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Isolation an diagnosis of Proteus Spp. from Urine of Diabetic Patient in Salah Aldeen Provence in Iraq

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Abstract:

This study concentrated on the isolation and identification of Proteus mirabilis isolates from diabetic mellitus patients with urinary tract infections. 100 clinical specimens of mid-stream urine were collected from male and female Iraqi patients who visited Salah al-Din general hospital. On the other hand, all urine specimens from patients were cultured on blood agar and MacConkey agar. The results of the samples were distributed as 71% and 29% of females and males, respectively. The results of the current study showed that the highest rate of UTIs was for the age group between 30 and 40 years. According to the cultural, morphological, biochemical, only 23 isolates (23%) were identified as Proteus mirabilis and and the remaining percent (77%) represent other species in the current work.

Keywords: UTI, Proteus mirabilis, Diabetic type 2

Urinary tract infections (UTIs) are acute or chronic illnesses of the urinary system caused by microorganisms such as bacteria and fungi that multiply abnormally (Amali et al. 2009, Prakash and Saxena 2013).

UTIs can be occur in the community or acquired in a hospital setting. (Moyo et al. 2010).

Several risk factors have been associated with an increased incidence of UTIs in women, including family history of the disease, physiological anatomy, sexual intercourse, obesity and diabetes (Flores-Mireles et al. 2015).

A bacterial urinary tract infections (UTIs) is one of the most widespread human infections worldwide. (Al-Gasha *et al.* 2020).

UTIs are caused by both Gram-negative and Gram-positive bacteria. The most widely known causative agent of urinary tract infection is Escherichia coli. Other reported organisms include members of the Enterobacteriaceae family (Mahato *et al.* 2018). Increased of urinary frequency, dysuria, cloudy of urine and in some cases hematuria are common symptoms of UTIs (Dason et al. 2011). Observed *P. mirabilis*, *P. penneri*, and *P. vulgaris* were commonly isolated as clinical isolates with UTIs in patients with indwelling catheters (Jacobsen *et al.* 2008).

Inflammation of the lining of the bladder mucosa, commonly known as cystitis that causes several symptoms such as painful urination, malodourous urine, dysuria or difficult urination, stranguria, incontinence, haematuria (Lane and Takhar 2011). In addition, stones in the bladder and kidneys, acute pyelonephritis, cystitis, bacteriuria, and stone accumulation in the catheter that causes it to become obstructed were developed in humans infected with P. mirabilis (Burall et al. 2004).

P. mirabilis is a common cause of upper UTIs, which can include urothelial invasion of host cells (Jen liaw et al. 2000). *P. mirabilis* is Gram negative bacterium, rod shape, characterized by a swarming phenomenon, motile on agar plates, and urease production. *P. mirabilis* is a member of

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Enterobacteriaceae family, and belongs to the class Gammaproteobacteria (Adeolu et al. 2016).
P. mirabilis is a zoonotic pathogen causing a variety of human disorders, involving, diarrhea, UTIs, and keratitis. It is the second most prevalent zoonotic bacteria in human medicine after enteropathogenic Escherichia coli (Fonseca et al. 2018).
Pathogenic bacteria frequently produce pore-forming toxins that are secreted hemolysins(Cabezas et al. 2017). In **P. mirabilis**, the urease enzyme plays a vital role in the pathophysiology as it catalyzes the production of stones in the kidney and bladder, as well as inhibiting urinary tract function (Flannery et al. 2009).

UTI disease is higher in patients with type 2 diabetes than other cases, with the UT is the most common region of infection (Boyko et al. 2005). *P. mirabilis* was among the most dangerous diabetic foot ulcer infectious agents, properly accounted for about 18% of all isolates (Sekhar *et al.* 2014).

Aim of Study

Assess the frequency of P. mirabilis as one of the important pathogens that cause UTIs and its relationship with diabetic patients.

Materials and Methods

Study Groups

A total of 100 clinical samples of males and females presented type 2 diabetes with UTI visiting hospitals in Tikrit governorate Salah al-Din general hospital during the period of November 2021 to February 2022 and distributed according to gender into 29 male and 71 female.

Specimen collection

Mid-stream urine was obtained from People with diabetes. Mid-stream urine samples were collected by directing the patients to clean genitalia before collecting urine and to discard the first and last collect urine. As a result, the mid-stream urine is collected in a sterile wide hole in the top of the container to isolation bacteria.

Culture media was prepared for use

Samples were

transferred to the lab for isolation and identification of Proteus .by using sterile equipment and media. All samples were streaked on Blood agar, Mac Conkey agar and XLD agar plates. The plates were incubated aerobically at 37^{o} C for 24 hours. The isolates were identified depending on the microscopical feature by using Gram stain to detect their response to stain, shape and arrangement [11, 12]. In addition, the morphological features on culture media such as Swarming on blood agar, Non lactose fermented growth on MacConkey agar and color less growth on Xylose lysine deoxychoclate agar (XLD) agar were examined, also several of biochemical assays were used to identify the Proteus isolates, such as catalase, oxidase tests, Indole, Methyl Red/ Voges-Proskauer (MR-VP) test, citrate utilization tests, Urea test, Motility test, gelatin liquefaction test and triple sugar iron agar test [2].

