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PROTEUS MIRABILIS AND URINARY UROLITHIASIS

Priyeshkumar Patel[1], Shashank D. Patil[1], *Rajendra B. Nerli[1] and Shridhar Ghagane[2]
[1]Department of Urology, JN Medical College, KLE Academy of Higher Education and Research (Deemed-to-be-University), JNMC Campus, Nehru Nagar, Belagavi-590010, Karnataka, India
[2]Urinary Biomarkers Research Centre, KLES Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi-590010, Karnataka, India
**Author for Correspondence: rbnerli@gmail.com*

ABSTRACT
Proteus mirabilis is a Gram-negative rod-shaped bacterium. Most noted for its urease activity. It frequently causes catheter-associated urinary tract infections (CAUTI) that are often polymicrobial. These infections may be accompanied by urolithiasis, development of bladder or kidney stones due to alkalinization of urine from urease-catalyzed urea hydrolysis. Adherence of the bacterium to epithelial and catheter surfaces is mediated by 17 different fimbriae, most notably MR/P fimbriae. During infection, histological damage is caused by cytotoxins including hemolysin and a variety of proteases.

***Keywords**: Proteus mirabilis, Urolithiasis, Urease Enzyme*

INTRODUCTION

Proteus mirabilis is a Gram-negative rod-shaped bacterium (Figure 1a), that is well-known for its urease production. It has a distinctive ability to differentiate into elongated swarm cells and produce characteristic bull's-eye pattern of motility on agar plates (Figure 1b). It belongs to the class *Gammaproteo* bacteria and has long been recognized as a member of the order *Enterobacteriales*, family Enterobacteriaceae (Armbruster *et al.,* 2018). A recent publication however has suggested that Proteus be placed within a new *Morganellaceae* family (Adeolu, 2016).

P. mirabilis is found in a wide variety of environments, that includes soil, water sources, and sewage, but it is predominantly a commensal of the gastrointestinal tracts of humans and animals (Armbruster and Mobley, 2012). The bacteria are responsible for a variety of human infections, including those of wounds, the eye, the gastrointestinal tract, and the urinary tract (Armbruster *et al.*, 2018). *P. mirabilis* has been most noted for infections associated with the catheterized urinary tract, known as catheter-associated urinary tract infections (CAUTI) (Warren *et al.,* 1982; Mobley and Warren, 1987; Breitenbucher, 1984; Jacobsen, 2008; Nicolle, 2005; and Ambruster *et al.,* 2017). CAUTI are commonly seen in long-term catheterized patients, such as those who reside in nursing homes and chronic care facilities (Hung *et al.,* 2005). Urinary tract infections involving *P. mirabilis* are typically complicated by the formation of bladder and kidney stones (urolithiasis) and permanent renal damage (Griffith *et al.,* 1976; Li *et al.,* 2002; and Foxman and Brown, 2003) and are often known to progress to bacteremia and sepsis. Bacteremia and sepsis secondary to *P. mirabilis* carry a high mortality rate (Kim *et al.,* 2003; Watanakunakorn and Perni, 1994; Daniels *et al.,* 2014; and Hooton *et al.,* 2009).

P. mirabilis is known to form a biofilm, fouling the surface of a newly inserted urinary catheter. Surface organelles such as fimbriae and other adhesins play a significant role in this process of biofilm formation. The enzyme urease also contributes dramatically to this process of biofilm formation. Urea, which is excreted through urine, is present in high concentrations in urine (~400 mM) and is the substrate of the enzyme urease, to hydrolyze into CO_2 and NH_3. The liberated ammonia raises the pH of the urine (making it alkaline) and initiates the precipitation of otherwise soluble polyvalent anions and cations present in urine. This result in the formation of struvite ($MgNH_3PO_4$) or apatite ($CaPO_4$) stones and the process is called urolithiasis. These crystals can form on and within the lumen of catheters, blocking urine flow and necessitating catheter removal and replacement. Stones may also form in the renal tubules or renal pelvis, causing inflammation and often requiring surgical removal. This bacterium is capable of

invading bladder epithelial cells and produces a variety of cytotoxins that damage the epithelium, leading to significant histopathology.

Infection Stones

Urinary stones that originate secondary to infection are composed primarily of magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$) but may also contain calcium phosphate in the form of carbonate apatite ($Ca_{10}[PO_4]_6 \cdot CO_3$) (Pearle *et al.,* 2016). Brown was the first to theorize that bacteria split urea, thereby setting up the condition for stone formation. He also isolated *Proteus vulgaris* from a stone (Brown, 1901). It was Hager and Magath (1925) who postulated that a bacterial enzyme hydrolyzed urea into carbon dioxide and ammonia and it was Sumner (1926) who isolated urease from *Canavalia ensiformis.*[21] It is now well established that struvite stones (magnesium ammonium phosphate) occur only in association with urinary infection by urea-splitting bacteria.

