Research Article

Down-Regulation of fliL Gene Expression by Ag Nanoparticles and TiO$_2$ Nanoparticles in Pragmatic Clinical Isolates of *Proteus mirabilis* and *Proteus vulgaris* from Urinary Tract Infection

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Abstract

Ten isolates belonging to *Proteus* spp. were collected and obtained from Department of Biology, College of Science, the University of Baghdad. The diagnosis was done by polymerase chain reaction (PCR) technique using 16S rRNA gene and urease C gene. All isolates (100%) were sensitive to meropenem, imipenem, ciprofloxacin, gentamycin, amoxicillin, clavulanic acid and levofloxacin. These isolates also showed 60% sensitivity to cefixime and nitrofurantoin. However, both species of *P. mirabilis* and *P. vulgaris* showed the lowest sensitivity when treated with tetracycline (60%) and amikacin (20%). Cephalothin had a variable effect on the species under study as *P. mirabilis* isolates were 100% sensitive in comparison with the 80% sensitivity of *P. vulgaris* isolates. The antibacterial activities of Ag and TiO$_2$ nanoparticles (NPs) were investigated. The minimum inhibitory concentration (MIC) value of Ag NPs against both species isolates was 10 mg/mL, while the MIC value of TiO$_2$ NPs was 14 mg/mL against *P. mirabilis* and 15 mg/mL against *P. vulgaris* isolates. *P. mirabilis* isolates showed larger swarming diameter than *P. vulgaris*, but this motility phenomenon of *P. vulgaris* was arrested rapidly after incubation with sub-MIC of TiO$_2$ NPs and Ag NPs comparatively with control. All isolates showed shifting to down-regulation in the fliL gene expression under the effect of the NPs using TiO$_2$ NPs and Ag NPs. In conclusion, down-regulation of the fliL gene expression is directly linked to the inhibition of swarming movement of *Proteus* species. We encourage using these inhibitors (after tests to ensure minimal toxicity to human) in combination with antibiotics to ensure bactericidal/bacteriostatic effect to treat *Proteus* infections.

Keywords: *P. mirabilis*; *P. vulgaris*; Swarming phenomenon; fliL gene expression; TiO$_2$ NPs; Ag NPs

Introduction

The genus of *Proteus* classification belongs to the family enterobacteriaceae and the swarming phenomenon is the most recognizable characteristics of *Proteus* spp compared to other members of the family [1]. This genus is gram negative pathogen, habitating in the intestinal tract of both animals and...
human, sewage, manure and environments [2]. *Proteus vulgaris* is an opportunistic bacterial pathogen in this genus coming after *Proteus mirabilis* and ranking as the third cause of UTI infection after *E. coli* and *Klebsiella pneumoniae* [3]. *Proteus* spp have many virulence factors helping in their adhesion, growth, colonization and invasion into infected tissues and thus progressing of the pathogenesis. These virulence factors include flagella, capsule, fimbrae, outer membrane proteins, lipopolysaccharides (LPS), biofilm formation, and several enzymes such as, haemolysin, metalloprotease, amino acid deaminase, urease [4] and chondroitinase [5, 6]. A lot of data about antibiotic resistance are obtainable about *Proteus* spp [7]. *Proteus* spp are resistant to different types of antibiotics such as polymyxin and tetracycline. Furthermore, multidrug-resistant strains were observed to be resistant towards antibiotics including aminoglycoside, streptothricin, fluoroquinolone, β-lactams, phenicol and trimethoprim-sulfamethoxazole [8]. In general, the 6–10 tiny peritrichous flagella on the surface of *Proteus* spp have been conceived as a catalyst for the colonization and transmission from the initial site to new location, and this movement is defined as swarming [9]. The swarming phenomenon is considered an important virulence factor differing from swimming; it is a multi-cellular operation that occurs on solid surface and needs the discrimination of vegetative cells into a special form of cell type termed as swarmer cells [10]. The swarming phenomenon is a flagellum-dependent movement style associated with increasing chance of infectious diseases caused by *Proteus* spp under suitable conditions, such as bacteremia, wound infections, meningitis in infants, rheumatoid arthritis and others [11]. Colonization of *Proteus* spp on surfaces such as urinary tract is encouraged by differentiation of swarmer individual cell, which is established by hesitancy in flagellar spinning when first contacting with surface by the bacteria [12]. In general, the bacterial flagellum is built from: Firstly the basal body, followed by the hook, and then the helical filament, and the assembly of all is harmonious with a finely controlled regulatory rotation [13]. A mutation, disorder or other events lead to variations in fliL gene, encoding a flagellum structural protein may lead to improper creation of swarmer cells, known as pseudo-swarmer cells, indicating the sharing of fliL protein in the surface sensing process under non-motivational conditions [12]. Nanotechnology is one of the sciences that deal with the production of nanoparticles which are very small in size, ranging from 1 to 100 nm and possessing a high surface area compared with huge examples. Nanoparticles can be synthesized from many metals including silver, platinum, titanium, gold, copper, etc. Nanoparticles have unique chemical, physical and biological properties [14]. Biogenic nanoparticles have been applied in bactericidal applications, which may be attributed to their biocompatibility and long-term stability [15]. Metal nanoparticles have become one of the hopeful alternatives to defeat the microbial resistance of MDR bacteria [16]. Mechanisms of these nanoparticles include metal ion release, and oxidative and non-oxidative stress being active at the same time [17]. These mechanisms caused membrane degradation, deterioration of cellular and so on [18]. Silver nanoparticles (Ag NPs) are among these inorganic agents, which is non-toxic, prepared from silver metal, and having bactericidal, antiviral and anti-fungal effects at low concentrations [19]. Titanium dioxide nanoparticles (TiO₂ NPs) are another one with stability in their physical and chemical structure, and optical, electrical and biocompatible properties. They are considered safe and non-toxic as confirmed by the American Food and Drug Administration (FDA). They are used in many applications such as cosmetics, tooth pastes and detergents, showing powerful germicidal feature and removing unpleasant odours [20]. These nanoparticles have wide spectra of antimicrobial agents; therefore, they can be used as alternative treatments for many diseases caused by bacteria [21]. Titanium is usually used in treating some skin infections as a sun blocker among patients who suffer from dermal damage, due to its safety and better ability to absorb UV radiation than other nanoparticles [2]. It has been applied in environmental usage to remove contaminants from both air and water. It has also been used as a semiconductor photo-catalyst [23].