Results and Discussions

A total of 100 clinical specimens were collected from patients, male and female, who suffered from diabetic mellitus 2. The clinical specimens were divided into 71 females (71%) and 29 males (29%), as shown in Figure 4.1.

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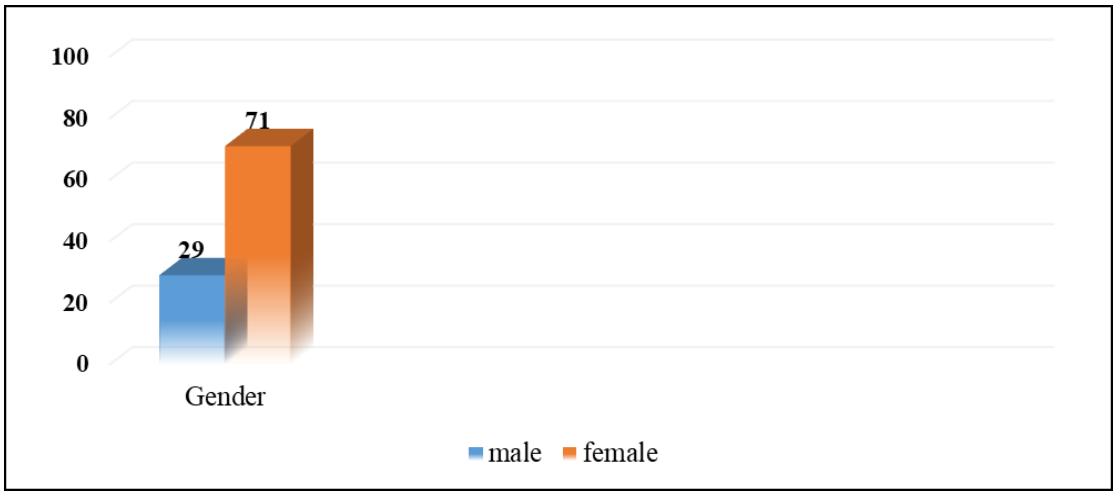

Figure 4.1 Clinical specimen's distribution according to gender

Similarly, close results were reported claimed that the females showed a much higher prevalence of UTI with diabetic type 2 than the males (88.5% and 11.5%, respectively) (Sewify et al. 2016).

Female diabetic patients had a UTI prevalence of 67.98%, which was significantly greater than male diabetic patients' 32.02%. This is in line with existing findings, which show a high prevalence of UTI in females (Ramana and Chaudhury 2012).

In Table 4.1 showed the distribution of samples in the current study according to age groups in the present work. The results in male group were 2 (6.89%), 5 (17.24%), 9 (31.03%), 9 (31.03%) and 4 (13.79%) for the age group (10-20, 20-30, 40-50, and over 50 year respectively). On the other hand, the results in female group were 5 (7.04%), 18 (25.35%), 23 (32.39%), 16 (22.53%) and 9 (12.67%) for the age group (10-20, 20-30, 40-50, and over 50 year respectively).

Table 4.1 Distribution of clinical samples according to age groups

Age group/years	10 – 20	20 – 30	30 – 40	40 – 50	Over 50 year	Total
Male	2 (6.89%)	5 (17.24%)	9 (31.03%)	9 (31.03%)	4 (13.79%)	29
Female	5 (7.04%)	18 (25.35%)	23 (32.39%)	16 (22.53%)	9 (12.67%)	71

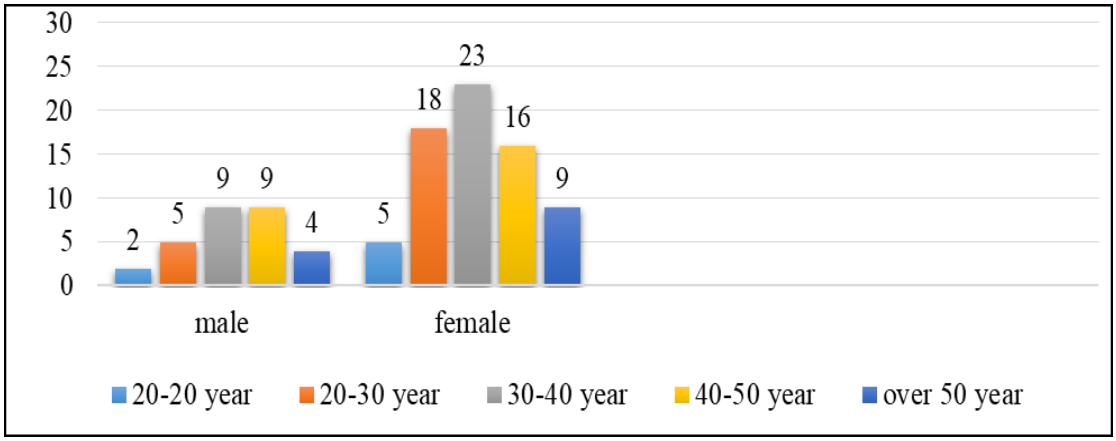

Figure 4.2 Distribution of samples in the current study according to age groups

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In Figure 4.2 depicts the age group distribution of the samples in the current study. According to the current study, the occurrence of UTI in diabetics was more common in patients between the ages of 31 and 40 years, followed by the age group 41-50 years. Explain the similarity in the prevalence of UTIs between patients with diabetes type 2 in the age group of 30 to 40 years as the highest prevalence rate. In addition, was recorded the same fact among the prevalence rate of UTI with diabetic patients in these age groups (Adeyeba and Adesiji 2007). Adults with diabetes who engage in sexual activity may be older, making them more susceptible to UTIs.

