Microbes and Infectious Diseases

Case report

A case oriented study: Whole genome sequencing on a wild urease negative *Proteus mirabilis* isolated from deep surgical site infection at El Hussein University Hospital, Al Azhar University Cairo, Egypt

Sawsan H.M. El Tayeb *[1], Mohamed R. Abdelhamed [1], Doaa Gamal [2], M.S. Soliman [3], Amani Ali El-Kholy [3]

1- Clinical Pathology Department, Faculty of medicine (Boys), Al Azhar University, Cairo, Egypt,
2- Microbiology Department, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt,
3- Clinical Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

A R T I C L E I N F O

A B S T R A C T

Background: *Proteus mirabilias (P. mirabilis)* strains are motile Gram negative rods that colonize the human gastrointestinal tract and could be a source of nosocomial infections especially in immune compromised cancer patients. *Proteus mirabilis* is identified by its urease production as a major bacterial virulence especially in urinary tract infections. **Aim:** This study aimed to characterize a case of a urease negative *P. mirabilis* isolated from a cancer patient suffering from deep surgical site infection. **Methods:** Conventional biochemical tests performed on swarming bacterial growth cultures were characteristic of *P.* mirabilis except for urease production. Identification was confirmed by Vitek-2 compact system and Vitek- MS. Dienes test proved the isolate is antigenically different from other urease positive *P. mirabilis.* Whole genome sequencing (WGS) identified this *P. mirabilis* strain with the seven encoding urease genes *ureABCDEFG* as well as their positive regulator gene *UreR*. **Results:** Mutations were found within amino acid sequences of UreB, UreE, UreF and UreG and UreR. Antibiotic sensitivity as well as WGS identified resistance genes to multiple classes of antibiotics. **Conclusion:** This is the first report of a wildly existed urease negative *P. mirabilis* due to mutations in the urease gene cluster (*UreABC*) as well as the regulator UreR. Questions are raised towards the urease enzyme as a key virulence factor to *P. mirabilis*. Other virulence factors that allow this species to cause other severe infections should be considered. The rate of isolation of urease negative *P. mirabilis* should be monitored and assessed in the future.

Introduction

Proteus species are members of the order Bacteriales, family Morganellaceae and genus Proteus. Many species are present within the genus Proteus of which *Proteus mirabilis (P. mirabilis), Proteus vulgaris* and *Proteus pennerie* are common human pathogens. *Proteus mirabilis* is the third common cause of nosocomial infection and accounting for 90% of all *Proteus* infections[1,2] .

Proteus mirabilis is a well-known ureolytic human's pathogen with urease as one of the major bacterial virulence factors especially during urinary tract infections caused by these bacteria. Urease has

* *Corresponding author:* Sawsan El Tayeb

E-mail address: sawsaneltayeb@gmail.com

a toxic effect on human cells and is essential in colonization of a host organism and in maintenance of the bacterial cells in tissues. The *P. mirabilis* urease gene cluster consists of *ureABC*, which encode the apoenzyme structural subunits, and *ureDEFG*, which encode proteins that facilitate insertion of the essential nickel ions into the catalytic site. Urease enzyme in *P. mirabilis* composed mainly of three structural polypeptides subunits: UreA (subunit γ), UreB (subunit β) and UreC (subunit α) encoded by the three structural genes: *ureA*, *ureB* and *ureC* respectively. This is in addition to UreR, encoded by *UreR,* that positively activates expression of the *ure* gene cluster in the presence of urea and is essential for urease production in *P. mirabilis* [3].