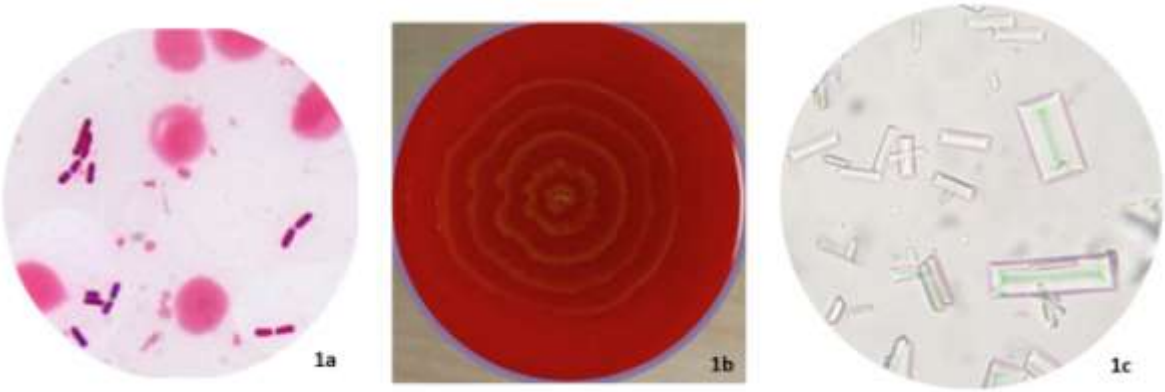

Figure 1a: H & E stain shows gram-negative rod-shaped bacteria – *P.Mirabilis;* 1b: Bull's-eye pattern of motility due to swarming effect on agar plates; 1c: Infection stones

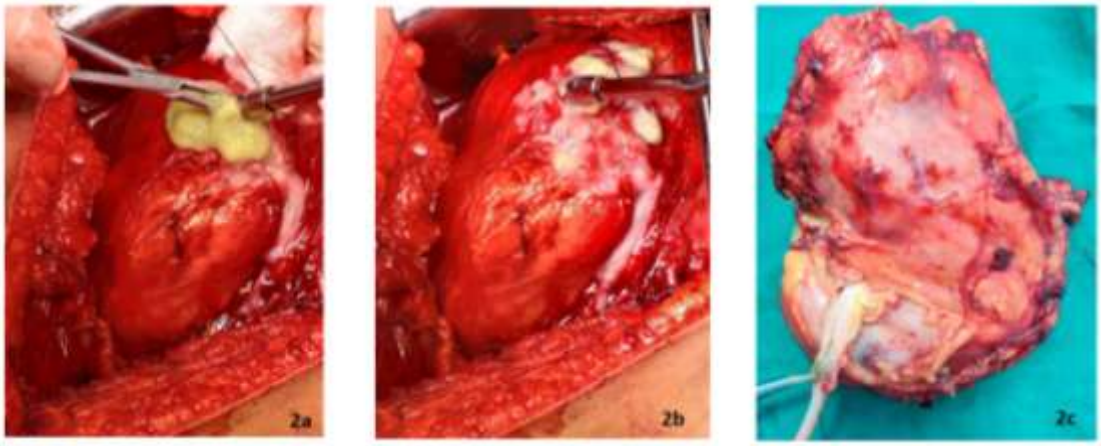

Figure 2a & b: Yellow pus oozing out from the kidney
2c: Cut-open specimen of kidney showing calculus in the upper ureter

The chemical process of urea lysis provides an alkaline urinary environment and sufficient concentrations of carbonate and ammonia to induce the formation of infection stones. As urease is not present in sterile human urine, infection with urease-producing bacteria is a prerequisite for the formation of infection stones. A cascade of chemical reactions generates the conditions conducive to the formation of infection

stones. Urinary urea, a constituent of normal urine, is first hydrolysed to ammonia and carbon dioxide in the presence of bacterial urease:

$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$

The alkaline urine that results from this reaction (pH 7.2 to 8.0) favours the formation of ammonium:

$NH_3 + H_2O \rightarrow NH_4 + OH^-$ (pK=9.0)

In the presence of urease, ammonia continues to be produced, further increasing urinary pH. The alkaline environment also promotes the hydration of carbon dioxide to carbonic acid, which then dissociates into HCO_3^- and H^+. This chemical cascade, along with physiologic concentrations of magnesium, provides the constituents necessary for precipitation of struvite. Besides, the concentrations of calcium, phosphate, and carbonate allow precipitation of carbonate apatite and hydroxy- apatite, thereby comprising the components of infection stones (Figure 1c).