**Experimental**

**Isolation, culture conditions, and identification**

In this study, 10 isolates of pathogenic bacteria belonging to *Proteus* spp were obtained in slant tubes containing brain heart infusion agar from post graduate students (Department of Biology, College of Science, Baghdad University). These isolates were activated by re-culturing on different media for primary diagnosis performed according to morphological and biochemical tests and then
maintained in brain heart infusion agar. The diagnosis was re-confirmed by PCR technique using specific set of primers’ composition for amplification of 16S rRNA gene, f5-’CACGCAGGGCGTCAATTAAG-3’ and r5-’TCTTTTGCAACCCACTCCTCAT-3’ [24]. In order to amplify the specific urease C gene, another set of primers were employed to confirm that isolates of Proteus spp belong to P. vulgaris including f5-’CGCCTTTGCGATGGCAAGTACAAC-3′ and r5-’GCAAATTGAGTGACTTTGGCTGGAC-3′ [6].

In PCR tube, reaction was performed on a total volume of 25 μL mixture which contained 12 μL of Green PCR Master Mix, 1 μL of each primer, and 2 μL of DNA template. The rest volume was achieved with sterile deionized distilled water, then mixed well by vortex. The suitable PCR process for 16S rRNA gene amplification was initial denaturation, denaturation, annealing, and extension, at 95 °C for 5 min, 30 cycles at 95 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 1 min respectively, where the final step (extension) was conducted at 72 °C for 10 min and then held at 4 °C. The above PCR process was identical to urease C gene amplification except that annealing temperature was 62 °C.

**Investigation effect of nanoparticles as anti-swarming**

**Phenotypic effect**

In Eppendorf tubes, 50 μL from the overnight culture of Proteus spp was mixed well with the sub-MIC watery solutions of Ag NPs and TiO2 NPs, separately, and then incubated at 37 °C for 24 h under monitoring. In the next incubation, 5 μL of the above culture was implanted on the centre of blood agar plates and allowed to incubate in the same condition as sated above. It was observed that the waves of swarming diameter started from the central spot of inoculation, were metric in millimetre, and matched with the control (bacterial plat swarming without nanoparticles’ treatment). The nanoparticles that made a movement of diameter wider than the control was categorized as stimulatory action; however, if the zone was smaller than the reference colony, it was labelled as inhibitory [29].

**Genotypic effect**

fliL gene expression analysis was monitored before and after treatment of Proteus spp isolates with Ag NPs and TiO2 NPs.

**Incubation of bacteria with nanoparticles**

The isolates were left to grow in BHI broth for 18 h at 37 °C. Then, 100 μL (0.5 McFarland) of fresh bacterial suspension was transferred to the Eppendorf tubes containing sub-MIC of both nanoparticles independently, mixed well by pipetting for few 2-5 min and then incubated for 24 h at 37 °C until used in the next step.
RNA extraction

The bacterial growth was collected and harvested from BHI, and then the total RNA of isolates was taken away according to instructions of TRizol™ kit (Thermo Scientific, USA). All extraction was treated with the DNase for 1 h. The purification and concentration values were detected by using a NanoDrop™ 1000 spectrophotometer and a Quants™ fluorometer (Promega, USA). In Eppendorf tubes, 1 μL of each RNA sample was mixed together with 199 μL of water-quality flour colorant and incubated in dark place at room temperature for 5-10 min.

cDNA synthesis, Evaluation of RT-PCR

The fliL gene expression shot was estimated by one step quantitative RT-qPCR using sets of primer: fliLF5'-GGTGATCGCCATTATTGCAAG-3', fliLR5'-AGCGTAACGTGATCCCTATG-3'. And rpoA acting as housekeeping gene was F5'-GCGTGTTATAGCCCAGTTGA-3' and R5'-AGGCTGACGACATCACTGTA-3' [12]. The thermocycling requirements were 25 °C for 10 min, 37 °C for 2 h, and 85 °C for 5 min, employed to convert about 1 μg of total RNA into cDNA by cDNA reverse transcription kit containing RNase inactivate agent. For RT-PCRs, 50 μL for each reaction solution contained 2 μL (200 μM) of deoxynucleoside triphosphates (dNTPs), 1 μL (200 nM) of each primer, 1 μL of ThermoPol buffer and 2 μL (25 ng) of cDNA as template were mixed well with 1μL of Taq polymerase (5 IU). The experimental conditions were 95 °C for 3 min, 30 cycles at 95 °C for 1 min, 60 °C for 30 sec, and 72 °C for 30 sec, and 72 °C for 2 min; qRT-PCR was run with the SYBR green kit. For each reaction, the mixture was brought up to 25 μL as final volume with nuclease-free water, incorporating 2×SYBR green master mix, 1 μL (200 nM) of each primer, and then pipetted well with 2 μL (25 ng) of cDNA as template. And to follow, the reaction was carried out on a Mic qPCR Cycler (Bio MolecularSystem, Australia) using GoTag qPCR Master MixGoTaq® 1-Step RT-qPCR System, (Promega, USA), following the optimized conditions: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 20 sec, and 60 °C for 60 sec. A separation curve interpretation was achieved for test to maintain harvest (95 °C for 20 sec, 60 °C for 60 sec, and 95 °C for 20 sec). The quantum of fold (change) was calculated using the 2^ΔΔCT formula:

\[
\text{Folding} = 2^{\Delta \Delta CT}, \quad \Delta \Delta CT = \Delta CT \text{ treated} - \Delta CT \text{ control}, \quad \Delta CT = CT \text{ gene} - CT \text{ housekeeping gene} [30].
\]

The threshold cycle (CT) method was employed in relative expressions calculation for quantitative RT-qPCR.

