Genome sequencing and analysis of the first spontaneous Nanosilver resistant bacterium *Proteus mirabilis* strain SCDR1

Amr T. M. Saeb 1*, Khalid A. Al-Rubeaan 1, Mohamed Abouelhoda 2,3, Manojkumar Selvaraju 3,4 and Hamsa T. Tayeb 2,3

**Abstract**

**Background:** *P. mirabilis* is a common uropathogenic bacterium that can cause major complications in patients with long-standing indwelling catheters or patients with urinary tract anomalies. In addition, *P. mirabilis* is a common cause of chronic osteomyelitis in Diabetic foot ulcer (DFU) patients. We isolated *P. mirabilis SCDR1* from a Diabetic ulcer patient. We examined *P. mirabilis SCDR1* levels of resistance against Nanosilver colloids, the commercial Nanosilver and silver containing bandages and commonly used antibiotics. We utilized next generation sequencing techniques (NGS), bioinformatics, phylogenetic analysis and pathogenomics in the characterization of the infectious pathogen.

**Results:** *P. mirabilis SCDR1* was the first Nanosilver resistant isolate collected from a diabetic patient polyclonal infection. *P. mirabilis SCDR1* showed high levels of resistance against Nanosilver colloids, Nanosilver chitosan composite and the commercially available Nanosilver and silver bandages. The *P. mirabilis -SCDR1* genome size is 3,815,621 bp. with G + C content of 38.44%. *P. mirabilis-SCDR1* genome contains a total of 3533 genes, 3414 coding DNA sequence genes, 11, 10, 18 rRNAs (5S, 16S, and 23S), and 76 tRNAs. Our isolate contains all the required pathogenicity and virulence factors to establish a successful infection. *P. mirabilis SCDR1* isolate is a potential virulent pathogen that despite its original isolation site, the wound, can establish kidney infection and its associated complications. *P. mirabilis SCDR1* contains several mechanisms for antibiotics and metals resistance, including, biofilm formation, swarming mobility, efflux systems, and enzymatic detoxification.

**Conclusion:** *P. mirabilis SCDR1* is the first reported spontaneous Nanosilver resistant bacterial strain. *P. mirabilis SCDR1* possesses several mechanisms that may lead to the observed Nanosilver resistance.

**Keywords:** *Proteus Mirabilis*, Multi-drug resistance, Silver nanoparticles, Genome analysis, Pathogenomics, Biofilm formation, Swarming mobility, Resistome, Glutathione S-transferase, Copper/silver efflux system

**Background**

The production and utilization of nanosilver is one of the primary and still growing applications in the field of nanotechnology. Nanosilver is used as the essential antimicrobial ingredient in both clinical and environmental technologies. Nanosilver is utilized in the formulation of dental resin amalgams, medical device coatings, water filter antimicrobial coating, antimicrobial agents in air sanitizers, textiles, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines, bone cement, wound dressings, hospital beds and furniture to control infection and support anti-biofilm activity [1–8]. Nanosilver is known to exert inhibitory and bactericidal effects against many Gram-positive, Gram-negative and fungal pathogens [9]. Latest studies suggest that the use of nanosilver-containing wound dressings prevents or reduces microbial growth in wounds, and may improve the healing process [10]. Moreover, antibacterial nanosilver-containing wound dressing gels may be important for patients that are at risk of non-healing of diabetic foot wounds and traumatic/surgical wounds [11].
Increased usage of nanosilver in both medical and environmental products has generated concerns about the development of bacterial resistance against the antimicrobial ingredient. Bacterial resistance against metallic silver has been documented with several bacterial strains such as *E. coli* [*Enterobacter cloacae*, *Klebsiella pneumoniae* and *Salmonella typhimurium*] [12, 13]. However, information about bacterial resistance against Nanosilver is scarce. Only Gunawan et al., (2013) reported the occurrence of induced adaptation, of non-targeted environmental *Bacillus* species, to antimicrobial Nanosilver [14]. In this study, we report on a spontaneous nanosilver-resistant *Proteus mirabilis* isolate (“SCDR1”). *Proteus mirabilis* is a motile gram-negative bacterium that is characterized by its swarming behavior [15, 16]. *P. mirabilis* is a common uropathogen that can cause major complications. In addition, *P. mirabilis* can cause respiratory and wound infections, bacteremia, and other infections [16–21]. In fact, *P. mirabilis* is a common cause of chronic osteomyelitis in Diabetic foot ulcer (DFU) patients along with *Bacteroides fragilis*, *E. coli*, and *Klebsiella pneumoniae* [22]. Generally, *P. mirabilis* is responsible for 90% of genus *Proteus* infections, and can be considered as a community-acquired infection [23]. As a pathogen *P. mirabilis* acquires many virulence determinants that enable it to establish successful infections [24–26]. A lot of information concerning antibiotic resistance is available for *P. mirabilis* [27–35]. *P. mirabilis* is intrinsically resistant to tetracyclines and polymyxins. Moreover, multidrug-resistant (MDR) *P. mirabilis* strain resistance to β-lactams, aminoglycosides, fluoroquinolones, phenicols, streptothricin, tetracycline, and trimethoprim-sulfamethoxazole has been reported [36]. However, limited information about heavy metals, including silver, is available. In this study, we present the first report and genome sequence of the nanosilver-resistant bacterium *P. mirabilis* strain SCDR1, isolated from diabetic foot ulcer (DFU) patient.

Methods
Bacterial isolate
*Proteus mirabilis* strain SCDR1 was isolated from a diabetic ulcer patient in the diabetic foot unit at the University Diabetes Center, King Saud University. *P. mirabilis SCDR1* was the first nanosilver-resistant isolate to be collected from a diabetic patient’s polyclonal infection. A Proper wound swab was obtained from the patient and was sent for further microbiological study and culture. Wounds needing debridement were debrided before swabbing the surface of the wound. The specimen was inoculated onto blood agar (BA; Oxoid, Basingstoke, UK) and MacConkey agar (Oxoid) and incubated at 37 °C for 24–48 h. The Vitek 2 system and its advanced expert system were used for microbial identification, antibiotic sensitivity testing, and the interpretation of results. ID GN cards were used to identify the bacterial isolate, and AST-N204 was used for the antimicrobial susceptibility testing of gram-negative rods. Manual disk diffusion and MIC method for AgNPs and antibiotic sensitivity testing were performed when required. Results were categorized according to EUCAST 2.0 VITEK 2 MIC breakpoints.

Preparation of colloidal and composite Nanosilver and commercial products for antimicrobial activity testing
Colloidal silver nanoparticles were prepared and characterized, and their concentration was determined as described by Saeb et al., 2014 [9]. Nanosilver chitosan composite preparations were made by chemical reduction method, as described by Latif et al., 2015 [37]. Moreover, the following commercially silver and nanosilver containing wound dressing bandages were used for antimicrobial activity testing: Silvercel non-adherent antimicrobial alginate Dressing (Acetly L.P. Inc., San Antonio, Texas, USA), Sorbsan Silver dressing made of Calcium alginate with silver (Aspen Medical Europe Ltd., Leicestershire, UK), ColActive® Plus Ag (Covalon Technologies Ltd., Mississauga, Ontario, Canada), exsalt®SD7 wound dressing (Exciton Technologies, Edmonton, Alberta, Canada), Puracol Plus AG+ Collagen Dressings with Silver (Medline, Mundelein, Illinois, USA) and ACTISORB® silver antimicrobial wound dressing 220 (Acetly L.P. Inc., San Antonio, Texas, USA).

Antimicrobial susceptibility test
Antimicrobial activities were performed against the following strains: *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Proteus mirabilis* ATCC 29906, *Klebsiella pneumoniae* ATCC 700603, *E. coli* ATCC 25922 and *Enterobacter cloacae* ATCC 29212.

Disk diffusion antimicrobial susceptibility testing
Disk diffusion antimicrobial susceptibility testing was performed as described by Matuschek et al. [38]. Briefly, Mueller–Hinton (MH) agar plates were inoculated with agar with an inoculum corresponding to a McFarland 0.5 turbidity with a sterile cotton swab to prepare bacterial lawns of the abovementioned bacterial test strains. Sterile discs were loaded with different concentrations (50–200 ppm) of colloidal silver nanoparticles solutions and the Nanosilver chitosan composite (composite concentration ranged from 0.1% and 0.01 M to 3.2% and 0.16 M from chitosan and Silver nitrate respectively) and then placed on Mueller–Hinton (MH) agar plates with bacterial lawns. Within 15 min of application of antimicrobial disks, the plates were inverted and incubated at 37 °C for 16 h. All experiments were done in aseptic conditions in a laminar air flow cabinet. After incubation, inhibition zones were read at the point where no apparent growth was detected. The inhibition zone diameters were measured to the nearest millimeter. Similarly, 5 mm discs from the commercially available bandages were prepared in aseptic conditions and tested for antimicrobial activity, as previously described.
Minimum bactericidal (MBC) and minimal inhibitory concentration (MIC) test

MBC and MIC testing were performed as described by Holla et al., [39]. Briefly, a dilution with $1 \times 10^5$ CFU/ml (equivalent to 0.5 McFarland) was used as an inoculum for MIC testing. Different volumes that contained a range of silver Nanoparticles (50–700 ppm) were delivered to 7.5 ml of Muller-Hinton (MH) broth, each inoculated with 0.2 ml of the bacterial suspensions. Within 15 min of application of silver nanoparticles, the tubes were incubated at 37°C for 16 h in a shaker incubator at 200 rpm. We included a positive control (tubes containing inoculum and nutrient media without silver nanoparticles) and a negative control (tubes containing silver nanoparticles and nutrient media without inoculum).

Biofilm formation

In order to test the ability of P. mirabilis SCDR1 isolate to form biofilm, a culture was prepared by inoculation on Columbia agar, supplemented with 5% blood and incubated at 37°C for 24 h. The culture was then used to prepare 0.5 McFarland standard bacterial suspension. Wells of sterile 96-well flat-bottomed plastic microplates were filled with 250 μL of the Brain-heart infusion broth. Negative control wells contained the broth only. Twenty μL of bacterial suspension were then added to each well. The plate was incubated at 37°C for 24 h. Following the incubation, the content of each well was aspirated and washed three times with 300 μL of sterile distilled water. The remaining attached bacteria were fixed with 200 μL of methanol per well, and after 15 min the plates were emptied and left to dry air. After this, the plates were stained for 5 min with 160 μl per well of crystal violet used for gram stain. Excess stain was rinsed off by placing the microplates under running tap water. After the plates were air dried, the dye which was bound to the adherent cells was re-solubilized with 160 μL of 33% (v/v) glacial acetic acid per well. The optical density (OD) was measured at 570 nm [40].

Molecular genomics analysis

DNA purification, sequencing, bioinformatics and phylogenetic analysis

DNA isolation, purification, genome sequencing, bioinformatics and phylogenetic analysis were performed as described by Saeb et al., 2017 [41]. In addition, we used Mauve [42] and CoCoNUT [43] to generate the whole genome pairwise and multiple alignments of the draft P. mirabilis strain SCDR1 genome against selected reference genomes. Furthermore, we performed whole genome phylogeny based proteomic comparison among P. mirabilis SCDR1 isolate and other related Proteus mirabilis strains using Proteome Comparison service which is a protein sequence-based comparison using bi-directional BLASTP available at (https://www.patricbrc.org/app/SeqComparison) [44].