Missing informations on whole genome sequencing (WGS) is a laboratory procedure that determines the order of bases in the genome of an organism in one process. Whole genome sequencing provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

Using WGS technology, multiple isolates can be analyzed simultaneously to find out the evolution of the pathogen (phylogenetics) and transmission events (who infected whom) as well as information about resistance to certain antibiotics, which has potential to guide antibiotic treatment. Bacterial and viral genomes are less complex unlike human genome, and the sequencing cost is less than one-tenth that for a human genome. Whole genome sequencing can lead to reduced transmission and infection rates and lower overall costs [4]

Materials and Methods

Bacterial strain and culture conditions: A patient of 18 years old suffered from deep surgical site infection after operation removing malignant tumor involved the right side of his face causing damage of the right cheek with severe tissue necrosis. The patient was operated 52 days in his governor hospital before attending to the oncology unit at El Hussein hospital for chemical therapy. The patientwas referred to Microbiology outpatient clinic laboratory for culture and sensitivity at El Hussein University Hospital, Al Azhar University Cairo, Egypt. Multiple swabs were taken immediately at admission from different sites for culture and sensitivity from the infected wound. The swabs went so deep inside the lesion without any resistance due to severe tissue damage. Swabs

cultures yielded heavy growth of one organism which was lactose non-fermenter pale colonies on MacConkey agar plates and swarming growth of motile Gram negative rods on Blood and Muller Hinton agar plates forming the bull`s eye pattern appearance. Conventional biochemical reactions revealed a catalase positive, oxidase negative, indole negative, urease negative, citrate positive, triple sugar iron (TSI) with acid/alkaline reaction and hydrogen sulphide production, and lysine iron agar (LIA) deamination **(Figure 1),** thus phenotypic tests (except for urease) gave a presumptive identification of *P. mirabilis*. This identification was confirmed by Vitek-2 compact system and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI -TOF MS) by Vitek-MS (Biomerieux, France).

Dienes phenomenon for typing different strains of Proteus species was done on on a Mueller Hinton agar plate. The Dienes phenomenon is based on the principle that when two different strains of *Proteus* species inoculate at different places of the same non-inhibitory medium (blood agar or Meller-Hinton agar), the swarming of the two strains remains separated by a narrow visible furrow. However, in the case of two identical strains of *Proteus*, swarming of two coalesce without signs of demarcation. In the current study our test strain was tested against six different urease positive *P. mirabilis* strains obtained from different samples of different specimens (urine, stool and wounds) of different patients. Agar plates are incubated at 37°C for 24h [5] .

Antibiotic susceptibility tests were done preliminary with disk diffusion by Kirby-Bauer technique and then confirmed by determining minimum inhibitor concentrations (MIC)s using Vitek-2 compact system (BioMerieux, France). Results were interpreted according to Clinical laboratory Standards Institute (CLSI) [6] .

Genomic DNA preparation and WGS: Whole Genome Sequencing was done to the query isolate. In which bacterial DNA was extracted from freshly sub-cultured colonies using the QIAamp DNA Mini Kit (Qiagen, cat # 51304) according to the manufacturer's instructions, and the DNA concentration was measured using a Denovix Fluorometer (Denovix, USA). A total of bacterial DNA (1 ng) was used in the library preparation. The library was prepared with Nextera XT DNA Library Preparation Kit (FC-131-1096, Illumina, USA),

according to the manufacturer's instructions. In brief, transposons were used to fragment the DNA, subsequently adapter sequences were added onto the DNA template, the product was then size-selected for optimum insert length, enriched and quantified. Sequencing was carried out with the MiSeq reagent kit 600 v3 (Illumina, USA) on the Illumina MiSeq, generating, on average301 basepair paired –end reads.

FastQ files were uploaded on the Pathosystems Resource Integration Center

(PATRIC) for assembly and annotation [7]. Alignments was done using the basic local alignment search tool (BLAST) [8] .