Statistical analysis

Data analysis in this article was completed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and calculated by student’s t-test (t) and ANOVA test. P-value < 0.05 was deemed statistically significant.

Results and Discussion

Isolation, culture conditions and identification

The ten isolates of Proteus spp., obtained from post graduate students, were re-activated on BHI agar. Results of the primary diagnosis were that all the isolates (100%) were non-lactose fermenter, pale colonies on MacConky agar. Also, all the isolates exhibited the swarming phenomenon clearly when cultured on blood agar plates, which is the main characteristics to distinguish Proteus spp. from other members of Enterobacteriaceae. Our results using molecular techniques showed that all isolates in this study were Proteus species. Amplification of 16S rRNA gene and urease C gene were separated on agarose gel by electrophoresis with amplicon size 857 bp and 263 bp, respectively under UV-spector, confirming identification of the isolates as P. mirabilis and P. vulgaris, respectively (Fig. 1 and 2).

Our findings are similar to those obtained by Al-Imam, et al. and Al-Saadi, et al. who used the same genes to identify P. mirabilis and P. vulgaris [6, 31]. On the other hand, Mukhtar et al. and Adnan et al. applied 16S rRNA PCR gene amplification technique for the identification of P. mirabilis [32, 33]. The latter technique has many advantages over phenotypic and biochemical techniques. It is extremely

![Gel electrophoresis of amplified 16S rRNA (857 bp) for Proteus spp. isolates on agarose (1%), TBE buffer (1×), stained with ethidium bromide. M: DNA ladder (100 bp); Lanes 1-10 were positive.](image-url)
preserved within types and among species of the same genus, present in all bacteria and considered a fixed structural gene (little variation frequency) [34]. The lack of functional mutations in this gene leads to fine identification compared with other genes that may be exposed to different mutations which may cause dysfunction. And it is highly accurate compared with phenotypic assays by bacteriological and biochemical methods [35] which may be changed due to environmental conditions, growth conditions, temperature and pH levels, etc. [36]. Using PCR technique in bacterial diagnosis has been proved by all means to be a reliable, easy, rapid and accurate procedure for identification and diagnosis [37], overcoming the difficulties and unpleasant results compared with conventional methods in diagnosis. Since the conventional identification of bacteria is usually achieved by a series of biochemical tests, the major disadvantage of this approach is it takes long time and is expensive for laboratory. Its close relation with other individuals of Proteus genus and other enterobacteriaceae has also made the identification of Proteus spp. difficult.

Antibiotics susceptibility

The results are illustrated in Fig. 3, showing variable patterns of susceptibility against different antibiotics. The antiprogram pattern displayed that all isolates of *P. mirabilis* and *P. vulgaris* were sensitive (100%) to meropenem (MEM), imipenem (IPM), ciprofloxacine (CIP), gentamycin (GEN), amoxicillin + clavulanic (AUG) acid and levofloxacine (LEV). In addition, these isolates were 60% sensitive under the effect of cefixime (CEF) and nitrofurantoin (NIT). However, both species exhibited the lowest sensitivity patterns of 60% and 20% toward tetracycline (TET) and amikacin (AMK), respectively. On the other hand, cephalothin (CEP) had a variable effect on the two species as *P. mirabilis* isolates were 100% sensitive in comparison with 20% by *P. vulgaris*. It is interesting to find that our results were similar to other studies reported by several authors who noticed all isolates of *P. vulgaris* were sensitive against meropenem, ciprofloxacine, levofloxacine, gentamycin, cephalothin and amoxicillin + clavulanic acid.

The swarmer cells showed an increase in their resistance to different antibiotics, but re-culturing these cells in broth media may cause them to be normal planktonic cells and to thereby exhibit different antibiotic susceptibility patterns [40]. The results of our study did not match with other reports which showed that *vulgaris* was 100% resistant to cephalothin and 93.3% to nitrofurantoin, but showed that 96.6%, 80%, and 65.7% of isolate were sensitive to ciprofloxacine, gentamycin, and amikacin, respectively [41]. Other studies of *P. vulgaris* isolates showed it was resistant only to ampicillin and cefuroxime [42]. However, extended spectrum beta-lactamas (ESBLs) play a role in Proteus spp. resistance to cefotaxime, ceftazidime, and ceftriaxone, as well as aztreonam, monobactam and the cephemycins (cefmetazole, cefoxitin and cefotetan), and the carbapenems (meropenem and imipenem) which are generally not
hydrolysed by ESBLs [43]. *Proteus* spp. can win resistance to antibiotics such as ampicillin through plasmid and chromosomal beta-lactamase expression [44]. The shifting in the regulatory (responsible) genes of the beta-lactamase was observed to produce high activity of the enzyme and cause resistance to cefotaxime, ceftriaxone, cefuroxime and penicillin [45]. And the change might occur in penicillin binding proteins which are responsible for synthesis of cellular wall peptidoglycan [46]. There are many reasons for bacterial resistance, including modified enzyme production which is able to inhibit activity of antibiotics. The occurrence of safe mutation leads to change in the target site of antibacterial drugs, reducing permeability behaviour. Efflux pumps, R-plasmids, integrons and transposons are probably acquired from other microorganisms that are found in the same environment [47, 48]. *Proteus* spp. can be naturally resistant to oxacillin, benzylpenicillin and macrolides [49]. The antibiotic susceptibility tests illustrated that *Proteus* spp. had a variety style, which may be attributed to lipid lipoproteins, bilayer, polysaccharides and LPS found in the extra outer cytoplasmic membrane. Additionally, misuse and abuse of different antimicrobial agents in medicine (medical and veterinary) may lead to the spreading and development of antibiotic resistance genes strategies [50, 51]. Given all the information above, we can consider *Proteus* spp. as an ESBL producing bacteria due to their ability to produce a chromosomally encoded beta-lactamase. This conclusion is in agreement with results achieved by Bush et al. [52].

