA restriction analysis (ARDRA) need treatment of the amplified region with five different restriction enzymes in addition to computer-assisted gel analysis of its gel pattern (Fig. 4). Although sequence analysis of the 16S–23S rRNA gene spacer region or 16S rRNA gene is considered the gold standard method for reliable *Acinetobacter* species identification, their high cost and complexity restrict their routine use and development of point of care testing. Because of the high sensitivity and specificity of AuNP-based assays for detection of nucleic acid targets, AuNPs-based methods have been established for detection of

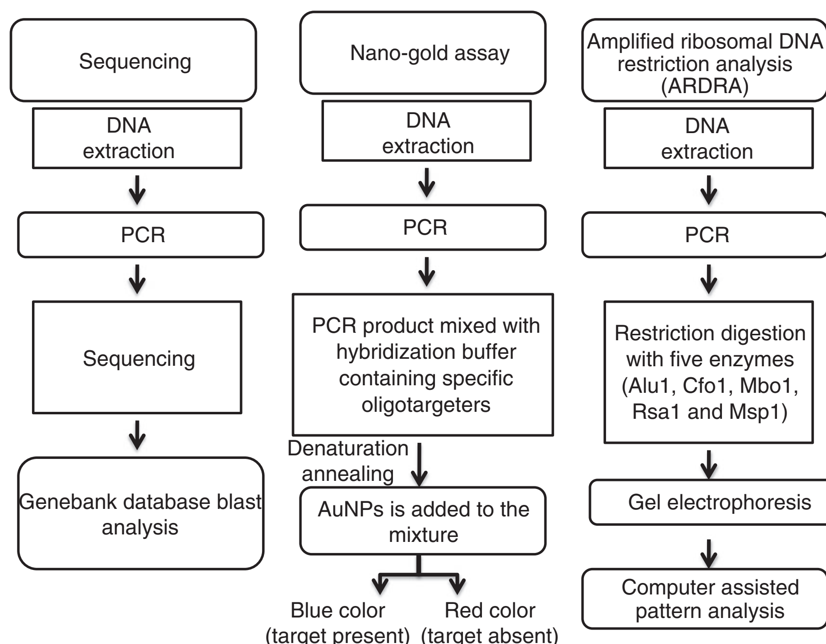


**Figure 4** Schematic illustration of different methods for detection of *Acinetobacter baumannii* using sequencing, unmodified AuNPs and amplified ribosomal DNA restriction analysis (ARDRA).

pathogenic bacteria such as *Mycobacterium tuberculosis* (Baptista *et al.* 2006; Soo *et al.* 2009; Hussain *et al.* 2013). The present study is the first nano-gold prototype for detection of *Ac. baumannii*.

Emanation of *Ac. baumannii* in healthcare units, particularly multidrug-resistant strains, is a major cause of concern, which requires immediate interventions of strict infection control measures. Biochemical assays cannot be used for species identification of *Ac. baumannii*. Complexity and high cost of many molecular techniques restrict its use in routine laboratory identifications. The need for simple, rapid and precise method for *Acinetobacter* identification is a critical step for detection and subsequent appropriate selection of antimicrobial therapy and infection control measures implementation. A colorimetric assay has been designed for rapid detection of *Ac. baumannii* using unmodified AuNPs. Direct detection of amplified ITS region by spherical unmodified AuNPs was performed on 53 clinical *Ac. baumannii* strains in addition to nine reference strains. The results from the nano-gold colorimetric assay were in concordance with biochemical and $bla_{\text{Oxa-51}}$ identification results. Furthermore, the higher sensitivity level of the assay was confirmed with a detection limit that reached down to 0·8125 ng $\mu l^{-1}$. The assay is considered inexpensive, sensitive, reliable and rapid that can compete with standard methods. This assay is the first AuNPs-based prototype for detection of *Ac. baumannii.* Our future plans include development of optimized form of the assay for direct detection of unamplified *Acinetobacter* DNA isolated from clinical specimens, in addition to test the assay on a large number of samples.

## Acknowledgements

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## Conflict of interest

Dr. Hassan Azzazy is an author on several patent applications on development of nano-gold assays for detection of infectious agents and tumour markers in clinical specimens. He is also a co-founder and CTO of D-Kimia, LLC, which develops nanoparticle-based assays for detection of infectious agents and disease biomarkers.

## References

Al-Hassan, L., El Mehallawy, H. and Amyes, S.G. (2013) Diversity in *Acinetobacter baumannii* isolates from paediatric cancer patients in Egypt. *Clin Microbiol Infect* **19**, 1082–1088.

Azzazy, H.M., Mansour, M.M., Samir, T.M. and Franco, R. (2012) Gold nanoparticles in the clinical laboratory: principles of preparation and applications. *Clin Chem Lab Med* **50**, 193–209.

Baptista, P.V., Koziol-Montewka, M., Paluch-Oles, J., Doria, G. and Franco, R. (2006) Gold-nanoparticle-probe-based assay for rapid and direct detection of *Mycobacterium tuberculosis* DNA in clinical samples. *Clin Chem* **52**, 1433–1434.

Bergogne-Berezin, E. and Towner, K.J. (1996) *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* **9**, 148–165.