Gene annotation and Pathogenomics analysis

P. mirabilis SCDR1 genome contigs were annotated using the Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) available at NCBI (http://www.ncbi.nlm.nih.gov/). In addition, contigs were further annotated using the bacterial bioinformatics database and analysis resource (PATRIC) gene annotation service (https://www.patricbrc.org/app/Annotation) [44]. The PathogenFinder 1.1 pathogenicity prediction program available at (https://cge.cbs.dtu.dk/services/PathogenFinder/) was used to examine the nature of P. mirabilis SCDR1 as a human pathogen [45]. Virulence gene sequences and functions, corresponding to different major bacterial virulence factors of Proteus mirabilis were collected from GenBank and validated using virulence factors of the pathogenic bacteria database available at (http://www.mgc.ac.cn/VFs/) [46], the Victors virulence factors search program available at (http://www.phidias.us/victors/) and the PATRIC_VF tool available at https://www.patricbrc.org/= [44].

Resistome analysis

P. mirabilis SCDR1 genome contigs were investigated manually for the presence of antibiotic resistance loci using the PGAAP and PATRIC gene annotation services. Antibiotic resistance loci were further investigated using specialized search tools and services, namely Antibiotic Resistance Gene Search available at (https://www.patricbrc.org/=) [44], Genome Feature Finder (antibiotic resistance) available at (https://www.patricbrc.org/=) [44], ARDB (Antibiotic Resistance Genes Database) available at (https://ardb.cbcb.umd.edu/) [47], CARD (The Comprehensive Antibiotic Resistance Database) available at (https://card.mcmaster.ca/) [48, 49], Specialty Gene Search available at (https://www.patricbrc.org/=) and ResFinder 2.1 available at (https://cge.cbs.dtu.dk/services/ResFinder/) [50].

The heavy metal resistance gene search for P. mirabilis SCDR1 contigs were investigated using PGAAP and PATRIC gene annotation services, PATRIC Feature Finder searches tool and BacMet (antibacterial biocide and metal resistance genes database) available at (http://bacmet.biomedicine.gu.se/) [44, 51].

Results

Initial identification and antimicrobial susceptibility test

The Vitek 2 system showed that our isolate belongs to the Proteus mirabilis species. Antibiotic sensitivity testing using Vitek 2 AST-N204 card showed that our isolate P. mirabilis SCDR1 is resistant to ampicillin, nitrofurantoin, and Trimethoprim/ Sulfamethoxazole. In addition, P. mirabilis SCDR1 was resistant to ethidium bromide,
tetracycline, tigecycline, colistin, polymyxin B, rifamycin, doxycycline, vancomycin, fusidic acid, bacitracin, metronidazole, clarithromycin, erythromycin, oxacillin, clindamycin, trimethoprim, novobiocin, and minocycline. P. mirabilis SCDR1 was intermediate resistant against nalidixic acid, Imipenem, and Cefuroxime. Conversely, it was sensitive to chloramphenicol, amoxicillin/clavulanic Acid, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, cefaclor, cephalothin, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tobramycin, streptomycin, and fosfomycin.

P. mirabilis SCDR1 isolate showed high resistance against colloidal and composite Nanosilver and metallic silver compared with other tested Gram positive and negative bacterial species. For instance, Table 1, shows the resistance of P. mirabilis SCDR1 against colloidal Nanosilver assessed by the disk diffusion method, in comparison with S. aureus ATCC 29213, P. aeruginosa ATCC 27853, E. coli ATCC 25922 and E. cloacae ATCC 29212. Generally, P. mirabilis SCDR1 showed high resistance (0.0 cm), while K. pneumoniae showed the highest sensitivity (1.5–1.9 cm) against all tested silver nanoparticle concentrations (50–200 ppm). S. aureus also showed high sensitivity (1.4–1.6 cm) against all tested silver nanoparticle concentrations. None of the tested bacterial isolates except for P. mirabilis SCDR1 showed any resistance against silver-nanoparticles, even against the lowest concentration (50 ppm). Furthermore, Table 2 shows the resistance of P. mirabilis SCDR1 against colloidal Nanosilver assessed by a minimal inhibitory concentration method, compared with other tested Gram positive and negative bacterial species. Once more, P. mirabilis SCDR1 showed high resistance against the gradually increased concentrations of colloidal nanosilver. We observed P. mirabilis SCDR1 bacterial growth to colloidal Nanosilver concentration up to 500 ppm. On the other hand, K. pneumoniae showed the highest sensitivity against silver nanoparticles, with no observed growth at only 100 ppm colloidal nanosilver concentration. In addition, both E. coli and P. aeruginosa showed high sensitivity against silver nanoparticles, with no observed growth at 150 ppm colloidal Nanosilver concentration. Conversely, S. aureus tolerated only 200 ppm colloidal Nanosilver concentration. Similarly, Table 3 shows the resistance of P. mirabilis SCDR1 against silver and Nanosilver composite assessed by disk diffusion method. Nanosilver chitosan composites, with a concentration ranging from between 0.1% and 0.01 M to 3.2% and 0.16 M from chitosan and Silver nitrate respectively, had a comparable killing effect on both Gram positive and negative bacterial, namely S. aureus and P. aeruginosa. Meanwhile, none of the tested Nanosilver chitosan composites had any killing effect on P. mirabilis SCDR1. Similarly, all the tested commercially available silver and Nanosilver containing wound dressing bandages showed the enhanced killing effect on both S. aureus and P. aeruginosa. However, all these wound dressing bandages failed to inhibit P. mirabilis SCDR1 growth. P. mirabilis SCDR1 was able to produce strong biofilm with OD of 0.296.

**Table 1** Resistance of P. mirabilis SCDR1 against colloidal Nano-Silver assessed by disk diffusion method

| S. No | Sample ID | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) | Zone Of Inhibition (cm) |
|-------|-----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1     | 200 ppm   | 1.6 cm                  | 1.5 cm                  | 1.4 cm                  | 1.1 cm                  | 1.9 cm                  | 0.0 cm                  |                         |
| 2     | 150 ppm   | 1.5 cm                  | 1.2 cm                  | 1.3 cm                  | 1.0 cm                  | 1.7 cm                  | 0.0 cm                  |                         |
| 3     | 100 ppm   | 1.5 cm                  | 1.2 cm                  | 1.3 cm                  | 1.0 cm                  | 1.6 cm                  | 0.0 cm                  |                         |
| 4     | 50 ppm    | 1.4 cm                  | 1.1 cm                  | 1.1 cm                  | 0.9 cm                  | 1.5 cm                  | 0.0 cm                  |                         |

**General genome features**

Data from our draft genome of P. mirabilis SCDR1 was deposited in the NCBI-GenBank and was assigned accession number LUFT0000000. The bacterial bioinformatics database and analysis resource (PATRIC) gene annotation analysis showed the presence 308 unique genes of the biosynthesis of secondary metabolites such as tetracycline, Streptomycin, Novobiocin, and Betalain. It is also noteworthy that Xenobiotics Biodegradation and Metabolism pathways also maintained a high number of dedicated unique gene (245) (Additional files 1 and 2: Tables S1 and S2).

**Pathogen identification and phylogenetic analysis**

As previously stated, biochemical identification of the isolate confirmed the identity of our isolate as belonging to the *Proteus mirabilis* species. Moreover, Primary analysis of Metaphlan showed that *Proteus mirabilis* is the most dominant species in the sample (Fig. 1). The appearance of other bacterial species in the Metaphlan diagram is explained by the genomic homology similarity of other bacteria to *Proteus mirabilis*. *P. mirabilis* SCDR1 genome showed high similarity, 92.07%, to the genome of *P. mirabilis* strain BB2000 followed by *P. mirabilis* strain C5028 (90.99%) and *P. mirabilis* strain PR03 (89.73%) (Table 4). A similar scenario was observed when constructing the phylogenetic relationship between our isolate and other *Proteus mirabilis* available in the NCBI-GenBank. 16Sr DNA-based maximum likelihood phylogenetic tree (Fig. 2) showed that our isolate is located within a large clade that contains the majority of *Proteus mirabilis* strains and isolates. In addition, *P. mirabilis* SCDR1 was
shown to be closely related to the reference strain *P. mirabilis* HI4320 compared with *P. mirabilis* BB2000, which is located in another clade of four *Proteus mirabilis* taxa. On the contrary, the whole genome Neighbor-joining phylogenetic tree of *Proteus mirabilis* spices including *P. mirabilis* SCDR1 isolate (Fig. 3), showed that our isolate was more closely related to *P. mirabilis* BB2000 compared with the reference strain/genome *P. mirabilis* HI4320. However, Fig. 4 showed that *P. mirabilis* SCDR1 exhibited obvious genetic divergence from other *Proteus mirabilis* species. Similar results were observed when performing pairwise pair-wise whole genome alignment of *P. mirabilis* strain SCDR1 against reference genomes (Fig. 4). This was also confirmed with the clear divergence among *P. mirabilis* SCDR1 *Proteus mirabilis* species at the proteomic level (Fig. 5).

**Bacterial pathogenic and virulence factors**

Pathogenomics analysis using PathogenFinder 1.1 showed that our input organism was predicted as a human pathogen, and the probability of being a human pathogen was 0.857. *P. mirabilis* SCDR1 comparative proteome analysis showed 35 matched hits from pathogenic families and only one hit from non-pathogenic families (Additional file 3: Table S3). In addition, genome analysis showed that *P. mirabilis* SCDR1 isolate contains numerous virulence factor genes and/or operons that marks it out to be a virulent pathogenic bacterium. These virulence factors include swarming behavior, mobility (flagellae), adherence, toxin and hemolysin production, Urease, Quorum sensing, iron acquisition systems, proteins that function in immune evasion, cell invasion and biofilm formation, stress tolerance factors, and chemotaxis related factors (Additional file 4: Table S4).

**Proteus Mirabilis SCDR1 Resistome**

**Antibiotic resistance**

Antibiotic resistance identification Perfect and Strict analysis using Resistance Gene Identifier (RGI) showed that *P. mirabilis* SCDR1 isolate contains 34 antibiotic resistance genes that serve in 21 antibiotic resistance functional categories (Additional file 5: Table S5 and Fig. 6). Meanwhile, using the less strict (Loose) antibiotic resistance identification criteria identified 3750 hits in *P. mirabilis* SCDR1 genome that represent potential AROs (Antibiotic Resistance Ontology) that fall into 59 antibiotic resistance functional categories (Fig. 7) of which 38 are considered to lose antibiotic resistance functional categories. Modified loose antibiotic resistance identification criteria, by removing all hits with objectionable e-values, lead to a number of 366 antibiotic resistance related hits (Additional file 6: Table S6 and Fig. 7). Manual genome annotation based mining resulted in the identification of 64 drug resistance related proteins in *P. mirabilis* SCDR1 genome (Additional file 7: Table S7).

| AgNPs (concentration in ppm) | S. aureus ATCC 29213 | P. aeruginosa ATCC 27853 | E. cloacae ATCC 29212 | E. coli ATCC 25922 | K. pneumoniae ATCC 700603 | P. mirabilis SCDR1 | P. mirabilis ATCC 29906 |
|-----------------------------|----------------------|--------------------------|-----------------------|---------------------|--------------------------|-----------------------|--------------------------|
| 50                          | Growth               | Growth                   | Growth                | Growth              | Growth                   | Growth                | Growth                   |
| 100                         | Growth               | Growth                   | Growth                | Growth              | Growth                   | Growth                | Growth                   |
| 150                         | Growth               | No Growth                | Growth                | No Growth           | No Growth                | Growth                | Growth                   |
| 200                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 250                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 300                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 350                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 400                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 450                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 500                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 550                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 600                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 650                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |
| 700                         | No Growth            | No Growth                | No Growth             | No Growth           | No Growth                | No Growth             | Growth                   |

*S. aureus*: 250 ppm/7.5  
*P. aeruginosa*: 150 ppm/7.5  
*E. cloacae*: 250 ppm/7.5  
*P. mirabilis* SCDR1: 550 ppm/7.5  
*P. mirabilis* ATCC: 500 ppm/7.5
We performed a species-wide antibiotic resistome constituent analysis of *P. mirabilis*. All available *P. mirabilis* genomes, including the *P. mirabilis* SCDR1 genome, were included in this analysis (Table 5). Results of our analysis (Table 6 and Fig. 8) showed that the number of the observed antimicrobial resistance based ontologies (AMRO) in *P. mirabilis* genomes is 61. Only 16 AMROs were observed amongst all the studied 56 *P. mirabilis* genomes. Meanwhile, 13 AMROs were observed amongst 55 *P. mirabilis* genomes. In addition, only four AMROs were observed amongst 54 *P. mirabilis* genomes and two AMROs were observed amongst 48 *P. mirabilis* genomes. This suggests that the core constituent of antibiotic resistome of *P. mirabilis* species is made up of 35 AMROs (Table 6). On the other hand, eight AMROs were detected only in one *P. mirabilis* genome. For example, the membrane fusion component of tripartite multidrug resistance system was only observed in our *P. mirabilis* SCDR1 genome.