**Determination of antibacterial activity and MIC**

The augmentation in bacterial resistance to antibiotics became an overall health question. A short time ago, nanoparticles have become an agent at odds with multidrug-resistant (MDR) bacteria. Many in-vitro and in-vivo results reported that metal nanoparticles had bactericidal activities towards a wide spectrum of bacterial species. However, in this study, the antibacterial activity for both Ag NPs and TiO$_2$ NPs was investigated by dilution method in agar wells. The MIC values of Ag NPs against *P. mirabilis* and *P. vulgaris* isolates were detected at 10 mg/mL (Table 2 and 3). However, the highest effective concentrations of TiO$_2$ NPs causing the total perishing of *P. mirabilis* was 14 mg/mL and 14-15 mg/mL for *P. vulgaris* isolates (Table 4 and 5). We can conclude that *Proteus* spp. isolates may be able to switch on some mechanical resistance associated with gradual increase in the concentration of TiO$_2$ NPs less than in the case of silver, which caused the loss of all isolates.

Our results were in disagreement with results

### Table 1 MIC values for Ag NPs vs. *P. mirabilis*

| Isolate  | Ag NPs concentration (mg/mL) |
|---------|-----------------------------|
|         | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| *P. mirabilis* 1 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 2 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 3 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 4 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 5 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

*Positive (+) means inhibition of bacterial growth.
Negative (-) means bacterial growth.

### Table 2 MIC values for TiO$_2$ vs. *P. mirabilis*

| Isolate  | TiO$_2$ concentration (mg/mL) |
|---------|-----------------------------|
|         | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| *P. mirabilis* 1 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 2 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 3 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 4 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| *P. mirabilis* 5 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

*Positive (+) means inhibition of bacterial growth.
Negative (-) means bacterial growth.

http://www.nanobe.org
about MIC values of Ag NPs against *Proteus* spp. at 10 μg/mL and 16 μg/mL as reported by Parveen et al. and Raheem et al. [53, 54], and at 0.015 mg/mL as investigated by Juniﬁcius and co-workers [55]. But the MIC values reached to 50 mg/mL when *Proteus* spp. were treated with Ag NPs [56]. There has been no article available that detected the MIC values of titanium dioxide nanoparticles against *Proteus* spp. However, Chatterjee and co-workers observed that *Proteus mirabilis* treated with titanium dioxide nanoparticles gave the inhibition zone about 3.4 mm around colonies [57]. On the other hand, MIC values of titanium nanoparticles were 128 μg/mL [58] and 20 mg/mL, 72 mg/mL, and 100 mg/mL for *Shigella dysenteries*, multidrug-resistant *E. coli*, and *Aeromonas hydrophilic*, respectively [59]. The mechanism of action for both antibiotics and nanoparticles was the same in way of interference in the synthesis of macromolecules such as DNA, RNA, protein, as well as membrane destroyed [60]. However, the antibacterial mechanism of Ag NPs was described by many articles, including (i) Ag NPs can attach with cell membrane of bacteria causing disruption of its permeability and causing the formation of many pores and slots in the cell membrane and then the influx of intra-cellular contents [61, 62]; (ii) free radicals and reactive oxygen species (ROS) such as H₂O₂ are liberated by Ag NPs on the surface of nanoparticles and cause deactivation of DNA molecule by effectiveness in replication enzymes’ activity [63]; (iii) the Ag ions which are released by Ag NPs react with the thiol group of some bacterial proteins that are responsible for many important cellular functions and inactivation of them, such as damaged DNA molecules [64, 65]; and (vi) disruption of the ATP production [66]. However, the action of titanium nanoparticles against

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**Table 3** MIC values for Ag NPs vs. *P. vulgaris*

| Isolate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|
| *P. vulgaris* 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |

*Positive (+) means inhibition of bacterial growth.  
Negative (-) means bacterial growth.

**Table 4** MIC values for TiO₂ vs. *P. vulgaris*

| Isolate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|
| *P. vulgaris* 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| *P. vulgaris* 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |

*Positive (+) means inhibition of bacterial growth.  
Negative (-) means bacterial growth.