Results

Dienes test revealed a distinct line was observed between urease negative *P.mirabilis* and each of the six urease positive strains indicating that the isolate under the study is different from the other urease positive strains **(Figure 2).**

The isolate was found sensitive to amikacin (MIC ≤ 2), meropenem (MIC 0.5), piperacillin/ tazobactam (MIC ≤4), ceftriaxone and cefepime (both of MIC ≤1) and ceftazidime (MIC= 4). It was resistant to ampicillin, ampicillin/sulbactam, cefazolin, ciprofloxacin, levofloxacin and sulfa/trimethoprim and intermediate to cefoxitin **(Table 1).**

General features of the *P. mirabilis* genome.

The *P. mirabilis* genome consists of a 4.063-Mb chromosome and a 36,289-nucleotide (nt) plasmid (pHI4320) containing 3,685 and 55 CDSs, respectively. The origin of replication was assigned based on the GC deviation of the genome and was found to be between *mioC* (PMI3054) and *gidA* (PMI3055).

Genome of *P. mirabilis* is 4,063MB length with 37.7% GC content. Alignments of the amino acid sequences using *P. mirabilis* strain HI4320 showed mutations within the β-subunit as well as in UreE, UreF and UreG and UreR **(Figure3)**. In addition to multiple resistance genes that explain multiple resistance phenotype exhibited by the isolate **(Table 1)**.

Certainly, in the life cycle of *P. mirabilis* environments that lack urea are encountered. It is therefore interesting that there is a pathway by which urea can be synthesized de novo (The fig below) from ammonia and carbon dioxide. This is a figure to show:

Predicted generation of putrescine and urea from ammonia. *P. mirabilis* HI4320 possesses seven genes potentially encoding a pathway that converts ammonia, ATP, carbon dioxide, and water into putrescine and urea in six steps [9].
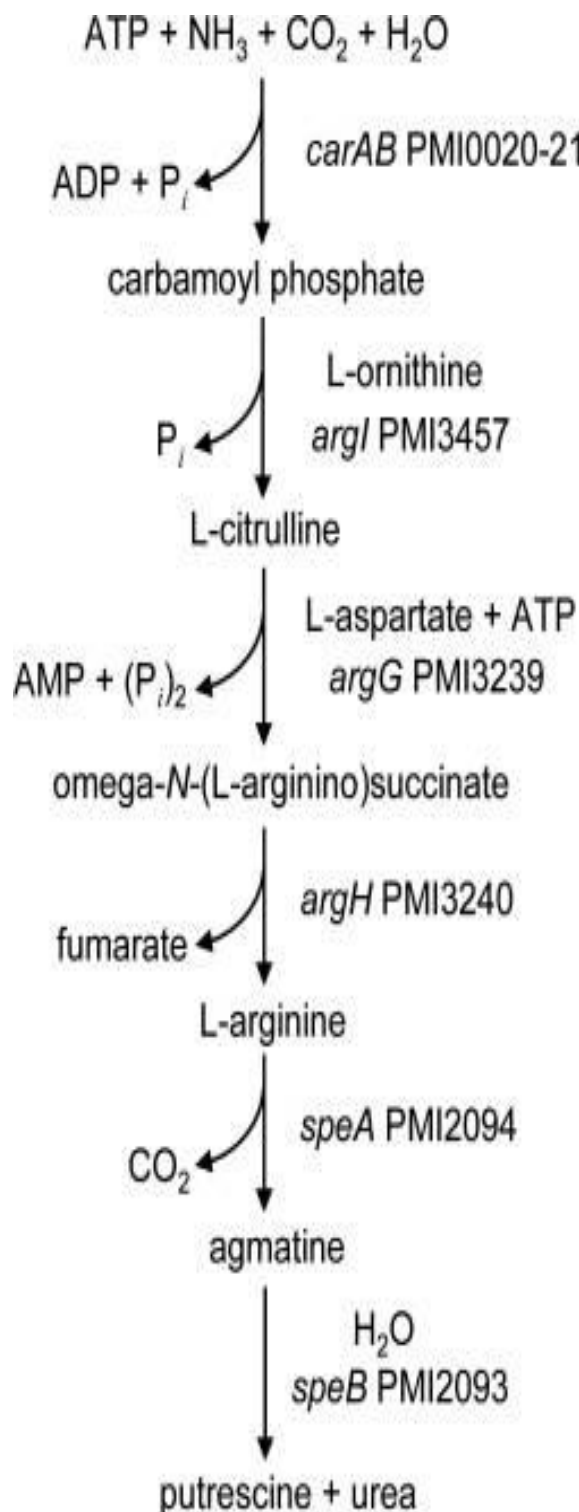
Table 1. Antibiotic sensitivity results of urease negative *P. mirabilis* strain according to CLSI, 2020.