### Proteus mirabilis comparative genomics based resistome analysis

We performed a species-wide antibiotic resistome constituent analysis of *P. mirabilis*. All available *P. mirabilis* genomes, including the *P. mirabilis* SCDR1 genome, were included in this analysis (Table 5). Results of our analysis (Table 6 and Fig. 8) showed that the number of the observed antimicrobial resistance based ontologies (AMRO) in *P. mirabilis* genomes is 61. Only 16 AMROs were observed amongst all the studied 56 *P. mirabilis* genomes. Meanwhile, 13 AMROs were observed amongst 55 *P. mirabilis* genomes. In addition, only four AMROs were observed amongst 54 *P. mirabilis* genomes and two AMROs were observed amongst 48 *P. mirabilis* genomes. This suggests that the core constituent of antibiotic resistome of *P. mirabilis* species is made up of 35 AMROs (Table 6). On the other hand, eight AMROs were detected only in one *P. mirabilis* genome. For example, the membrane fusion component of tripartite multidrug resistance system was only observed in our *P. mirabilis* SCDR1 genome.

#### Consensus *P. mirabilis*-SCDR1 antibiotic Resistome

Table 7 displays the consensus *P. mirabilis*-SCDR1 antibiotic resistome. Genomics analysis of *P. mirabilis*-SCDR1 63 contigs showed that our isolates contained genetic determinants for tetracycline resistance (tetAJ), fluoroquinolones resistance (gyrA, parC and parE), sulfonamide resistance (folP), daptomycin and rifamycin resistance (rpoB), elfamycin antibiotics resistance (tuB), Chloramphenicol (cpxR, cpxA and cat), ethidium bromide-methyl viologen resistance protein (emrE) and polymyxin resistance (phoP). In addition, several multidrug resistance efflux systems and complexes such as MdtABC-TolC, MacAB-TolC, AcrAB-TolC, EmrAB-TolC, AcrEF-TolC and MATE.

#### Heavy metal resistance

Table 8 presents *P. mirabilis* SCDR1 heavy metal resistance/binding factors. Numerous genetic determinants for metal resistance were observed in the *P. mirabilis* SCDR1 genome. Several Copper resistance genes/proteins were detected, namely, copA, copB, copC, copD, cueO, cueR, cutC, cutF and CuRO_2_CopA_like1. In addition, gene determinants of Copper/silver efflux system were observed, namely, oprB, oprM and cusC_1. Moreover, several heavy metal resistance proteins and efflux systems were observed, such as magnesium/cobalt efflux protein CorC, metal resistance proteins (AGS59089.1, AGS59090.1 and AGS59091.1), nickel-cobalt-cadmium resistance protein NccB, arsenical pump membrane protein (ArsB permease), Lead, cadmium, zinc and mercury transporting ATPase, outer membrane component of tripartite multidrug resistance system (CusC) and complete *P. mirabilis* tellurite resistance loci (terB, terA, terC, terD, terE, terZ). Furthermore, enzymes involved in heavy metal resistance were also observed such as glutathione S-transferase (gst1, gst, Delta and Uncharacterized), arsenite S-adenosylmethytransferase (Methyltransferase type 11) and alkylmercury lyase (MerB).
Fig. 1 Metaphlan primary identification of the tested taxon
Table 4. Comparison of Proteus mirabilis SCDR1 to complete and draft reference genomes of Proteus mirabilis

| NCBI ID       | Reference     | Ref Size | Gaps sum length | Gaps > = 100 bp | Bases sum length | Bases >500 bp | % Reference |
|---------------|---------------|----------|-----------------|-----------------|-----------------|---------------|-------------|
| Completed Genomes |               |          |                 |                 |                 |               |             |
| NC_010554.1   | Proteus mirabilis Hi4320 | 4,063,606 | 555,251         | 549,285         | 3,508,355       | 3,472,919     | 86.33       |
| NC_01055.1    | Proteus mirabilis plasmid pH4320 | 36,289   | 36,289          | 36,289          | 0               | 0             | 0           |
| NC_022000.1   | Proteus mirabilis BB2000 | 3,846,754 | 304,708         | 298,947         | 3,542,046       | 3,510,682     | 92.07       |
| Draft Genomes |               |          |                 |                 |                 |               |             |
| NZ_ACLE00000000 | Proteus mirabilis ATCC_29,906 | 4,027,100 | 565,180         | 560,679         | 3,461,920       | 3,432,786     | 85.96       |
| NZ_ANBT00000000 | Proteus mirabilis C05028 | 3,817,619 | 343,688         | 338,218         | 3,473,931       | 3,445,432     | 90.99       |
| NZ_AORN00000000 | Proteus mirabilis PR03 | 3,847,612 | 394,926         | 390,203         | 3,452,686       | 3,430,536     | 89.73       |
| NZ_AMGU00000000 | Proteus mirabilis WGLW4 | 3,960,485 | 474,704         | 469,864         | 3,485,781       | 3,458,264     | 88.01       |
| NZ_AMGT00000000 | Proteus mirabilis WGLW6 | 4,101,891 | 606,773         | 601,555         | 3,495,118       | 3,461,467     | 85.20       |

Discussion

Proteus mirabilis isolate was observed as mixed culture along with S. aureus isolate while testing our produced silver Nanoparticles against several pathogenic S. aureus isolates [9]. Whereas other tested Gram positive and negative bacteria showed great sensitivity against silver Nanoparticles, P. mirabilis, SCDR1 isolate exhibited extreme resistance. P. mirabilis SCDR1 isolate resistant against at least one antibiotic belonging to ansamycins, glycopeptides, fucidanes, cyclic peptides, nitroimidazoles, macrolides, lincosamides, folate pathway inhibitors and aminocoumarin antimicrobial categories. Moreover, our isolate exhibited intrinsic resistance against tetracyclines and polymyxins specific to P. mirabilis species [36, 52, 53]. However, fortunately, our isolate was sensitive to several operational antimicrobial categories such as penicillins with b-lactamase inhibitors, extended-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones and phosphonic acids. In addition, our P. mirabilis SCDR1 isolate showed high resistance against colloidal and composite Nanosilver and metallic silver when compared to other tested Gram positive and negative bacterial species, both qualitatively and quantitatively. To the best of our knowledge, this is the first reported case of bacterial spontaneous resistance to colloidal and composite nanosilver. However, Gunawan et al., (2013) reported the occurrence of induced adaptation, of non-targeted environmental Bacillus species to antimicrobial Nanosilver [14]. In addition, it was found that bacteria can straightforwardly develop resistance to AgNPs, and this occurs by relatively simple genomic changes [54]. They both showed that a Bacillus sp. environmental isolate and an E.coli isolate were able to adapt to Nanosilver cytotoxicity upon continued exposure. Nonetheless, as previously stated, P. mirabilis SCDR1 exhibited instantaneous resistance against nanosilver without the need for any prolonged exposure. P. mirabilis SCDR1 demonstrated resistance against colloidal nanosilver assessed either by disk diffusion or by minimal inhibitory concentration methods. While all used concentrations of colloidal Nanosilver have shown strong effects on all tested microorganisms (Table 1), there was no effect on the bacterial growth of P. mirabilis SCDR1 even at the highest used concentration (200 ppm). Similarly, P. mirabilis SCDR1 was able to resist ten fold (500 ppm) higher than K. pneumoniae (50 ppm), five fold higher than P. aeruginosa and E. coli (100 ppm) and two and a half fold (200 ppm) higher than S. aureus and E. cloacae (Table 2). Moreover, while both laboratory prepared and commercially available silver and Nanosilver composite showed a clear effect against both S. aureus and P. aeruginosa, the most common pathogens of diabetic foot ulcer, no effect was observed against P. mirabilis SCDR1 (Table 3). Although chitosan nanosilver composites have documented combined effect against both Gram positive and negative pathogens [37] no effect was observed against P. mirabilis SCDR1. Silver is a highly toxic element for microbes. The Nanosilver exhibits high surface to volume ratio, which shows increased antimicrobial power in comparison to the same bulk silver material [55]. It is suggested that the antimicrobial mechanism of silver ions involves the disruption of phospholipids of cytoplasmic, and the disruption of DNA replication, impairing the function of ribosomes to transcribe messenger RNA and/or inactivation of cytochrome b by binding with sulfhydryl group [56]. P. mirabilis SCDR1 genome analysis showed that our isolate contains a large number of genes (245) responsible for xenobiotics biodegradation and metabolism (Additional file 2: Table S2).
Although *P. mirabilis SCDR1* does not contain the chitinase genes responsible for Chitin and chitosan degradation, it contained Chitin binding protein (cbp, 203 amino acid protein). This may justify the ability of *P. mirabilis SCDR1* to resist the antimicrobial effect of chitosan. Chitin-binding protein even without any catalytic domain can facilitate the degradation of β-chitin by means of disrupting the crystalline chitin polymer structure [57, 58]. Microbial ability to produce proteins with high specific affinity to a certain crystalline chitin structure could be pivotal for the capability of bacteria to differentiate and react to specific crystalline chitin structures [59]. In addition, these chitin-binding domains may affect chitin degradation by facilitating adhesion of cells to the chitosinous materials [57]. Thus, although we did not detect chitinase genes in *P. mirabilis SCDR1*, the presence of Chitin-binding protein suggests that *P. mirabilis SCDR1* has some mechanisms of protection against chitin and the chitosan antimicrobial effect. In addition, the presence of genes encoding for the members Chitosanase family GH3 of N, N'-diacetylchitobiose-specific 6-phospho-beta-glucosidase (EC 3.2.1.86), Beta N-acetyl-glucosaminidase (nagZ, beta-hexosaminidase) (EC 3.2.1.52), and Glucan endo-1,4-beta-glucosidase (EC 3.2.1.-) in *P. mirabilis SCDR1* suggests that it can hydrolyze chitosan to glucosamine [60–62]. This justifies the lack of antimicrobial effect of chitosan against *P. mirabilis SCDR1*. Likewise, *P. mirabilis SCDR1* showed resistance against all the tested commercially available silver and Nanosilver containing wound dressing bandages. These silver containing commercially available bandages (wound dressing material) use different manufacturing technology and constituents. For example, Silvercel wound dressing contains high G calcium alginate in addition to 28% Silver-coated fibers (dressing contains 111 mg silver/100 cm²). The silver-coated fibers encompass elemental silver, which is converted to silver oxide upon contact with oxygen. Silver oxide dissolves in fluid and releases ionic silver (Ag⁺) that has antimicrobial action [63]. Clinical studies showed that Silvercel wound dressing is effective against many common wound pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant Staphylococcus epidermidis (MRSE) and vancomycin-resistant Enterococcus (VRE). In addition, these studies showed that Silvercel wound dressing prevented and disrupted the formation of bacterial biofilms [64, 65]. However, this was not the case with our *P. mirabilis SCDR1* isolate. Similarly, Sorbsan Silver wound dressing which is made of the fiber of the calcium salt of the alginate acid in addition to 28% Silver-coated fibers (dressing contains 111 mg silver/100 cm²). The Silver oxysalts offer greater oxidation states of silver*
(Ag^{2+}, Ag^{3+}) capable of interacting with microbial DNA, proteins and lipids, as well as providing potent oxidizing action through the increased power of Ag^{2+}, Ag^{3+} for advanced biocidal activity. Exsalt® SD7 showed high antimicrobial activity against tested Gram-negative and positive bacteria and fungi tested [69]. P. mirabilis SCD1 isolate showed high resistance against Exsalt® SD7. In addition, P. mirabilis SCD1 isolate showed high resistance against Puracol® Plus Ag, which is made of 100% Collagen in addition to antimicrobial silver. Furthermore, Actisorb® Silver 220, which is a sterile primary dressing encompassing an activated charcoal cloth, impregnated with silver within a spun bonded perforated nylon sleeve [70] was not active against P. mirabilis SCD1 isolate.