**Table 5** Effect of Ag NPs and TiO₂ NP at sub-MIC on the swarming of *P. mirabilis.*

| Isolates | Swarming control (mm) | Ag NPs sub-MIC (8 mg/mL) | TiO₂ NPs sub-MIC (10 mg/mL) |
|----------|------------------------|--------------------------|-----------------------------|
| *P. mirabilis* 1 | 21 | 13 | 12 |
| *P. mirabilis* 2 | 27 | 22 | 19 |
| *P. mirabilis* 3 | 40 | 32 | 30 |
| *P. mirabilis* 4 | 44 | 35 | 32 |
| *P. mirabilis* 5 | 42 | 23 | 20 |
bacteria is dissolving the outer membranes of microbes due to the presence of hydroxyl groups that may cause change in the permeability of the cellular membrane and finely lead to the death of pathogen, which may be attributed to the ability of interaction with O2 and -OH are adsorbed on the surface to obtain OH and O2 free radical [67, 68]. The photocatalytic operation of titanium nanoparticles triggers the reduced expression of a wide range of genes encoded proteins set for regulatory control, signalling and progressive roles in tantamount with the next chosen events on cell wall texture, co-enzyme independent respiration, and ion homeostasis [69]. Also, titanium dioxide nanoparticles are able to cause damage of DNA, formation of reactive oxygen species (ROS) and superoxide radicals [70, 71]. Some reports demonstrated that exposing bacteria to TiO2 photocatalysis quickly disrupted the regulatory signalling level, reduced the coenzyme-independent respiratory chains, downregulated the facility to take up and carry phosphorous and iron, and inhibited the ability of bio-synthesis and declination of heme Fe-S cluster [16, 72]. For example, TiO2 NPs display antimicrobial activity by different strategies given that the chance of expansion of reluctance against these nanoparticles is depressed [73]. However, in order to enhance the resistance against these metal nanoparticles, the bacteria may need to gain numerous gene mutations, which is not very likely [74]. On the other hand, Proteus spp. genome carries antibiotic resistance loci towards different mechanisms of antimicrobial agents and metals, including swarming mobility, biofilm formation, enzymatic detoxification and efflux systems by employing the PATRIC and PGAAP gene explanation services [75-77].

The impact of nanoparticles on swarming phenomenon

Results showed all isolates varied in their behaviour of swarming. It was observed that P. mirabilis isolates’ motility ranged from 21-42 mm, larger and faster than P. vulgaris which ranged from 14-35 mm (p-value < 0.05). The restraint and obstruction role for sub-MIC of TiO2 NPs on the diameter of swarming waves’ motion of P. vulgaris isolates was observed as more active

| Isolates      | Swarming control (mm) | Ag NPs sub-MIC (8 mg/mL) | TiO2 NPs sub-MIC (10 mg/mL) |
|---------------|-----------------------|--------------------------|-----------------------------|
| P. vulgaris 1 | 14                    | 9                        | 8                           |
| P. vulgaris 2 | 18                    | 13                       | 10                          |
| P. vulgaris 3 | 26                    | 23                       | 21                          |
| P. vulgaris 4 | 31                    | 28                       | 23                          |
| P. vulgaris 5 | 35                    | 30                       | 24                          |

Fig. 4 Down-regulation in fliL gene expression of P. mirabilis exposed to sub-MIC of nanoparticles.

Fig. 5 Down-regulation in fliL gene expression of P. vulgaris exposed to sub-MIC of nanoparticles.

Table 6 Effect of Ag NPs and TiO2 NP at sub-MIC on the swarming of P. vulgaris.
than the sub-MIC of Ag NPs of P. mirabilis (p-value < 0.05) (Table 5 and 6).

These two sub-MIC might increase the impairment of flagellar synthesis or rotation and thus the activity in cellular movement of Proteus spp. It was concluded that the swarming was of dose-dependent pattern reduced by both TiO$_2$ NPs and Ag NPs. There are many reasons behind the deviation in swarming patterns of Proteus spp. that may be associated with the bacteria themselves, such as strain variation, growth condition, source, incubation condition (the containing medium, pH, temperature, moisture, etc.), and expression of particular related swarming genes. Our results may be confirmed by Senior’s view that infectious diseases were encouraged more by P. mirabilis than by P. vulgaris due to their efficiency to produce many virulence factors such as protease and haemolysin, etc. that synchronized with the swarming motility and then moved out of urinary tract to another site in host tissues [78]. Most previous articles lacked the effect of TiO$_2$ NPs and Ag NPs on the swarming phenomenon presented by Proteus spp., but there are some other articles dealing with the arresting effect of synthesized Ag NPs on rhl regulation system which controls the swarming movement exhibited by P. aeruginosa [79]. The synthesized Ag NPs demonstrated a reducing effect on the swarming behaviour of E. coli, P. aeruginosa PAO1, and K. pneumoniae [80]. Also, Ag NPs showed to have reduced about 98% activity of the swarming motility associated with the arresting effect in the expression of fliL flagellar gene in E. coli [81]. The swarming movement was the reason of combination sensory transduction and universal control operations. The swarming cells called for sensing and coupling of an assortment of cell-to-cell, environmental and intracellular signals, and involved adjusting expression of gene networks key to physiological and morphological alterations [82]. Alternations in gene flaA encoding flagellin protein [83], and flhDC gene contributed to the upregulation of flagellin protein production, C and wad genes needed for the core region of LPS [84]. Other genes implicated in flagellum construction drove the repression of Proteus rods’ segregation and inhibition of swarming.

**Effect of nanoparticles on fliL gene expression**

The results proved that incubation of isolates with both sub-MIC of TiO$_2$ NPs and Ag NPs caused alternation in the expression manner of the fliL gene after 24 h. In this study, the Ct values of fliL gene in all isolates of P. mirabilis and P. vulgaris increased significantly (p-values < 0.05) after exposed to nanoparticles (Fig. 1 and 2), and the fold change $2^{-\Delta\Delta Ct}$ was less than 1 (Table 7 and 8). The threshold cycle Ct method was employed in relative expressions calculation for quantitative RT-qPCR; all the results were compared to the control (which was not treated with any nanoparticles under the same experimental conditions). Hence, we could conclude that gene expression was 100% down-regulated, and our results may reveal a regulatory link between fliL gene and swarming prominence. In other words, the isolates may not have an antioxidant preservation strategies to remain off ROS created by TiO$_2$ NPs and Ag NPs, which may be integrated with fliL gene and then reduce their regulation, that is to say, inhibition of movement is synchronized with the reduction of fliL gene expression. The down regulation may occur due to interfering TiO$_2$ NPs and Ag NPs with different extra and entry cellular proteins such as flagellar protein and cause arrest in their movement function. As can be said, reasons beneath the lowering in gene expression may belong to the same antimicrobial mechanisms of nano-material against pathogens, including damage of bacterial membrane and cell wall, deterioration to bacterial proteins and internal parts of bacteria, ions’ liberation, and oxidative stress and DNA damage [85], or that ribosomal loss subunits ability to expression of cellular proteins [86]. Any disorder or any treatment