Bacteriology: The most common urease-producing pathogens are *Proteus*, *Klebsiella*, *Pseudomonas*, and *Staphylococcus* species, with *Proteus mirabilis* the most common organism associated with infection stones. Although *Escherichia coli* is a common cause of urinary tract infections, only rare species of *E. coli* produce urease. [22] Bacteria may be involved in stone formation by damaging the mucosal layer of the urinary tract, resulting in both increased bacterial colonization and crystal adherence (Pearle, 2016).

Urolithiasis

Urolithiasis is the process of stone formation, and the urease of *P. mirabilis* is unambiguously associated with the development of infection-induced stone formation (Mobley andWarren, 1987; and Griffith and Musher, 1976). Indeed, Proteus species have been isolated in 70% of cases of bacteria-induced stone formation (Prywer and Olszynski, 2017). It has been noted that GFP-expressing *P. mirabilis* could be observed within the matrix of urinary stones in mice experimentally infected with *P. mirabilis* (Li et al, 2002). Genome sequences of *P. mirabilis* have also been detected in urinary calculi by PCR (Huang *et al.*, 1999). Using confocal microscopy, Schaffer and colleagues (2016) found, that *P. mirabilis* formed extracellular clusters in the bladder lumen that served as the basis for focused mineral deposition, consistent with nascent stone formation.

Cytotoxicity, and histopathology

P. mirabilis can invade and lyse host cells that have been explored for decades and have been demonstrated to contribute to infection progression and the severity of disease in animal models. The level of host cell invasion and cytotoxicity achieved by *P. mirabilis* in vitro varies dramatically by the bacterial strain. The pathological changes in the bladder and kidneys of infected animals vary to some extent based on the bacterial strain, inoculating dose, and infection model. Several virulence factors have been implicated in contributing to cell invasion and cytotoxicity *in vitro*, as well as histopathological changes *in vivo*. For instance, flagella contribute to invasion in part by allowing the bacterial cells to come into proximity to the host cells, and mutants lacking flagella are unable to invade cells unless centrifuged directly onto the host cell monolayer (Mobley *et al.*, 1996, Allison *et al.*, 1992).

Bladder invasion and histopathology: Internalization of *P. mirabilis* by bladder epithelial cells has been directly demonstrated in vitro using a hemolysin (hpmA) mutant to avoid confounding from the effects of the cytolytic toxin. In addition to internalization, *P. mirabilis* is capable of lysing bladder epithelial cells using a combination of the Proteus toxic agglutinin (Pta) and hemolysin (Alamuri *et al.*, 2009). Intracellular bacteria are uncommonly observed at later times post-inoculation, and *P. mirabilis* appears to instead form large, extracellular clusters within the bladder lumen and adjacent to the urothelium after this initial invasion phase rather than establishing the intracellular communities that are characteristic of uropathogenic *E. coli* (Schaffer and Norsworthy, 2016). Formation of these clusters requires urease activity and the mannose-resistant Proteus-like (MR/P) fimbriae and protects from infiltrating neutrophils (Schaffer and Norsworthy, 2016).

Kidney invasion and histopathology: Infection with *P. mirabilis* tends to result in unique kidney pathology (Figure 2 a, b, c). For instance, *P. mirabilis* is the only species that causes a high incidence of

kidney stone formation in a rat model of pyelonephritis (Braude and Siemienski, 1960), and it causes more kidney stones and greater kidney damage than other urease-positive organisms such as *P. stuartii* in murine models of ascending UTI and CAUTI (Li *et al.*, 2002; and Armbruster *et al.*, 2017. Specifically, kidney colonization by *P. mirabilis* in the ascending model of UTI is most often associated with moderate pyelonephritis, including neutrophilic interstitial nephritis within the peri-pelvic renal cortex and occasional damage to the surrounding renal parenchyma (Alamuri *et al.*, 2009; Armbruster *et al.*, 2017). In addition to directly damaging kidney tissue and inducing inflammation, *P. mirabilis* proliferates within the tubular epithelium of the kidneys in both mice and rats, resulting in necrosis and nephrosis (Braude and Siemienski, 1960).

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