Pathogenomics analysis showed that P. mirabilis SCD1 isolate is a potential virulent pathogen (Additional files 3 and 4: Tables 3 and 4). P. mirabilis SCD1 shows that it possesses the characteristic bull’s eye pattern of swarming behavior. Presenting swarmer cells form is associated with the increase in expression of virulence genes [71]. Swarming is important to P. mirabilis uropathogenesis. It has been shown that swarming bacteria that move in multicellular groups exhibit adaptive resistance to multiple antibiotics [72]. Swarming behavior promotes the survival of bacteria in harsh environments or in unfavorable conditions. Moreover, migrating swarm cells display an increased resistance to many of antimicrobial agents. Therefore antimicrobial resistance could be a general feature of bacterial multicellular social behavior [73]. For example, the swarm cells of P. aeruginosa were able to migrate very close to the disc containing arsenite, indicating resistance to this heavy metal [73]. It has been suggested that high densities promote bacterial survival, the ability to move, as well as the speed of movement, confers an added advantage, making swarming an effective strategy for prevailing against antimicrobials including heavy metals [72, 73]. Furthermore, altruism or self-sacrifice is a suggested phenomenon associated with swarming, which involves risk of wiping out some individuals upon movement of bacteria to a different location, allowing the remaining individuals to continue their quest [72, 74]. Another suggested phenomenon associated with swarming is selfish behavior, in which the survival may be highest on top cells that are furthest from the antimicrobial agent while the lower cells in the swarm die because of the proximity to antimicrobial agents [72, 75]. Thus, selfish cells within the swarm sense where the best location is to avoid the toxic effect of the antimicrobial agent. Swarming behavior may indeed be one main reason for the observed nanosilver resistance of P. mirabilis SCD1. Thus, maintaining high cell density, through the observed quorum sensing ability (Additional file 4: Table S4) and the circulation within the multilayered colony to minimize exposure to the heavy metal in addition to the death of individuals that are directly exposed, could be the key to the observed nanosilver resistance.

P. mirabilis SCD1 isolate exhibited the ability of biofilm formation and also our pathogenomics analysis showed that it contains the genes responsible for this,
Fig. 4 (See legend on next page.)
such as glpC gene coding for anaerobic glycerol-3-phosphate dehydrogenase subunit C (EC 1.1.5.3), pmrI gene coding for UDP-glucuronic acid decarboxylase and baaS gene coding for biofilm formation regulatory protein BssS. We believe that the ability of *P. mirabilis* SCDR1 to form biofilm may also assist in the observed Nanosilver resistance. Biofilm formation can reduce the metal toxic effect by trapping it outside the cells. It was found that in the relative bacteria *Proteus vulgaris* XC 2, the biofilm cells showed considerably greater resistance to Chloromycetin compared to planktonic cells (free-floating counterparts) [76]. Moreover, it is suggested that the ability of biofilm formation may play a pivotal role in Polymyxin B antibiotic resistance in *P. mirabilis* [77]. Furthermore, it was found that biofilm formation is very important for heavy metal resistance in *Pseudomonas* sp. and that a biofilm lacking mutant was less tolerant to heavy metals [78]. Furthermore, it was found that both Extracellular Polysaccharides and Biofilm Formation is a resistance mechanism against toxic metals in *Sinorhizobium meliloti*, the nitrogen-fixing bacterium [79]. In addition, several reports claimed that the minimum inhibitory concentration (MIC) of some antibiotics for biofilms can be 1000-fold higher than that for planktonic bacteria [80].

It is well known that there are several mechanisms for metal resistance. These include physicochemical interactions, efflux, intracellular sequestration and extracellular precipitation by the excreted polymeric compounds [79]. Indeed, additional to swarming activity, Polysaccharides and biofilm formation (Additional file 4: Table S4), *P. mirabilis* SCDR1 contains several genes and proteins that also facilitate metal resistance including silver and Nanosilver (Table 8). Our results indicate the presence

---

**Fig. 4** Pair-wise Whole Genome Alignment of *P. mirabilis* strain SCDR1 against reference genomes. *a* *P. mirabilis* BB200 and *P. mirabilis* SCDR1, Mauve whole genome alignment, *b* *P. mirabilis* HI4320 and *P. mirabilis* SCDR1, *c* *P. mirabilis* AOUC001 and *P. mirabilis* SCDR1, *d* *P. mirabilis* CYPM1 and *P. mirabilis* SCDR1, *e* *P. vulgaris* CYPV1 and *P. mirabilis* SCDR1, *f* *P. mirabilis* SAS71 and *P. mirabilis* SCDR1 Mauve whole genome alignment

**Fig. 5** Whole genome phylogeny based proteomic comparison among *Proteus mirabilis* strains

---

List of tracks, from outside to inside: *Proteus mirabilis* BB200, *Proteus mirabilis* H4320, *Proteus mirabilis* strain SAS71, *Proteus mirabilis* strain CYPM1, *Proteus vulgaris* strain CYPV1 and *Proteus mirabilis* SCDR1

---

Percent protein sequence identity

| Value | 100 | 99.9 | 99.8 | 99.5 | 99 | 98 | 96 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
|-------|-----|------|------|------|----|----|----|----|----|----|----|----|----|----|----|----|---|
|       | 100 | 99.9 | 99.8 | 99.5 | 99  | 98 | 96 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |

---

**Fig. 5** Whole genome phylogeny based proteomic comparison among *Proteus mirabilis* strains
of endogenous silver and copper resistance mechanism in *P. mirabilis* SCDR1. We observed the presence of gene determinants of Copper/silver efflux system, oprB encoding for Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB), oprM encoding for Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB), oprM encoding for Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB).
outer membrane protein CusC (outer membrane efflux protein OprM), cusC_1 encoding for Copper/silver efflux system outer membrane protein CusC (RND efflux system outer membrane lipoprotein), cpxA encoding for Copper sensory histidine kinase and outer membrane component of tripartite multidrug resistance system (CusC). In addition, we observed the presence of several Copper resistance genes/proteins were detected, namely, copA, copB, copC, copD, cueO, cueR, cutC, cutF and CuRO_2_CopA_like1. A similar endogenous silver and copper resistance mechanism has been described in *E. coli* and has been associated with the loss of porins from the outer membrane and up-regulation of the native Cus efflux mechanism, which is capable of transporting silver out of the cell [81, 82]. However, the genetic basis resistant phenotypes are still not fully known, and it is not known if they are obligatory or sufficient to exhibit resistance to silver [83]. Thus, we suggest a comprehensive study for this endogenous silver resistance mechanism within the *Proteus mirabilis* as well as *E. coli*.

Furthermore, we observed the presence of genes encoding to enzymes involved in heavy metal resistance such as Glutathione S-transferase (EC 2.5.1.18) (gst1, gst, Delta and Uncharacterized) in *P. mirabilis* SCDR1 genome. Thus, we propose a role of Glutathione S-transferases of *P. mirabilis* SCDR1 in the observed Nanosilver resistance. Glutathione S-transferases (GSTs) are a family of multifunctional proteins that play an important role in the detoxification of harmful physiological and xenobiotic compounds in organisms [84]. Moreover, it was found that a Glutathione S-transferase is involved in copper, cadmium, Lead and mercury resistance [85]. Furthermore, it was found that GST genes are differentially expressed in defense against oxidative stress caused by Cd and Nanosilver exposure [85].

Moreover, we observed the presence of a complete tellurite resistance operon (terB, terA, terC, terD, terE, terZ) which was suggested as contributing to virulence or fitness and protection from other forms of oxidative
**Table 5** Proteus mirabilis genomes represented in the species wide comparative genomics antibiotic resistance analysis (Continued)

| Genome/Strain Name | GenBank Accessions |
|---------------------|--------------------|
| P. mirabilis strain 292_PMIR | WGS JVMQ01000000 |
| P. mirabilis strain 360_PMIR | WGS JVKD01000000 |
| P. mirabilis strain 373_PMIR | WGS JVQO01000000 |
| P. mirabilis strain 418_PMIR | WGS JVK01000000 |
| P. mirabilis strain 429_PMIR | WGS JVKI01000000 |
| P. mirabilis strain 430_PMIR | WGS JVFU01000000 |
| P. mirabilis strain 50,664,164 | WGS LNHT01000000 |
| P. mirabilis strain 51_PMIR | WGS JVEH01000000 |
| P. mirabilis strain 646_PMIR | WGS JUET01000000 |
| P. mirabilis strain 672_PMIR | WGS JUXR01000000 |
| P. mirabilis strain 68_PMIR | WGS JUXK01000000 |
| P. mirabilis strain AOCU-001 | Complete CP015347 |
| P. mirabilis strain ATCC 7002 | WGS JOVJ00000000 |
| P. mirabilis strain CYPM1 | Complete CP012674 |
| P. mirabilis strain FDAARGOS 60 | Complete JTBW01000000 |
| P. mirabilis strain FDAARGOS 67 | Complete JTPB01000000 |
stress or agents causing membrane damage, such as silver and Nanosilver, in *P. mirabilis* [86]. Several other heavy metal resistance genes and proteins were observed in the *P. mirabilis* SCDR1 genome. These included arsM encoding for arsenite S-adenosylmethyltransferase (Methyltransferase type 11), which play an important role in prokaryotic resistance and detoxification mechanism to arsenite [87, 88] and merB encoding for alkylmercury lyase that cleaves the carbon-mercury bond of organomercurials, such as phenylmercuric acetate [89]. Moreover, numerous heavy metal resistance proteins were observed, such as magnesium/cobalt efflux protein CorC, metal resistance proteins, nickel-cobalt-cadmium resistance protein NccB, arsenical pump membrane protein (ArsB permease), Lead, cadmium, zinc and mercury transporting ATPase (Table 8).