| Table 7 | The folding change of treated fliL gene expression in P. mirabilis |
| Isolates | Sliver (8 mg/mL) | Titanium (10 mg/mL) |
|----------|-----------------|---------------------|
| P. mirabilis 1 | 0.000403 | 0.000281 |
| P. mirabilis 2 | 0.000539 | 0.000105 |
| P. mirabilis 3 | 0.002013 | 0.000250 |
| P. mirabilis 4 | 0.000129 | 0.000102 |
| P. mirabilis 5 | 0.000532 | 0.000100 |

| Table 8 | The folding change of treated fliL gene expression in P. vulgaris |
| Isolates | Sliver (8 mg/mL) | Titanium (10 mg/mL) |
|----------|-----------------|---------------------|
| P. vulgaris 1 | 0.000183 | 0.000112 |
| P. vulgaris 2 | 0.000105 | 0.000154 |
| P. vulgaris 3 | 0.000213 | 0.000144 |
| P. vulgaris 4 | 0.000752 | 0.000117 |
| P. vulgaris 5 | 0.006104 | 0.000115 |
with exogenous compounds, such as chemicals, may lead to change in the function of fliL gene, which is responsible for encoding a flagellar structural protein and causes creation of pseudo swarmer cells, suggesting the sharing of fliL in the surface sensing passage under no stimulating conditions [12]. FliL protein is necessary for swarming movement, but not for swimming motion [87]. The relative expressions calculation of quantitative RT-qPCR method for bacterial genes function was employed widely in different reports. Shehab and co-workers marked the alternation in stable-state mRNA levels of a gene towards numerous samples and described it as comparative to levels of a domestic control RNA [88].

Conclusions

Our study showed that both Ag NPs and TiO$_2$ NPs caused swarming reduction by the down-regulation of fliL gene expression of $P$. mirabilis and $P$. vulgaris in vitro. Nanomaterials can be employed in curing many infections caused by Proteus spp. as the chance of colonization and reaching to other sites of urinary tract are reduced. We encourage using these inhibitors, unless their toxicity for human is proven, in combination with antibiotics to ensure bactericidal/bacteriostatic effect to treat risky Proteus infections.

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References

[1] R. Belas, J. Manos, and R. Suvanasuthi, Proteus mirabilis Zap-A metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. Infect Immun. 2004, 72: 5159-5167.

[2] V. Sosa, G. Schlapp, and P. Zunino, Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiol. 2006, 152: 2149-2157.

[3] S.M. Jacobsen, D.J. Stickler, H.L.T. Mobley, et al., Complicated catheter associated urinary tract infections due to Escherichia coli and Proteus mirabilis. ClinMicrobiol Rev., 2008, 21: 26-59.

[4] K. Struble, M.S. Bronze, and G. Gonzalez, Proteus infections: Overview. eMedicine, 2009.

[5] M.N. Abdul-Gani, B.A. Laftaa, Purification and characterization of chondroitinase ABC from Proteus vulgaris, an Iraqi clinically isolate. Curr Sci, 2017, 113(11): 2134-2140.

[6] M.J.K. Al-Imam, B.A.L. Al-Rubaii, The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by Proteus vulgaris caused urinary tract infection. Iraqi J Sci, 2016, 57(4A): 2412-2421.

[7] K. Bush, Alarming $\beta$-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol., 2010, 13: 558-564.

[8] L. Chen, N. Al Laham, K.D. Chavda, et al., First report of an OXA-48-producing multidrug-resistant Proteus mirabilis strain from Gaza, Palestine. Antimicrob Agents Chemother, 2015, 59: 4305-4307.

[9] R. Belas, The swarming phenomenon of Proteus mirabilis. Am Soc Microbiol, 1992, 58: 15-22.

[10] D.B Kearns, R. Losick, Swarming motility in undomesticated Bacillus subtilis. Mol Microbiol, 2003, 49: 581-590.

[11] J. Bloch, X. Lemaire, L. Legout, et al., Brain abscesses during Proteus vulgaris. Bacteremia Neurol Sci, 2010, 32: 661-663.

[12] K. Cusick, Y.Y. Lee, B. Youchak, et al., Perturbation of fliL interferes with Proteus mirabilis swarmer cell gene expression and differentiation. J Bacteriol, 2012, 194(2): 437-447.

[13] R.M. Macnab, Flagella and motility. Escherichia coli and Salmonella: Cellular and molecular biology. ASM Press, 1996: 123-145.

[14] R Snigh, S. Nadhe, S. Wadhwani, et al., Nanoparticles for control of Biofilms of Acinetobacter species. Material, 2016, 9(5): 383.

[15] G. Franci, A. Falango, S. Galdiero, et al., Silver nanoparticulas as potential antibacterial agents. Molecules, 2015, 20(5): 8856-8874.

[16] A.J. Huh, Y.J. Kwon, “Nanoantibiotics”: A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release: Off. J. Control Release Soc, 2011, 156: 128-145.