Antibiotic	MIC[a] (µg/ml)	Interpretation[b]	Resistance genes by WGS[c]
Ampicillin	≥32	R	*bla*$_{cmy-2}$
Ampicillin/sulbactam	≥32/16	R	*bla*$_{cmy-2}$
Cefoxitin	16	R	*bla*$_{cmy-2}$
Ceftazidime	4	S	(*bla*$_{cmy-2}$)
Cefepime	≤1	S	---
Piperacillin/tazobactam	≤4	S	---
Amikacin	≤2	S	---
Gentamicin	8	R	aac(3)
Tobramycin	8	R	aac(3)
Meropenem	0.5	S	---
Levofloxacin	≥8	R	qnrA
Ciprofloxacin	≥4	R	qnrA
Sulfa/trimethoprim	≥320	R	sul (1,2,) and dfrA

[a]MIC: Minimum inhibitory concentration, [b] S: sensitive; I: intermediate; R: resistant;[c] WGS: whole genome sequencing.

Figure 1. Results of biochemical reactions: 1: triple sugar iron (TSI), 2: urease, 3: motility indole ornithine medium (MIO), 4 : lysine iron agar (LIA) & 5: Citrate.

Figure 2. The Dienes test showing distict line between urease negative *P. mirabilis* and other urease positive *P. mirabilis* isolates.
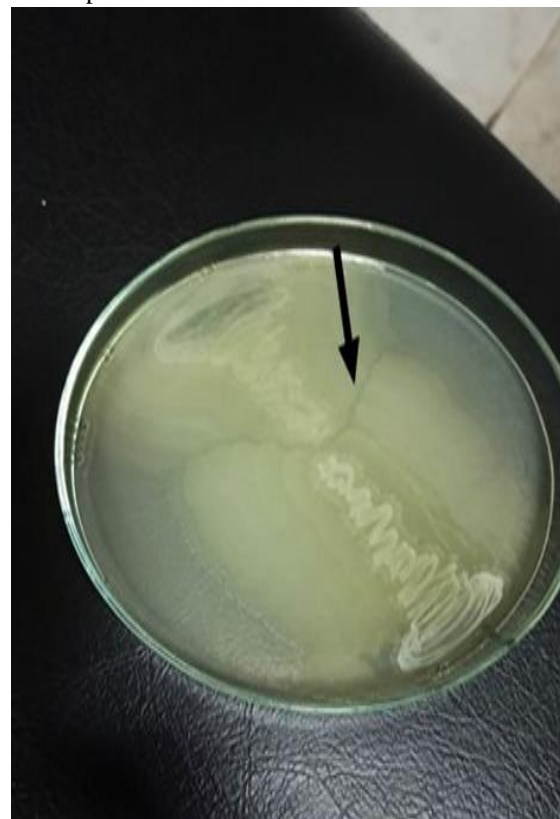
Figure 3. Mutations in amino acid sequence within (A) Beta subunit, (B) UreE, (C) UreF, (D) UreG, and (E) UreR. Our strain is the query strain compared to *P. mirabilis* strain HI4320 (sbjct). The genome sequence was deposited in the NCBI under bioproject number PRJNA719581 and biosample SAMN18613398.