In order to gain information about antimicrobial resistome constituents in *P. mirabilis* species, we performed comparative genomics analysis amongst all available 56 *P. mirabilis* genomes, including the *P. mirabilis* SCDR1 genome. As stated before, all *P. mirabilis* genomes shared 16 AMROs (Table 6). For example, all genomes contained the AMRO of copper sensory histidine kinase CpxA in cpxA mutant confer resistant to amikacin, copper-sensing two-component system response regulator CpxR, which is a regulator that promotes acrD expression when phosphorylated by a cascade involving CpxA, a sensor kinase and linked to cefepime and chloramphenicol resistance in *Klebsiella pneumoniae* [90]. However, different *P. mirabilis* genomes varied in the remaining 45 studied AMRO (Table 6). For example, genomics analysis of *P. mirabilis*-SCDR1 showed that our isolates contained genetic determinants for fluoroquinolones resistance (gyrA, parC and parE) [91, 92], Daptomycin and Rifamycin resistance (rpoB) [93], Chloramphenicol (cpxA and cat) [90, 94], Ethidium bromide-methyl viologen resistance protein (emrE) [95] and Polymyxin and colistin resistance (phoP) [96]. In addition, several multidrug resistance efflux systems and complexes were observed. These include MdtABC-TolC, which is a multidrug efflux system in Gram-negative bacteria, including *E. coli* and *Salmonella* that confer resistance against β-lactams, novobiocin and deoxycholate. It is noteworthy that MdtABC-TolC and AcrD plays a role in metal resistance (copper and zinc), along with their BaeSR regulatory system [97] which was also was found in our *P. mirabilis* SCDR1 genome [Table 7], and thus may also play an additional role in silver resistance. MdtABC-TolC contains MdtA, which is a membrane fusion protein, TolC, which is the outer membrane channel and...
| Antimicrobial Resistance based ontology (AMRO)                                                                 | Number of Genomes shared AMRO |
|----------------------------------------------------------------------------------------------------------------|------------------------------|
| $\theta$-$N$-acetyltransferase                                                                                 | 4                            |
| Aminoglycoside 3'-phosphotransferase @ Streptomycin 3'-kinase StrA                                            | 13                           |
| Aminoglycoside 3'-phosphotransferase                                                                            | 16                           |
| Putative transport protein ARO:3,001,215, ARO:1,000,001                                                          | 48                           |
| Beta-lactamase                                                                                                 | 14                           |
| Bicyclomycin resistance protein                                                                                  | 3                            |
| Chloramphenicol acetyltransferase                                                                                | 54                           |
| Aminoglycoside 3'-phosphotransferase 16                                                                         | 16                           |
| Putative transport protein ARO:3,001,215, ARO:1,000,001                                                          | 48                           |
| Beta-lactamase                                                                                                 | 14                           |
| Bicyclomycin resistance protein                                                                                  | 3                            |
| Chloramphenicol acetyltransferase                                                                                | 54                           |
| COG0488: ATPase components of ABC transporters with duplicated ATPase domains                                    | 1                            |
| Copper sensory histidine kinase CpxA                                                                             | 56                           |
| Copper-sensing two-component system response regulator CpxR                                                     | 56                           |
| Cyclic AMP receptor protein                                                                                     | 56                           |
| Dihydropteroate synthase                                                                                       | 56                           |
| Dihydropteroate synthase type-2 @ Sulfonamide resistance protein                                               | 16                           |
| DNA gyrase subunit A                                                                                            | 56                           |
| DNA-binding protein H-NS                                                                                       | 55                           |
| DNA-directed RNA polymerase beta subunit                                                                          | 56                           |
| Ethidium bromide-methyl viologen resistance protein EmrE                                                        | 55                           |
| Gentamicin 3'-N-acetyltransferase                                                                                 | 2                            |
| Hypothetical protein ARO: 3,000,230, ARO: 1,000,001                                                               | 2                            |
| Streptomycin 3'-O-adenylyltransferase @ Spectinomycin 9-O-adenylyltransferase                                    | 5                            |
| Macrolide export ATP-binding/permease protein MacB                                                               | 56                           |
| Macrolide-specific efflux protein MacA                                                                            | 55                           |
| Membrane fusion component of tripartite multidrug resistance system                                            | 1                            |
| MFS superfamily export protein YceL                                                                              | 55                           |
| Mobile element protein ARO: 3,000,903, ARO: 1,000,001                                                              | 9                            |
| Multi antimicrobial exclusion protein (Na (+)/drug antiporter), MATE family of MDR efflux pumps                 | 56                           |
| Multidrug resistance protein D. ARO: 3,000,309, ARO: 1,000,001                                                  | 56                           |
| Multidrug resistance protein ErmA                                                                                 | 55                           |
| Multidrug resistance protein ErmB                                                                                 | 56                           |
| Multidrug transporter MdtB                                                                                      | 56                           |
| Multidrug transporter MdtC                                                                                      | 56                           |
| Multidrug-efflux transporter, major facilitator superfamily (MFS)                                               | 54                           |
| N-3-oxohexanoyl-L-homoserine lactone quorum-sensing transcriptional activator                                    | 1                            |
| Outer membrane porin OmpF                                                                                        | 54                           |
| Outer membrane protein F precursor                                                                               | 1                            |
| Probable RND efflux membrane fusion protein                                                                       | 1                            |
| Putative transport protein ARO: 3,001,215, ARO: 1,000,001                                                          | 48                           |
| Redox-sensitive transcriptional activator SoxR                                                                     | 55                           |
| Response regulator BaeR                                                                                        | 56                           |
| Ribosomal RNA methyltransferase                                                                                  | 1                            |
| Rifampin ADP-ribosyl transferase                                                                                 | 3                            |
| RND efflux system, inner membrane transporter ARO: 3,000,216, ARO: 1,000,001                                      | 2                            |
MdtBC that forms a drug transporter. In the absence of MdtB, the MdtAC-TolC has narrower drug specificity, leading to the loss of novobiocin resistance [98]. The MdtABC and AcrD systems may be related to bacterial metal homeostasis by transporting metals directly. This is to some extent similar to the copper and silver resistance mechanism by cation efflux of the CusABC system belonging to the RND protein superfamily [97, 99].

As stated before, this is the first report for spontaneous resistance against nanosilver. However, Gunawan et al., (2013) reported the natural ability of Bacillus sp. to adapt to nanosilver cytotoxicity under prolonged cellular oxidative stress mechanism by cation efflux of the CusABC system belonging to the RND protein superfamily [97, 99].

As stated before, this is the first report for spontaneous resistance against nanosilver. However, Gunawan et al., (2013) reported the natural ability of Bacillus sp. to adapt to nanosilver cytotoxicity under prolonged cellular oxidative stress mechanism by cation efflux of the CusABC system belonging to the RND protein superfamily [97, 99].

As stated before, this is the first report for spontaneous resistance against nanosilver. However, Gunawan et al., (2013) reported the natural ability of Bacillus sp. to adapt to nanosilver cytotoxicity under prolonged cellular oxidative stress mechanism by cation efflux of the CusABC system belonging to the RND protein superfamily [97, 99].

Increasing antimicrobial nanosilver usage could prompt a silver resistance problem in Gram-negative pathogens, particularly since silver resistance is already known to exist in several such species [81, Table 6 Species wide Proteus mirabilis antibiotic resistome constituents (Continued)

| Antimicrobial Resistance based ontology (AMRO)                                                                 | Number of Genomes shared AMRO |
|---------------------------------------------------------------------------------------------------------------|------------------------------|
| RND efflux system, inner membrane transporter: Aminoglycoside, Glycylcycline, Beta_lactam, Macrolide, Acriflavin | 3                            |
| RND efflux system, inner membrane transporter Aminoglycoside, Glycylcycline, Beta_lactam, Macrolide, Acriflavin ARO: 3,000,216, ARO: 1,000,001 | 3                            |
| RND efflux system, membrane fusion protein (acrA, ARO: 1,000,001, ARO: 3,000,207) OR (mdtA, ARO: 1,000,001, ARO: 3,000,792) | 56                           |
| RND multidrug efflux transporter; Acriflavin resistance protein                                           | 2                            |
| Sensor histidine kinase PhoQ                                                                                 | 55                           |
| Sensory histidine kinase BaeS                                                                                 | 56                           |
| SSU rRNA (adenine (1518)-N (6)/adenine (1519)-N (6))-dimethyltransferase                                   | 1                            |
| Streptomycin 3′-Oadenylyltransferase @ Spectinomycin 9-O-adenylyltransferase (spectinomycin, streptomycin) (ARO: 1,000,001, ARO: 3,000,232) (tobramycin, gentamicin, dibekacin, sisomicin, kanamycin) | 9                            |
| Tetracycline efflux protein TetA                                                                               | 55                           |
| Topoisomerase IV subunit A                                                                                    | 54                           |
| Transcription repressor of multidrug efflux pump acrAB operon, TetR (AcrR) family                           | 3                            |
| Transcriptional regulator of acrAB operon, AcrR                                                             | 56                           |
| Transcriptional regulatory protein PhoP                                                                       | 55                           |
| Transcriptional repressor MrpA                                                                               | 55                           |
| Translation elongation factor Tu                                                                              | 55                           |
| TrkA-N: Sodium/hydrogen exchanger                                                                            | 3                            |
| Two-component system response regulator OmpR                                                                  | 55                           |
| Type I secretion outer membrane protein, TolC precursor                                                      | 55                           |
| UDP-4-amino-4-deoxy-L-arabinose formyltransferase/ UDP-glucuronic acid oxidase (UDP-4-keto-hexauronic acid decarboxylating) | 1                            |
Fig. 8 Species wide *Proteus mirabilis* antibiotic resistome constitutents
| Source | Source Organism | Gene | Product | Function | Query Coverage | Identity | E-value |
|--------|----------------|------|---------|----------|----------------|----------|---------|
| ARDB   | *P. mirabilis* ATCC 29906 | tetAJ | Tetracycline efflux protein TetA | Major facilitator superfamily transporter, tetracycline efflux pump. | 97 | 95 | 0 |
| CARD   | *P. mirabilis* BB2000 | tetAJ | Tetracycline efflux protein TetA | Major facilitator superfamily transporter, tetracycline efflux pump. | 97 | 94 | 0 |
| ARDB   | *P. mirabilis* HI4320 | tetAJ | Tetracycline efflux protein TetA | Major facilitator superfamily transporter, tetracycline efflux pump. | 80 | 99 | 2e-74 |
| CARD   | *P. mirabilis* BB2000 | gyrA | DNA gyrase subunit A (EC 5.99.1.3) | Point mutation of *Escherichia coli* gyrA resulted in the lowered affinity between fluoroquinolones and gyrA. Thus, conferring resistance | 98 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | baeR | Response regulator BaeR | BaeR is a response regulator that promotes the expression of MdtABC and AcrD efflux complexes. | 100 | 99 | 2e-171 |
| CARD   | *P. mirabilis* BB2000 | baeS | Sensory histidine kinase BaeS | BaeS is a sensor kinase in the BaeSR regulatory system. While it phosphorylates BaeR to increase its activity. | 100 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | mdtC | Multidrug transporter MdtC | MdtC is a transporter that forms a hetero-multimer complex with MdtB to form a multidrug transporter. MdtBC is part of the MdtABC-TolC efflux complex. | 100 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | mdtB | Multidrug transporter MdtB | MdtB is a transporter that forms a heteromultimer complex with MdtC to form a multidrug transporter. MdtBC is part of the MdtABC-TolC efflux complex. | 100 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | mdtA | RND efflux system, membrane fusion protein | MdtA is the membrane fusion protein of the multidrug efflux complex mdtABC. | 100 | 98 | 0 |
| CARD   | *P. mirabilis* BB2000 | folP | Dihydropteroate synthase (EC 2.5.1.15) | Point mutations in dihydropteroate synthase folP prevent sulfonamide antibiotics from inhibiting its role in folate synthesis, thus conferring sulfonamide resistance. | 100 | 100 | 0 |
| CARD   | *P. mirabilis* BB2000 | soxR | Redox-sensitive transcriptional activator SoxR | SoxR is a sensory protein that upregulates soxS expression in the presence of redox-cycling drugs. This stress response leads to the expression many multidrug efflux pumps. | 100 | 100 | 0 |
| CARD   | *Shigella dysenteriae* Sd197 | ompR | Two-component system response regulator OmpR | Transcriptional regulatory protein | 99 | 87 | 0 |
| CARD   | *P. mirabilis* BB2000 | emrR | Transcriptional repressor MprA | EmrR is a negative regulator for the EmrAB-TolC multidrug efflux pump in *E. coli*. Mutations lead to EmrAB-TolC overexpression. | 100 | 100 | 0 |
| CARD   | *P. mirabilis* BB2000 | emrA | Multidrug resistance protein ErmA | EmrA is a membrane fusion protein, providing an efflux pathway with EmrB and TolC between the inner and outer membranes of *E. coli*, a Gram-negative bacterium. | 95 | 96 | 0 |
| CARD   | *P. mirabilis* BB2000 | acrE | Membrane fusion component of tripartite multidrug resistance system | AcrEF-ToIC is a tripartite multidrug efflux system similar to AcrAB-ToIC and found in Gram-negative bacteria. AcrE is the membrane fusion protein, AcrF is the inner membrane transporter, and ToIC is the outer membrane channel protein. | 100 | 98 | 3e-44 |
| CARD   | *P. mirabilis* BB2000 | emrB | Multidrug resistance protein ErmB | EmrB is a translocase in the emrB-ToIC efflux protein in *E. coli*. It recognizes substrates including carbonyl cyanide m-chlorophenylhydrazone (CCCP), nalidixic acid, and thioloactomycin. | 100 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | rpoB | DNA-directed RNA polymerase beta subunit (EC 2.7.7.6) | Mutations in rpoB gene confers antibiotic resistance (Daptomycin and Rifamycin) | 100 | 99 | 0 |
| CARD   | *P. mirabilis* BB2000 | tuB | Translation elongation factor Tu | Sequence variants of elongation factor Tu confer resistance to elfamycin antibiotics. | 100 | 100 | 1e-43 |
| CARD   | *P. mirabilis* BB2000 | cpxA | Copper sensory histidine kinase CpxA | CpxA mutant confer resistant to amikacin | 94 | 99 | 0 |
Table 7 Consensus P. mirabilis-SCDR1 antibiotic Resistome (Continued)