[17] S. Zaidi, L. Misba, and A.U. Khan, Nano-therapeutics: A revolution in infection control in post antibiotic era. Nanomed Nanotechnol Biomed, 2017, 13: 2281-2301.

[18] S. Ahn, P. Singh, M. Jang, et al., Gold nanoflowers synthesized using Acanthopanacis cortex extract inhibit inflammatory mediators in LPS-induced RAW264.7 macrophages via NF-kappaB and AP-1 pathways. Colloids Surf B Biointerfaces, 2018, 162: 398-404.

[19] C. Marambio-Jones, E.M.V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanopart Res, 2010, 12: 1531-1551.

[20] J. Wist, J. Sanabria, C. Dieoff, et al., Evaluation of photocatalytic disinfection of crude water for drinking water production. Jphotochphotobio A, 2002, 147: 241-246.

[21] M. AlMatar, E.A. Makky, I. Var, et al., The role of nanoparticles in the inhibition of multidrug-resistant bacteria and biofilms. Curr Drug Deliv, 2017, 15: 470-494.

[22] S. Hosseinzadeh, H. Baharifar, and A. Amani, Efficacy of a model nano-TiO$_2$ sunscreen preparation as a function of ingredients concentration and ultrasonication treatment. Pharm Sci, 2017, 23 (2): 129-135.

[23] S. Pavasupree, J. Jitputti, S. Ngamsinlapasathian, et al., Hydrothermal synthesis, characterization, photocatalytic activity and dye sensitized solar cell performance of mesoporous anatase TiO$_2$ nanopowders. Mater Res Bull,
Prevalence of Proteus species with reduced susceptibility to imipenem isolated from a tertiary referral hospital in Malaysia. Malaya J Microbiol, 14(6): 513-518.

[43] A. Philippon, R. Labia, and G. Jacoby, Extended-spectrum beta-lactamases. Antimicrob Agents Chemother, 1989, 33: 1131-1136.

[44] W. Song, J. Kim, I.K. Bae, et al., Chromosome-encoded AmpC and CTX-M extended-spectrum β-lactamases in clinical isolates of Proteus mirabilis from Korea. Antimicrob Agents Chemother, 2011, 55: 1414-1419.

[45] D.M. Livermore, M.W. Carter, S. Bagel, et al., In vitro activities of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. Antimicrob Agents Chemother, 2001, 45: 1860-1867.

[46] P.A. Bradford, Extend-spectrum B-lactamase in the 21 century. Characterization, epidemiology, and detection of this important resistant threat. Clin Microbiol Rev, 2001, 14: 933-951.

[47] J.A. Karlowsky, M.E. Jones, C. Thronsberry, et al., Trend in Antimicrobials susceptibilities among Enterobacteriaceae isolated from hospitalized patients in United States from 1980-2001. Antimicrob Agents Chemother, 2003, 47(5): 1672-1680.

[48] G.F. Brooks, K. Carroll, J.S. Butel, et al., Adelberg’s medical microbiology, 24th ed. McGraw-Hill, 2007.

[49] I. Stock, Natural antibiotic susceptibility of Proteus spp., with special reference to P. mirabilis and P. penneri strains. J Chemother, 2003, 15: 12-26.

[50] R.M. Mordi, M.I. Momoh, Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. Afr J Biotechnol, 2009, 8: 725-730.

[51] I.O. Enabulele, S.C. Yah, E.O. Yusuf, et al., Emerging quinolones resistant transfer genes among gram-negative bacteria, isolated from faeces of HIV/AIDS patients attending some Clinics and Hospitals in the City of Benin, Edo State, Nigeria, Online J Health Allied Sc, 2006, 5(3).

[52] K. Bush, G.A. Jacoby, and A.A. Medeiros, A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother, 1995, 39: 1211-1233.

[53] A. Parveen, M.S. Yalagatti, V. Abbaraju, et al., Emphasized mechanistic antimicrobial study of biofunctionalized silver nanoparticles on model Proteus mirabilis. Journal of Drug Delivery, 2018, 2018: 1-10.

[54] H.Q. Raheem, A.A. Al-Tahir, T. Al-Abed, Antibacterial activity of silver nanoparticles extracted from Proteus mirabilis and healing the wound in rabbit. Biochem Cell Arch, 2018, 18: 97-104.

[55] J. Junevičius, J. Žilinskas, K. Cesaitis, et al., Antimicrobial activity of silver and gold in toothpastes: A comparative analysis. Stomatologia, Baltic Dental and Maxillofacial Journal, 2015, 17(1): 9-12.

[56] B. Buszewski, V. Railean-Plugaru, P. Pomastowski, et al., Antimicrobial activity of biosilver nanoparticles produced by a novel Streptacidiphilus durhamensis strain. J Microbiol Immunol Infect, 2018, 51: 45-54.

[57] A. Chatterjee, D. Nishanthini, N. Sandhiya, et al., Biosynthesis of titanium dioxide nanoparticles using Vigna Radiata. Asian J Pharm Clin Res, 2016, 9(4): 85-88.

[58] N.B.A. Abdulrahman, Z. Nssaif, Antimicrobial activity of zinc oxide, titanium dioxide and silver nanoparticles against mithicillin-resistant Staphylococcus aureus isolates. Tikkir J Pure Sci, 21(3) 2016: 49-53.

[59] S. Akhtar, I. Ali, S. Tauseef, et al., Synthesis, characterization and antibacterial activity of titanium dioxide (TiO2) nanoparticles, Fuumat J. Biol, 2016, 6(2): 141-147.

[60] J.M. Correa, M. Mori, H.L. Sanches, et al., Silver...
nanoparticles in dental biomaterials. *Int J Biomater*, 2015, (485275)2015.