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A|
Query    1         MIPGEIRVNAALGDIELNAGRETKNIQVANHGDRPVQVGSHYHFYEVNEALRFAREETLG    60
Sbjct    4025052   ......................T..............................K....     4025231

Query    61        FRLNIPAGMAVRFEPGQSRTVELVAFAGKREIYGFHGKVMGKLESEKK    108
Sbjct    4025232   ...............................................     4025375


B
Query    1         MKKFTQVIDQQKALELTSTEKPKLTLCLTMDERTKSRLKVALSDGQEAGLFLPRGTVLKE    60
Sbjct    4027106   ......I.....................................................     4027285

Query    61        GDILLSEEGDVVTIEAAKEQVSTVYSDDPLLLARVCYHLGNRHVPLQIEAGWCRYFHDHV    120
Sbjct    4027286   ............................................................     4027465

Query    121       LDDMARGLGATVVVGLEKYQPEPGAY    146
Sbjct    4027466   .........................     4027543


C
Query    1         MLADLRLYQLVSPSLPVGAFTYSQGLEWAIEKGWVCSAETLSDWLSAQMTGTLATLELPI    60
Sbjct    4027622   ............................................................     4027801

Query    61        LRQLQTSLAKGDSDTVKYWCDFMVASRETKELRQEERQRGIAFARLLPQLGIELDDTLQQ    120
Sbjct    4027802   ............................................................     4027981

Query    121       RVKQTQLMAFALAAVHWHIDSEKLCCAYVWGWLENTVMSGVKLVPLGQSAGQKILFALAE    180
Sbjct    4027982   ..........................................M......     4028161

Query    181       QIPAIVELSAHWPQEDIGSFTPAQVIASSRHETQYTRLFRS    221
Sbjct    4028162   .......................................     4028284


D
Query    1         MQEYNQPLRIGVGGPVGSGKTALLEVLCKAMRDSYQIAVVTNDIYTQEDAKILTRAQALD    60
Sbjct    4028297   ............................................................     4028476

Query    61        ADRIIGVETGGCPHTAIREDASMNLAAVEELAMRHKNLDIVFVESGGDNLSATFSPELAD    120
Sbjct    4028477   ............................................................     4028656

Query    121       LTIYVIDVAEGEKIPRKGGPGITHSDLLVINKIDLAPYVGASLEVMEAGYSQNASCETLC    180
Sbjct    4028657   ...........................................DTAK--------     4028812

Query    181       FYMRPVKPYVFTNLKEKVGLETIIDFIIDKGMLRR    215
Sbjct    4028813   --..............................     4028911

E
Query    1         MEYKHILSSNQISLKTFYIENPMIAMVYGAKGEICINGQtitvttnltliiPKYSQVSCD    60
Sbjct    4023401   .......................I....................................     4023222

Query    61        VTNFFPTKPIELHTLVLSETELQSVFSLLKPLIKSGAPITRHLPDYHLSTPEVVKTNFTL    120
Sbjct    4023221   ............................................................     4023042

Query    121       LQQCLPLEHGTPSQETLFMQQSLFFILLAVYHEGVDILNIFRFNYDEPKNQAITHLITQD    180
Sbjct    4023041   ............................................................     4022862

Query    181       PQRKWHLEDVAKTLYTTPSTLRRHLSKEGVSFCQLLLDVRMGIALNYLTFSNYSVFQISH    240
Sbjct    4022861   ............................................................     4022682

Query    241       RCGFGSNAYFCDAFKRKYGMTPSQFRTQSRQANDPNAIATMASQNDESIKKVF    293
Sbjct    4022681   ....................................................     4022523
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Discussion

Deep surgical site infection is the cause of mortality and morbidity on a global scope. Proteacae family may cause deep-seated infection particularly in the ears, mastoid sinus and other areas [10]. The organisms infecting these sites can multiply resulting in more tissue damage and necrosis. Patients in oncology wards are more vulnerable to develop complications as cancer and chemotherapy predispose them to infections [11]. In the present study the lesion occurred after operation to remove malignancy in the right patient`s cheek. As urease production is always considered as one of the important *P. mirabilis* virulence factor causing wound infection in literature and in many previous work, the negative urease result produced by this isolate by conventional tests and automated techniques raised questions about this strain.