| Source | Source Organism | Gene | Product | Function | Query Coverage | Identity | E-value |
|--------|-----------------|------|---------|----------|----------------|----------|---------|
| CARD   | P. mirabilis BB2000 | cpxR  | Copper-sensing two-component system response regulator CpxR | CpxR is a regulator that promotes acrD expression when phosphorylated by a cascade involving CpxA, a sensor kinase. Cefepime and chloramphenicol | 100 100 0 | 100 99 0 | 3e-177 |
| CARD   | P. mirabilis BB2000 | emrD  | Multidrug resistance protein D | EmrD is a multidrug transporter from the Major Facilitator Superfamily (MFS) primarily found in Escherichia coli. EmrD couples efflux of amphipathic compounds with proton import across the plasma membrane. | 100 99 0 | 100 99 0 | 3e-177 |
| CARD   | P. mirabilis BB2000 | macA  | Macrolide-specific efflux protein MacA | MacA is a membrane fusion protein that forms an antibiotic efflux complex with MacB and TolC. | 100 99 0 | 100 99 0 | 3e-177 |
| CARD   | P. mirabilis BB2000 | macB  | Macrolide export ATP-binding/permease protein MacB (EC 3.6.3.-) | MacB is an ATP-binding cassette (ABC) transporter that exports macrolides with 14- or 15-membered lactones. It forms an antibiotic efflux complex with MacA and TolC. | 100 99 0 | 100 99 0 | 3e-177 |
| ARDB   | P. mirabilis ATCC 29906 | cat   | Chloramphenicol acetyltransferase (EC 2.3.1.28) | Group A chloramphenicol acetyltransferase, which can inactivate chloramphenicol. | 99 93 6e-150 | 99 93 6e-150 | 6e-150 |
| CARD   | P. mirabilis BB2000 | cat   | Chloramphenicol acetyltransferase (EC 2.3.1.28) | Group A chloramphenicol acetyltransferase, which can inactivate chloramphenicol. | 99 93 6e-150 | 99 93 6e-150 | 6e-150 |
| CARD   | P. mirabilis BB2000 | acrR  | Transcription repressor of multidrug efflux pump acrAB operon, TetR (AcrR) family | AcrR is a repressor of the AcrAB-TolC multidrug efflux complex. AcrR mutations result in high level antibiotic resistance. | 100 95 2e-25 | 100 95 2e-25 | 9e-25 |
| CARD   | P. mirabilis BB2000 | acrR  | Transcriptional regulator of acrAB operon, AcrR | AcrR is a repressor of the AcrAB-ToIC multidrug efflux complex. AcrR mutations result in high level antibiotic resistance. | 93 95 2e-114 | 93 95 2e-114 | 2e-114 |
| CARD   | P. mirabilis BB2000 | acrA  | RND efflux system, membrane fusion protein | Protein subunit of AcrA-AcrB-ToIC multidrug efflux complex. AcrA represents the periplasmic portion of the transport protein. | 100 99 0 | 100 99 0 | 0 |
| CARD   | P. mirabilis BB2000 | mdtK  | Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps | A multidrug and toxic compound extrusions (MATE) transporter conferring resistance to norfloxacin, doxorubicin and acriflavine. | 98 99 3e-164 | 98 99 3e-164 | 3e-164 |
| CARD   | Salmonella enterica subsp. enterica serovar Agona str. SL483 | hns   | DNA-binding protein H-NS | H-NS is a histone-like protein involved in global gene regulation in Gram-negative bacteria. It is a repressor of the membrane fusion protein genes acrE, mdtE, and emrK as well as nearby genes of many RND-type multidrug exporters. | 100 80 0 | 100 80 0 | 0 |
| CARD   | P. mirabilis BB2000 | tufB  | Translation elongation factor Tu | Sequence variants of elongation factor Tu confer resistance to tetracycline antibiotics. | 100 99 0 | 100 99 0 | 0 |
| CARD   | Shigella dysenteriae 5d197 | cpr   | Cyclic AMP receptor protein | CRP is a global regulator that represses mdhEF multidrug efflux pump expression. | 100 98 0 | 100 98 0 | 0 |
| CARD   | P. mirabilis BB2000 | emrE  | Ethidium bromide-methyl viologen resistance protein EmrE | EmrE is a small multidrug transporter that functions as a homodimer and that couples the efflux of small polyaromatic cations from the cell with the import of protons down an electrochemical gradient. EmrE is found in E. coli and P. aeruginosa. | 100 99 6e-73 | 100 99 6e-73 | 6e-73 |
| CARD   | P. mirabilis BB2000 | mdtK  | Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps | A multidrug and toxic compound extrusions (MATE) transporter conferring resistance to norfloxacin, doxorubicin and acriflavine. | 100 100 2e-113 | 100 100 2e-113 | 2e-113 |
| CARD   | P. mirabilis BB2000 | NIA   | Putative transport protein | NIA | 100 94 7e-59 | 100 94 7e-59 | 7e-59 |
| CARD   | P. mirabilis BB2000 | NIA   | Multidrug resistance protein | NIA | 99 96 2e-112 | 99 96 2e-112 | 2e-112 |
| CARD   | P. mirabilis BB2000 | parC  | Topoisomerase I subunit A (EC 5.99.1.-) | ParC is a subunit of topoisomerase IV, which decatenates and relaxes DNA to allow access to genes for transcription or translation. Point mutations in ParC prevent fluoroquinolone antibiotics from inhibiting DNA synthesis, and confer low-level resistance. Higher-level resistance results from both gyrA and parC mutations. | 99 99 0 | 99 99 0 | 0 |
Both exogenous (horizontally acquired Sil system) endogenous (mutational Cus system) resistance to silver has been reported in Gram-negative bacteria [13, 81]. Li et al. [81] selected five *Escherichia coli* mutants that present a ≥64-fold decreases in silver susceptibility compared with their original strain. All the mutants exhibited loss of expression of outer membrane porins (OmpF or OmpF/C), which seemingly resulted in the reduction of outer membrane permeability. These findings implied that reduced silver susceptibility is a result of restricting silver entrance into the bacterial cell. Moreover, they found that these mutants express active efflux that pumps silver outside of the cell. It was found that the *cus* CFBA operon is the responsible of silver efflux pump. Similarly, in our case, we observed the presence of resistance operon with high similarity to the *cus* operon, which is a chromosomally encoded system because of the lack of any plasmid in *P. mirabilis SCDR1*. However, both endogenous and exogenous silver resistance systems, in Gram-negative bacteria, remain incompletely understood [83].

The occurrence of induced nanosilver resistance (in vitro) in *Bacillus sp.* and *E. coli* [14, 54], spontaneous resistance (in our case) and the frequent uses and misuses of nanosilver-containing medical products should suggest adopting an enhanced surveillance systems for nanosilver-resistant isolates in medical setups. In addition, there should be greater control over utilizing nanosilver-containing products in order to maintain nanosilver as a valuable alternative approach in the fight against multidrug resistant pathogens.

### Conclusion

In the present study, we introduced the *P. mirabilis SCDR1* isolate that was collected from a diabetic ulcer patient. *P. mirabilis SCDR1* showed high levels of resistance against nanosilver colloids, nanosilver chitosan composite and the commercially available nanosilver and silver bandages. Our isolate contains all the required pathogenicity and virulence factors to establish a successful infection. *P. mirabilis SCDR1* contains several physical and biochemical mechanisms for antibiotics and silver/nanosilver resistance, which are biofilm formation, swarming mobility, efflux systems, and enzymatic detoxification.
### Table 8  
*P. mirabilis* SCDR1 Heavy Metal Resistance/Binding factors