[61] P.R. Abreu, M.C. Almeida, R.M. Bernardo, et al., Guava extract *Psidium guajava* alters the labelling of blood constituents with technetium-99m, *J Zhejiang Univ Sci B*, 2006, (7): 429-435.

[62] M. Raffi, F. Hussain, T.M. Bhatti, et al., Antibacterial characterization of silver nanoparticles against *E. coli* TiO2-15224. *J. Mater. Sci. Technol.*, 2008, 24(2): 192-196.

[63] K.S. Santos, A.M. Barbosa, L.P. da Costa, et al., Silver nanocomposite biosynthesis: Antibacterial activity against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Molecules*, 2016, 21(9): Article no. 1255.

[64] M. Ahmadi, M. Adibhesami, The effect of silver nanoparticles on wounds contaminated with *Pseudomonas aeruginosa* in mice: An experimental study. *Iran J Pharm Res*, 2017, 16(2): 661-669.

[65] J.K. Patra, K.H. Beak, Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanospecies along with its antiscandial and antioxidan effects. *Front Microbiol*, 2017, 8(54): 167.

[66] C.Y. Chen, Y.H. Chen, P.L. Lu, et al., *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. *J Microbiol Immunol Infect*, 2012, 45: 228-236.

[67] G. Rajakumar, A.A. Rahuman, S.M. Roopan, et al., Fungus-mediated biosynthesis and characterization of TiO2 nanoparticles and their activity against pathogenic bacteria. *Spectrochim Acta Mol Biomol Spectrosc*, 2012, 91: 23-29.

[68] G. Rajakumar, A.A. Rahuman, B. Priyavvada, et al., *Ecliptaprostrata* leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles. *Mater Lett*, 2012, 68: 115-117.

[69] A. Kubacka, M. Diez, D. Rojo, et al., Understanding the antimicrobial mechanism of TiO2 based nano-composite films in a pathogenic bacterium. *Sci Rep*, 2014, 4: 4134-4143.

[70] A. Besinis, T. de Peralta, R.D. Handy, The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on *Streptococcus mutans* using a suite of biosays. *Nanotoxicology*, 2014, 8: 1-6.

[71] Y. Li, W. Zhang, J. Niu, et al., Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. *ACS Nano*, 2012, 6(6): 5164-5173.

[72] H.A. Foster, I.B. Ditta, S. Vaghese, et al., Photocatalytic disinfection using titanium dioxide: Spectrumanud mechanism of antimicrobial activity. *Appl Microbiol Biotechnol*, 2011, 90: 1847-1868.

[73] K. Blecher, A. Nasir, and A. Friedman, The growing role of nanotechnology in combating infectious disease. *Virulence*, 2011, 2: 395-401.

[74] K.M. Hindi, A.J. Ditto, M.J. Panzner, et al., The antimicrobial efficacy of sustained release silver-carbene complex-loaded L-tyrosine polyphosphate nanoparticles: Characterization, in vitro and in vivo studies. *Biomaterials*, 2009, 30: 3771-3779.

[75] A.T.M. Saeb, K.A. Al-Rubeaan, M. Abouelhoda, et al., Genome sequencing and analysis of the first spontaneous nanosilver resistant bacterium *Proteus mirabilis* strain SCDR1. *Antimicrob Resist Infect Control*, 2017, 6(1): 119.

[76] A.R. Wattam, D. Abrahm, O. Dalay, et al., PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res*, 2014, 42: 581-591.

[77] A.G. McArthur, G.D. Wright, Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. *Curr Opin Microbiol*, 2015, 27: 45-50.

[78] B.W. Senior, Investigation of the types and characteristics of the proteolytic enzymes formed by divers strains of *Proteus* species. *J Med Microbiol*, 1999, 48: 623-628.

[79] M. Arunkumar, K. Suhashini, N. Mahesh, et al., Quorum quenching and antibacterial activity of silver nanoparticles synthesized from *Sargassum pumilumphyllum*, *Bangl J Pharmacol*, 2014, 9: 54-59.

[80] A. Hussain, M.F. Alajmi, M.A. Khan, et al., Biosynthesized silver nanoparticle (AgNP) from *Pandanus odorifer* leaf extract exhibits anti-metastasis and anti-biofilm potentials. *Front Microbiol*, 2019, 10: 8.

[81] R.J. Osona, A. Akgul, I. Yazgan, et al., Flavonoid-derived anisotropic silver nanoparticles inhibit growth and change the expression of virulence genes in *Escherichia coli* SM10. *RSC Adv*, 2018, 8: 46409-4661.

[82] G.M. Fraser, C. Hughes, Swarming motility. *Curr Opin Microbiol*, 1999, 2: 630-635.

[83] R. Belas, Expression of multiple flagellin encoding genes of *Proteus mirabilis*. *J Bacterial*, 1994, 176: 7169-7181.

[84] R.M. Morgenstein, B. Szostek and P.N. Rather, Regulation of gene expression during swarmer cell differentiation in *Proteus mirabilis*. *FEMS Microbiol Rev*, 2010, 34(5): 753-763.

[85] V.K. Sharma, R.A. Ynggardard, and Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci*, 2009, 145(1-2): 83-96.

[86] U. Attmannspacher, B.E. Scharf, and R.M. Harshay, Fil is essential for swarming: motor rotation in absence of Fil. fractures the flagellar rod in swarmer cells of *Salmonella enterica*. *Mol Microbiol*, 2008, 68: 328-341.

[87] Z.H. Shehab, B.A.L. AL-Rubaii, Effect of D-mannose on *Pseudomonas aeruginosa* multidrug-resistant strains of *Acinetobacter baumannii*. *Antimicrob Resist Infect Control*, 2019, 8(54): 167.

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