Carr, E., Seviour, R.J. and Gurtler, V. (2002) Genomic fingerprinting of the 16S-23S gene spacer region suggests that novel *Acinetobacter* isolates are present in activated sludge. *Water Sci Technol* **46**, 449–452.

Chang, H.C., Wei, Y.F., Dijkshoorn, L., Vaneechoutte, M., Tang, C.T. and Chang, T.C. (2005) Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. *J Clin Microbiol* **43**, 1632–1639.

Dijkshoorn, L., Nemec, A. and Seifert, H. (2007) An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii. Nat Rev Microbiol* **12**, 939–951.

Dolzani, L., Tonin, E., Lagatolla, C., Prandin, L. and Monti-Bragadin, C. (1995) Identification of *Acinetobacter* isolates in the *A. calcoaceticus-A. baumannii* complex by restriction analysis of the 16S-23S rRNA intergenic-spacer sequences. *J Clin Microbiol* **33**, 1108–1113.

Ehrenstein, B., Bernards, A.T., Dijkshoorn, L., Gerner-Smidt, P., Towner, K.J., Bouvet, P.J., Daschner, F.D. and Grundmann, H. (1996) *Acinetobacter* species identification by using tRNA spacer fingerprinting. *J Clin Microbiol* **34**, 2414–2420.

Fouad, M., Attia, A.S., Tawakkol, W.M. and Hashem, A.M. (2013) Emergence of carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. *Int J Infect Dis* **17**, e1252–e1254.

Gales, A.C., Jones, R.N. and Sader, H.S. (2011) Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). *J Antimicrob Chemother* **66**, 2070–2074.

Gerner-Smidt, P. (1992) Ribotyping of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. *J Clin Microbiol* **30**, 2680–2685.

Gurtler, V. and Stanisich, V.A. (1996) New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. *Microbiology* **142**, 3–16.

Howard, A., O'Donoghue, M., Feeney, A. and Sleator, R.D. (2012) *Acinetobacter baumannii*: an emerging opportunistic pathogen. *Virulence* **3**, 243–250.

Hussain, M.M., Samir, T.M. and Azzazy, H.M. (2013) Unmodified gold nanoparticles for direct and rapid detection of *Mycobacterium tuberculosis* complex. *Clin Biochem* **46**, 633–637.

Janssen, P., Maquelin, K., Coopman, R., Tjernberg, I., Bouvet, P., Kersters, K. and Dijkshoorn, L. (1997) Discrimination of *Acinetobacter* genomic species by AFLP fingerprinting. *Int J Syst Bacteriol* **47**, 1179–1187.

Jin, R., Wu, G., Li, Z., Mirkin, C.A. and Schatz, G.C. (2003) What controls the melting properties of DNA-linked gold nanoparticle assemblies? *J Am Chem Soc* **125**, 1643–1654.

Ko, W.C., Lee, N.Y., Su, S.C., Dijkshoorn, L., Vaneechoutte, M., Wang, L.R., Yan, J.J. and Chang, T.C. (2008) Oligonucleotide array-based identification of species in the *Acinetobacter calcoaceticus-A. baumannii* complex in isolates from blood cultures and antimicrobial susceptibility testing of the isolates. *J Clin Microbiol* **46**, 2052–2059.

Li, H. and Rothberg, L. (2004) Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc Natl Acad Sci U S A* **101**, 14036–14039.

McGowan, J.E. Jr (2006) Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. *Am J Infect Control* **34**, S64–S73.

Millar, B.C., Jiru, X., Moore, J.E. and Earle, J.A. (2000) A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. *J Microbiol Methods* **42**, 139–147.

Prashanth, K. and Badrinath, S. (2000) Simplified phenotypic tests for identification of *Acinetobacter* spp. and their antimicrobial susceptibility status. *J Med Microbiol* **49**, 773–778.

Sato, K., Hosokawa, K. and Maeda, M. (2003) Rapid aggregation of gold nanoparticles induced by non-cross-linking DNA hybridization. *J Am Chem Soc* **125**, 8102–8103.

Shawky, S.M., Bald, D. and Azzazy, H.M. (2010) Direct detection of unamplified hepatitis C virus RNA using unmodified gold nanoparticles. *Clin Biochem* **43**, 1163–1168.

Shen, X.C., Jiang, L.F., Liang, H., Lu, X., Zhang, L.J. and Liu, X.Y. (2006) Determination of 6-mercaptopurine based on the fluorescence enhancement of Au nanoparticles. *Talanta* **69**, 456–462.

Soo, P.C., Horng, Y.T., Chang, K.C., Wang, J.Y., Hsueh, P.R., Chuang, C.Y., Lu, C.C. and Lai, H.C. (2009) A simple gold nanoparticle probes assay for identification of *Mycobacterium tuberculosis* and *Mycobacterium tuberculosis* complex from clinical specimens. *Mol Cell Probes* **23**, 240–246.

Turton, J.F., Woodford, N., Glover, J., Yarde, S., Kaufmann, M.E. and Pitt, T.L. (2006) Identification of *Acinetobacter baumannii* by detection of the *blaOXA-51*-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* **44**, 2974–2976.

Vaneechoutte, M., Dijkshoorn, L., Tjernberg, I., Elaichouni, A., de Vos, P., Claeys, G. and Verschraegen, G. (1995) Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. *J Clin Microbiol* **33**, 11–15.