| Annotation | Reference Genome | Accession Number | Gene | Protein ID | AA Length | Corresponding Protein |
|------------|------------------|------------------|------|------------|------------|------------------------|
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668580 | corC | ZP_03842837.1 | 293 | Magnesium/cobalt efflux protein CorC. |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | NA | AGS60530.1 | 305 | Cation efflux protein (Divalent metal cation (Fe/Ca/Zn/Cd) transporter). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | cueR | ZP_03840921.1 | 133 | MerR-family transcriptional regulator (copper efflux regulator). |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | arsB | AGS60689.1 | 429 | Arsenical pump membrane protein (ArsB_permease). |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | NA | AGS59089.1 | 129 | Metal resistance protein. |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | ahpF | ZP_03839875.1 | 521 | Protein-disulfide reductase. |
| PATRIC     | *P. mirabilis* strain 25,933 GTA | NZ_GG668578 | dsbB | ZP_03840198.1 | 174 | Protein disulfide oxidoreductase. |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | actP1 | ZP_03842696.1 | 803 | Metal resistance protein. |
| PATRIC     | *P. mirabilis* strain ATCC 7002 | JOV01000008 | grxA | KGA90223.1 | 87 | Glutaredoxin, GrxA family. |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | cueO | AGS58840.1 | 526 | Multicopper oxidase. |
| PATRIC     | *P. mirabilis* strain ATCC 7002 | JOV01000009 | yobA | ZP_03839688.1 | 130 | Copper resistance protein (Copper-binding protein CopC (methionine-rich)) (Inorganic ion transport and metabolism). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | copD | ZP_03839689.1 | 279 | Copper resistance protein. |
| PATRIC     | *P. mirabilis* strain SAS71 | LDU01000481 | NA | PGF_00419563 | 114 | Copper resistance protein D. |
| BRC1       | *P. mirabilis* H4320 | NC_010554 | NA | NA | 300 | Putative copper resistance protein, secreted. |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | cutC | ZP_03839779.1 | 250 | Copper homeostasis protein CutC (Cytosolic copper homeostasis protein CutC). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668576 | cutF | ZP_03841587.1 | 225 | Copper homeostasis protein CutF precursor/Lipoprotein NlpE involved in surface adhesion. |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | NA | AGS60771.1 | 904 | Copper exporting ATPase. |
| RefSeq     | *P. mirabilis* strain ATCC 7002 | JOV01000009 | kdpB | KGA9427.1 | 685 | Copper exporting ATPase (potassium-transporting ATPase subunit B). |
| RefSeq     | *P. mirabilis* | WP_0123687272.1 | copA-| WP_0123687272 | 984 | Copper exporting ATPase (Heavy-metal-associated domain (HMA)). |
| RefSeq     | *P. mirabilis* strain ATCC 7002 | JOV01000005 | cueF | KGA91278.1 | 135 | Copper-responsive transcriptional regulator (HTH_MerR-SF Superfamily). |
| RefSeq     | *P. mirabilis* BB2000 | CP004022 | cutF | ZP_03841587.1 | 154 | Copper homeostasis protein CutF precursor/Lipoprotein NlpE involved in surface adhesion. |
| Annotation | Reference Genome | Accession Number | Gene | Protein ID | AA Length | Corresponding Protein |
|------------|------------------|------------------|------|------------|-----------|-----------------------|
| PATRIC     | *P. mirabilis* BB2000 | CP004022         | terB | AGS60978.1 | 151       | *P. mirabilis* tellurite resistance loci. |
|            | RefSeq           |                  | terA | AGS60979.1 | 382       |                        |
|            |                  |                  | terC | AGS60977.1 | 341       |                        |
|            |                  |                  | terD | AGS60976.1 | 192       |                        |
|            |                  |                  | terE | AGS60975.1 | 191       |                        |
|            |                  |                  | terZ | AGS60980.1 | 194       |                        |
| PATRIC     | *Mycobacterium* sp. | YP_001705575.1  | ctpC | AEN01737.1 | 718       | Probable cation-transporting ATPase G (ATPase-Ib2_Cd). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668579     | yntB | ZP_03841770.1 | 325 | Nickel transport system permease protein nikB2 (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668579     | yntA | ZP_03841771.1 | 527 | Nickel ABC transporter, periplasmic nickel-binding protein nikA2 (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668583     | NA   | ZP_03839446.1 | 289 | Nickel transport system permease protein nikC (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668583     | NA   | ZP_03839447.1 | 269 | Nickel transport ATP-binding protein nikD (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668579     | yntD | ZP_03841768.1 | 267 | Nickel transport ATP-binding protein nikD2 (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668579     | yntE | ZP_03841767.1 | 203 | Nickel transport ATP-binding protein nikE2 (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668579     | yntC | ZP_03841769.1 | 270 | Nickel transport system permease protein nikC2 (TC 3.A.1.5.3). |
| PATRIC     | *P. mirabilis* BB2000 | CP004022         | hybF | AGS58541.1 | 113       | [NiFe] hydrogenase nickel incorporation protein HypA. |
| PATRIC     | *P. mirabilis* ATCC 29906 | NZ_GG668578     | hybB | ZP_03842517.1 | 282 | [NiFe] hydrogenase nickel incorporation-associated protein HypB. |
| RefSeq     | *C. crescentus* OR37 | APMP01000019     | NA   | ENZ81282.1 | 723       | Copper/silver/heavy metal-translocating P-type ATPase, Cd/Cu/Hg/Pb/Zn-transporting. |
| RefSeq     | Armactimonadetes bacterium OLB1 B. gilvus | JZC01000012     | arsM | KXX16912.1 | 283       | Arsene S-adenosylmethyltransferase (Methyltransferase type 11). |
| RefSeq     | *R. palustris* TIE-1 | NC_011004        | NA   | YP_001990857.1 | 973 | Heavy metal translocating P-type ATPase (ATPase-Ib1_Cu). |
| RefSeq     | *M. ulcerans* str. Harvey | EUA92940.1, | CuRO_2_CopA_like1 | EUA92940.1 | 552       | Multicopper oxidase family protein. |
| RefSeq     | B. mallei NCTC 10229 | NC_008835        | oprB | YP_001024205.1 | 553 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB). |
| RefSeq     | B. pseudomallei 576 | NA | oprM | ZP_03450560.1 | 558 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprM). |
| PATRIC     | Achromobacter sp. strain 27895TDY15608636 | CYTV01000008 | cusC_1 | ABAS2627.1 | 515 | Copper/silver efflux system outer membrane protein CusC (RND efflux system outer membrane lipoprotein). |
| RefSeq     | Achromobacter sp. strain 27895TDY15608623 | CYSV01000001 | NA | CUI29018.1 | 98 | Outer membrane component of tripartite multidrug resistance system (CusC). |
| RefSeq     | R. opacus TIE-1 | NC_011004 | NA | YP_001990857.1 | 973 | Heavy metal translocating P-type ATPase (ATPase-Ib1_Cu). |
| RefSeq     | B. ubiquitum str. Harvey | EUA92940.1, | CuRO_2_CopA_like1 | EUA92940.1 | 552 | Multicopper oxidase family protein. |
| RefSeq     | B. mallei NCTC 10229 | NC_008835 | oprB | YP_001024205.1 | 553 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB). |
| RefSeq     | B. pseudomallei 576 | NA | oprM | ZP_03450560.1 | 558 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprM). |
| PATRIC     | Achromobacter sp. strain 27895TDY15608636 | CYTV01000008 | cusC_1 | ABAS2627.1 | 515 | Copper/silver efflux system outer membrane protein CusC (RND efflux system outer membrane lipoprotein). |
| RefSeq     | Achromobacter sp. strain 27895TDY15608623 | CYSV01000001 | NA | CUI29018.1 | 98 | Outer membrane component of tripartite multidrug resistance system (CusC). |
| RefSeq     | R. opacus TIE-1 | NC_011004 | NA | YP_001990857.1 | 973 | Heavy metal translocating P-type ATPase (ATPase-Ib1_Cu). |
| RefSeq     | B. ubiquitum str. Harvey | EUA92940.1, | CuRO_2_CopA_like1 | EUA92940.1 | 552 | Multicopper oxidase family protein. |
| RefSeq     | B. mallei NCTC 10229 | NC_008835 | oprB | YP_001024205.1 | 553 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprB). |
| RefSeq     | B. pseudomallei 576 | NA | oprM | ZP_03450560.1 | 558 | Copper/silver efflux system outer membrane protein CusC (outer membrane efflux protein OprM). |

*PATRIC cross-genus families (PGfams)
Additional files

Additional file 1: Table S1. Distribution of unique gene counts amongst different metabolic pathways. (DOCX 11 kb)

Additional file 2: Table S2. Distribution of unique gene counts amongst pathways Classes and subclasses. (DOCX 16 kb)

Additional file 3: Table S3. P. mirabilis SCDR1 Pathogen Finder results. (DOCX 25 kb)

Additional file 4: Table S4. Major pathogenic virulence factors for Proteus mirabilis SCDR1. (DOCX 32 kb)

Additional file 5: Table S5. Strict Antibiotic resistance analysis of Proteus mirabilis SCDR1. (DOCX 17 kb)

Additional file 6: Table S6. Modified loose Antibiotic resistance analysis of Proteus mirabilis SCDR1. (DOCX 65 kb)

Additional file 7: Table S7. Drug Resistance related proteins and its corresponding genes or proteins GenBank access numbers. (DOCX 16 kb)

Abbreviations

ATMS: Involved in study conception and design, data analysis and interpretation. Involved in drafting the manuscript or revising it critically for important intellectual content. Preparing the final approval of the version to be published. MAH: Involved in study design. Involved in acquisition of data, or analysis and interpretation of data; preparation and involved in drafting the manuscript. MS: Involved in acquisition of data, or analysis and interpretation of data. HT: Involved in study conception and design. Involved in drafting the manuscript or revising it critically for important intellectual content. Preparing the final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by institutional review board in King Saud University, College of Medicine Riyadh, Kingdom of Saudi Arabia. The subject was provided written informed consent for participating in this study.

Consent for publication

All authors have consented for publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Genetics and Biotechnology Department, Strategic Center for Diabetes Research, College of medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia. 2Genetics Department, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia. 3Saudi Human Genome Project, King Abdulaziz City for Science and Technology (KACST), Riyadh, Kingdom of Saudi Arabia. 4Integrated Gulf Biosystems, Riyadh, Kingdom of Saudi Arabia.

Received: 11 July 2017 Accepted: 9 November 2017

Published online: 23 November 2017

References

1. Chen X, Schlüsener HU. Nanosilver: a nanoproduct in medical application. Toxicol Lett. 2008;176:1–12.
2. Dunn K, Edwards-Jones V. The role of Acticcoat with nanocrystalline silver in the management of burns. Burns J. Int. Soc. Burn Inj 2004;30 Suppl 1:S1–9.
3. Franci G, Falanga A, Gâldiero S, Falomba L, Rai M, Morelli G, et al. Silver nanoparticles as potential antibacterial agents. Mol. Basel Switz. 2015;20:856–74.
4. Lu L, Sun RW-Y, Chen R, Hui CK, Ho C-M, Luk JM, et al. Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther. 2008;13:253–62.
5. Microsoft Word - Final_Opinion_Health Effects of Exposure to nanosilver to be published on 13 06 2014.docx - scenery_o_039.pdf [Internet]. [cited 2016 Nov 3]. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenery_o_039.pdf
6. Oyandel-Craver VA, Smith JA. Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment. Environ Sci Technol. 2008;42:927–33.
7. Prabh S, Poulous EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int. Nano Lett. 2012;12:32.
8. Samuel U, Guggenbichler JP. Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. Int J Antimicrob Agents. 2004;23(Suppl 1):S57–8.
9. Saeb ATM, Alshammari AS, Al-Brahim H, Al-Rubeaan KA. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. ScientificWorldJournal. 2014;2014:704708.
10. Velaquez-Velasquez JL, Santos-Flores A, Araujo-Meléndez J, Sánchez-Sánchez R, Velasco-Ullo C, González C, et al. Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles. Mater Sci Eng C Mater Sci Eng C Mater Biol Appl. 2015;49:604–11.
11. Lulove EJ, Bernstein B. Use of SilvStat® in lower extremity wounds: a two center case series « Journal of Diabetic Foot Complications 2015;7:13–16.
12. Hendry AT, Stewart IO. Silver-resistant Enterobacteriaceae from hospital patients. Can J Microbiol. 1979;25:915–21.
13. McHugh GL, Moelering RC, Hopkins CC, Swartz MN. Salmonella typhimurium resistant to silver nitrate, chloramphenicol, and ampicillin. Lancet Lond Engl. 1975;1:235–40.
14. Gunawan C, Tech WY, Marquis CP. Amal R. Induced adaptation of Bacillus sp. to antimicrobial nanosilver Small Weinh Bergstr Ger. 2013;9:3554–60.
15. Jansen AM, Lockatell CV, Johnson DE, Mobley HLT. Visualization of Proteus Mirabilis morphotypes in the urinary tract: the elongated swarmer cell is rarely observed in ascending urinary tract infection. Infect Immun. 2003;71:3607–13.
16. Mobley HL, Belas R. Swarming and pathogenicity of Proteus Mirabilis in the urinary tract. Trends Microbiol. 1995;3:280–4.
17. Mathur S, Sabbubba NA, Suller MT, Sticker DJ, Fenley RCL. Genotyping of urinary and fecal Proteus Mirabilis isolates from individuals with long-term urinary catheters. Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol. 2005;24:643–4.
18. Nicolle LE. Catheter-related urinary tract infection. Drugs Aging. 2005;22:627–39.
19. Armbruster CE, Mobley HLT. Merging mythology and morphology: the multifaceted lifestyle of Proteus Mirabilis. Nat Rev Microbiol. 2012;10:743–54.
20. Jacobsen SM, Sticker DJ, Mobley HLT, Shrittif ME. Complicated catheter-associated urinary tract infections due to Escherichia Coli and Proteus Mirabilis. Clin Microbiol Rev. 2008;21:26-59.

21. Różański A, Sidorycz Z, Ketkeli K. Potential virulence factors of Proteus bacilli. Microbiol Mol Biol Rev. 1997;61:65-89.

22. Bronze MS, Cunha BA. Diabetic Foot Infections: Practice Essentials. Background, Pathophysiology [Internet]. 2016 [cited 2016 Nov 3]. Available from: http://emedicine.medscape.com/article/237378/overview

23. Gonzalez G, Bronze MS. Proteus Infections: Background, Pathophysiology, Epidemiology [Internet]. 2016 [cited 2016 Nov 3]. Available from: http://emedicine.medscape.com/article/226434-overview

24. Pearson MM, Sebaihia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, et al. Complete genome sequence of uropathogenic Proteus Mirabilis, a master of both adherence and motility. J Bacteriol. 2008;190:6027–33.