In the present study, several mutations were observed in the UreR, ureB, ureE, ureF and ureG; whereas amino acid sequence of the ureD, ureA, ureC showed complete similarity to the reference HI4320 strain as well as the nucleotide sequence of the polyA ureR-ureD intergenic area which appear to be the target of the ureR regulator [12]. Regarding the mutation in the ureR gene presented as amino acid substitution (I26M), it was only found in the strain HI4320 and other NCBI publicly available *Proteus mirabilis* genome sequences did not show this substitution and were similar to our sequence. One (K55E) of the 2 amino acid substitutions present in the ureB showed the same situation. Whereas the other one (T25N) was found in only another genome sequence of a model Proteus Mirabilis strain K670 isolated from Poland and submitted in March 2018 (ID: CP028356). On the other hand, our isolate showed two unique amino acid substitutions that were not found in any of the

publicly available sequences of Proteus Mirabilis: one in the ureE gene (I7V) and the other in the ureG (aa substitutions from 169 to 172 in addition to a gap from 173 to 182). Unlike the aa substitution found the ureF (M173I) which was also detected in 33 of the publicly available Proteus Mirabilis genome sequences. These genome sequences originated from different countries worldwide mainly from China, and to a lesser extent from USA, Italy and also from Canada, Russia and Singapore. They included clinical isolates from both human and animal sources. They were under the following bioproject numbers: PRJNA645205, PRJNA318385, PRJNA577780, PRJNA574564, PRJNA588589, PRJNA594246, PRJNA623736, PRJNA231221, PRJNA540912, PRJNA633229, PRJNA632650, PRJNA685919, PRJNA340922, PRJNA552673, PRJNA700516, PRJNA578857, PRJNA445794, PRJNA292901, PRJNA603518. The data from these sequences was mainly unpublished except for 2: one from Italy and the other from Singapore [13,14] .

In a previous study that evaluates the contribution of urease to virulence of *P. mirabilis* [15], a mutation was introduced into *P. mirabilis* H14320 in *UreR*, it was found that UreR is essential for basal urease activity in the absence of urea, for induction of urease by urea, and for virulence of *P. mirabilis* in the urinary tract. In the current study the isolate is wildly deficient of urease production and caused severe skin infection. The deficient urease production could be explained by the amino acid substitution in the ureR regulator, or by the mutations present in the other ure genes, that are known to inhibit the urease production (ureF, ureG) [16] , or possible inhibition of the urease production by the DNA binding histone-like nucleoid-structuring protein and a known transcriptional repressor H-NS gene especially that it's target [17] (ureR-ureD polyA intergenic area) is intact. These findings raise highlights towards the urease production as a key virulence factor in *Proteus*. and as a very important mechanism needed by this bacteria to establish infection. It also raises questions towards other virulence factors possessed by *P. mirabilis* in infections other than urinary tract infection. The rate of isolating and prevalence of urease negative *P. mirabilis* among total *Proteus* species should also be monitored and studied in the future to assess whether this case is just sporadic or it represents an emergence new trait in this species.

The discovery of mutation of certain amino acids within certain urease genes may raise the insights about the broader features of this population species and the possible clue to urinary tract infection caused by urease positive *P. mirabilis*.

Author contributions

All authors made a substantial contribution to the idea, study design, analysis of retrieved data; took part in drafting and revising the manuscript; gave final approval for the version to be published in the current journal

Disclosure

The authors declare no conflict of interest.

Consent

Written informed consent was obtained from the patient's family for publication of the case report. A copy of the written consent is available for review by the editor -in chief of the journal on request.

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