25. Habibi M, Asadi Karam MR, Bouzani S. In silico design of fusion protein of FimH from uropathogenic Escherichia Coli and MepH from Proteus Mirabilis against urinary tract infections. Adv. Biomed Res. 2015;4:217.

26. Baldo C, Rocha SPD. Virulence factors of Uropathogenic Proteus Mirabilis - a mini review. Int. J. Technol. Enhanc. Emerg. Eng. Res. 2014;3:24–7.

27. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol. 2010;13:558–64.

28. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17:1791–8.

29. Horner CS, Abbeley N, Denton M, Wilcox MH. Surveillance of antibiotic susceptibility of Enterobacteriaceae isolated from urine samples collected from community patients in a large metropolitan area, 2010-2012. Epidemiol Infect. 2014;142:399–403.

30. Miro E, Agüero J, Larrosa MN, Fernández A, Conejo MC, Bou G, et al. Prevalence and molecular epidemiology of acquired AmpC β-lactamases and carbapenemases in Enterobacteriaceae isolates from 35 hospitals in Spain. Eur J Clin Microbiol Infect Dis. 2013;32:253–9.

31. Sheng WH, Badal RE, Hsueh P-R, Program SMART. Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for monitoring antimicrobial resistance trends (SMART). Antimicrob Agents Chemother. 2013;57:2981–8.

32. Bouchillon SK, Badal RE, Hoban DJ, Hawser SP. Antimicrobial susceptibility of inpatient urinary tract isolates of gram-negative bacilli in the United States: results from the study for monitoring antimicrobial resistance trends (SMART) program: 2009-2011. Clin Ther. 2013;35:872–7.

33. Hawser SP, Badal RE, Bouchillon SK, Hoban DJ, Hackel MA, Biedenbach DJ, et al. Susceptibility of gram-negative aerobic bacilli from intra-abdominal pathogens to antimicrobial agents collected in the United States during 2011. J Inf Secur. 2014;68:71–6.

34. Karłowska JA, Adam HJ, Baxter MR, Lagacié-Weirs PRS, Walkty AJ, Hoban DJ, et al. Virto activity of cefotaxime–minocycline agent–negative and gram-positive pathogens isolated from patients in Canadian hospitals from 2010 to 2012: results from the CANDIAN surveillance study. Antimicrob Agents Chemother. 2013;57:6500–11.

35. Sader HS, Farell DJ, Flamm RK, Jones RN. Antimicrobial susceptibility of gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009-2011). Diagn Microbiol Infect Dis. 2014;78:443–8.

36. Chen L, Al Laham N, Chavda KD, Medivala JR, Jacobs MR, Bonomo RA, et al. First report of an OXA-48-producing multidrug-resistant Proteus Mirabilis strain from Gaza, Palestine. Antimicrob Agents Chemother. 2015;59:4305–8.

37. Latif U, Al-Rubeaan K, Saed AT, Voldy Larsen M, Møller MB. Are silver-containing dressings effective against multidrug-resistant bacteria in biofilms? [internet]. Orlando; 2010. Available from: www.mgc.ac.cn/VFs/

38. Liu B, Pop M. ARDB–antibiotic resistance genes database. Nucleic Acids Res. 2009;37:D443–7.

39. McArthur AG, Wright GD. Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. Curr Opin Microbiol. 2015;27:45–50.

40. McArthur AG, Waglechner N, Ninam F, Yan A, Azad MA, Baylaj AJ, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. 2013;57:3348–57.

41. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640–4.

42. Pal C, Bengtsson-Palme J, Rensing C, Kristiansson E, Lasson DGG. BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. 2014;42:D737–43.

43. Olatun AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014;5:643.

44. Magiogakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 2012;18:268–81.

45. Graves JL, Tajkari M, Cunningham Q, Campbell A, Nongha H, Harrison SH, et al. Rapid evolution of silver nanoparticle resistance in Escherichia coli. Front. Genet. [Internet]. 2015 [cited 2017 Oct 16]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4330922/

46. Lang T, Liu S, Song S, Wang E. Electrochemical synthesis of ag nanoparticles on functional carbon surfaces. J Electroanal Chem. 502:146

47. Tang Z, Liu S, Dong S, Wang E. Electrochemical synthesis of ag nanoparticles on functional carbon surfaces. J Electroanal Chem. 502:146

48. Sader HS, Farell DJ, Flamm RK, Jones RN. Antimicrobial susceptibility of gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009-2011). Diagn Microbiol Infect Dis. 2014;78:443–8.

49. McArthur AG, Wright GD. Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. Curr Opin Microbiol. 2015;27:45–50.

50. McInroy L, Cullen B, Clark R. Are silver-containing dressings effective against multidrug-resistant bacteria in biofilms? [internet]. Orlando; 2010. Available from: www.mgc.ac.cn/VFs/

51. Vajea-Kolstad G, Horn SJ, van Aalten DMF, Systad B, Elsijn VGH. The non-catalytic chitin-binding protein CBP21 from Seratia Marcescens is essential for chitin degradation. J Biol Chem. 2005;280:28492–7.

52. Swift AL, Chadaiah S, Moore JA, Kirmancham DL. Chitin degradation proteins produced by the marine bacterium Vibrio Harveyi growing on different forms of chitin. Appl Environ Microbiol. 1997;63:408–13.

53. Wiczkorek AS, Hetz SA, Kolb S. Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil sludges. Biogeosciences. 2014;11:3359–3362.

54. Gupta V, Prasanna R, Natarajan C, Srivastava AK, Sharma J. Identification, characterization, and regulation of a novel antifungal chitosanase gene (cho) in anabaena spp. Appl Environ Microbiol. 2010;76:2769–77.

55. Gupta V, Prasanna R, Srivastava AK, Sharma J. Purification and characterization of a novel antifungal endo-type chitosanase from anabaena fertilissima. Ann Microbiol. 2011;62:1089–98.

56. Cutting K, White R, Edmonds M. The safety and efficacy of dressings with silver - addressing clinical concerns. Int Wound J. 2007;4:177–84.

57. McInroy L, Cullen B, Clark R. Are silver-containing dressings effective against bacteria in biofilms? [Internet]. Orlando; 2010. Available from: www.oyagenerix.com/cms/uploads/McInroy_biofilms_SAWC_2010.pdf

58. Stephens S, Clark R, Del Boni M, Snyder R. Designing in vitro, in vivo and clinical evaluations to meet the needs of the patient and clinician: dressing wound adherence. Geneva; 2010.

59. Thomas S. Alginate dressings in surgery and wound management–part 1. J Wound Care. 2000;9:556–60.
67. Thomas S. Alginate dressings in surgery and wound management: part 2. J Wound Care. 2009;11:5–9.
68. Thomas S. Alginate dressings in surgery and wound management: part 3. J Wound Care. 2009;16:6–13.
69. Exalto® S7. Powerful and effective interaction with microbes. [Internet]. Accessed from: http://www.exalto-tech.com/images/docs/exalto%20science%20brochure%20spectrumpdf
70. Haycock S, Chadwick P. Using an activated charcoal dressing with silver for malodor, infection and overgranulation in diabetic foot ulcers: importance of appropriate dressing selection for diabetic foot ulcers. Diabet Foot J. 2014;7:4–7.
71. Allison C, Lai HC, Hughes C. Co-ordinate expression of virulence genes during swarming-cell differentiation and population migration of Proteus Mirabilis. Mol Microbiol. 1992;6:1583–91.
72. Butler MT, Wang Q, Harshay RM. Cell density and mobility protect swarming bacteria against antibiotics. Proc Natl Acad Sci U S A. 2010;107:3776–81.
73. Lai S, Tremblay J, Déziel E. Swarming motility: a multicellular behaviour conferring antimicrobial resistance. Environ Microbiol. 2009;11:126–36.
74. Gadagkar R. SURVIVAL STRATEGIES: COOPERATION AND CONFLICT IN ANIMAL SOCIETIES. [Internet]. Cambridge, Massachusetts: Harvard University Press, 1997. [cited 2016 Nov 3]. Available from: http://www.researchgate.net/publication/276283120_Gadagkar_R_1997_SURVIVAL_STRATEGIES_COOPERATION_AND_CONFLICT_IN_ANIMAL_SOCIETIES_Harvard_University_Press_Cambridge_Massachusetts_x_196_pp_ISBN_0-674-17055-S_price_hardcover_2200
75. Hamilton WD. Geometry for the selfish herd. J Theor Biol. 1971;31:295–311.
76. Wu YL, Liu KS, Yin XT, Fei RM. GlpC gene is responsible for biofilm formation and defense against phagocytes and imparts tolerance to pH and organic solvents in Proteus vulgaris. Genet Mol Res GMR. 2015;14:10619–29.
77. Jiang S-S, Liu M-C, Teng L-J, Wang W-B, Hsueh P-R, Liaw S-J. Proteus Mirabilis pmr, an RppA-regulated gene necessary for polymyxin B resistance, biofilm formation, and urothelial cell invasion. Antimicrob Agents Chemother. 2010;54:1564–71.
78. Chien C-C, Lin B-C. Biofilm WC-H. Formation and heavy metal resistance by an environmental pseudomonas sp. Biochem Eng J. 2013;7:812–7.
79. Nocelli N, Bogino PC, Banchoo E, Giordano W. Roles of extracellular polysaccharides and biofilm formation in heavy metal resistance of rhizobia. Materials. 2016;9:418.
80. Halby N, Bjamsholt T, Givkov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35:322–32.
81. Li XZ, Nkaido H, Williams KE. Silver-resistant mutants of Escherichia Coli display effective aig- and are deficient in porins. J Bacteriol. 1992;176:1327–32.
82. Lok C-N, Ho C-M, Chen R, Tam PK-H, Chiu J-F, Che C-M. Proteomic identification of the Cus system as a major determinant of constitutive Escherichia Coli Silver resistance of chromosomal origin. J Proteome Res. 2008;7:2351–6.
83. Randall CP, Gupta A, Jackson N, Busse D, O’Neill AJ. Silver resistance in gram-negative bacteria: a dissection of endogenous and exogenous mechanisms. J Antimicrob Chemother. 2015;70:1037–46.
84. Zhang W, Yin K, Li B, Chen LA. Glutathione S-transferase from Proteus Mirabilis involved in heavy metal resistance and its potential application in removal of Hg2+. J Hazard Mater. 2013;261:646–52.
85. Nair PMG, Choi J. Identification, characterization and expression profiles of Chironomus Riparius glutathione S-transferase (GST) genes in response to cadmium and silver nanoparticles exposure. Aquat Toxicol Amst Neth. 2011;101:550–60.
86. Toptchieva A, Sisson G, Bryden LI, Taylor DE, Hoffman PS. An inducible tellurium-resistance operon in Proteus Mirabilis. Microbiol Read Engl. 2003;49:1285–95.
87. Qin J, Lehr CR, Yuan C, Le XC, McDermott TR, Rosen BP. Biotransformation of arsenic by a Yellowstone thermophilic eukaryotic alga. Proc Natl Acad Sci U S A. 2009;106:5213–7.
88. Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenate S-adenosylmethionine methyltransferase. Proc Natl Acad Sci U S A. 2006;103:2075–80.
89. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, et al. CDD: NCBI’s conserved domain database. Nucleic Acids Res. 2015;43: D222–6.
90. Srivisvanas VB, Vaidyanathan V, Mondal A, Rajamohan G. Role of the two component signal transduction system CpxAR in conferring cefepime and chloramphenicol resistance in Klebsiella Pneumoniae NTUH-K2044. PLoS One. 2012;7:e33777.