4.2 Bacterial Isolation and Identification

In the current study, the bacterial isolates were identified using selective and differential media, microscopic examination, biochemical tests, and VITEK automated system. The initial identification, which included morphological features on culture media were done at the hospital *in situ*. All isolates in the current study were cultured on Blood agar, MacConky agar, and other selective media. A series of biochemical tests were performed on the isolates listed in Table 4.2.

The preliminary results of the bacterial culture and biochemical tests showed (23%) of the clinical isolates belonging to *P. mirabilis* and the remaining percent (77%) were distributed among other types (Figure 3.3).

In Figure 4.3 shows the percentage of *P. mirabilis* that collected from clinical samples in the present work. Only (23%) identified as *P. mirabilis*, and the remaining percent (77%) represent other species in the current work.

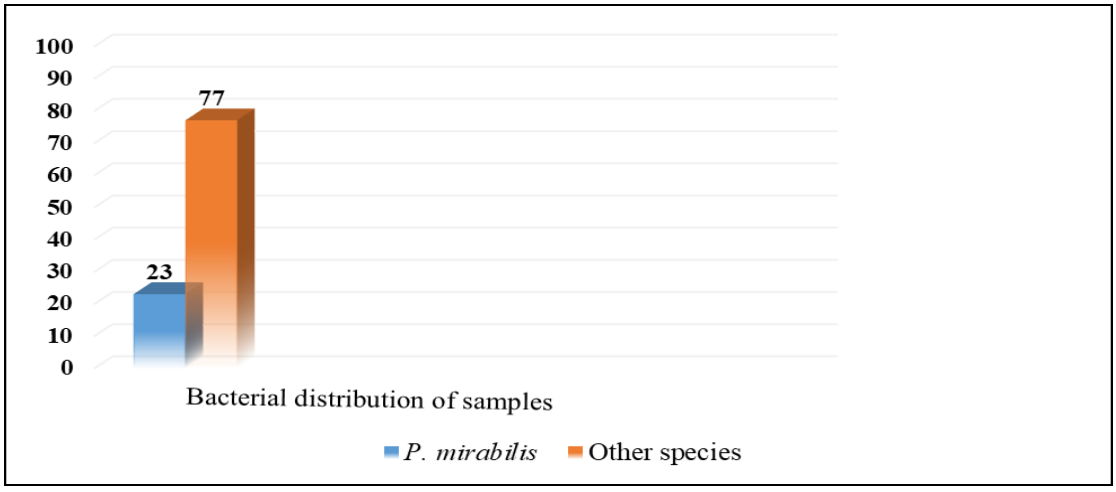

Figure 4.3 The percentage of *P. mirabilis* isolation from sample in the current study

Have claimed that the percentage of *P. mirabilis* were (8.3%) (Otajevwo 2013). Moreover, Illustrated that *P. mirabilis* causes 1 to 10% of all UTIs cases (Karlowsky *et al*. 2011). Other studies have shown that *P. mirabilis* was noted in 5% to 20% of UTIs (Adams-Sapper *et al*. 2012, Lubart *et al*. 2011).

These studies were agreement with results of the current study. The low percentage of *P. mirabilis* that cause UTIs may be due to the fact that these bacteria were observed in anatomical abnormality of urinary system, catheter-associated UTI and injuries of spinal cord (Jacobsen *et al*. 2008, Hung *et al*. 2007).

On the other hand, *P. mirabilis* is most common in elderly patients who are on long-term catheterization and cause UTI related long catheter (Lubart *et al*. 2011). Many uropathogens,

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including *P. mirabilis* were found to have encoded urease enzymes. Because the urease enzyme can catalyze urea hydrolysis to ammonia and carbon dioxide this process results in increased urine pH, the formation of calcium crystals (apatite), and the formation of magnesium ammonium phosphate ammoprecipitates (struvite) in urine and on catheters (Li *et al*. 2002). Importantly, ammonia accumulation is toxic to uroepithelial cells, causing direct tissue damage.

The urease enzyme of *P. mirabilis* is a vital and necessary enzyme for the invading and colonization of the bladder and kidneys, and it promotes stone formation (Armbruster and Mobley 2012). The urease enzyme of this bacteria is stimulated by urea and is constitutively expressed in urine during growth. Urea stimulates the urease enzyme, which is constitutively expressed in urine during growth.

Table 4.2 Results of growth of *P. mirabilis* on culture media and biochemical tests (no. = 23 isolates)

Isolate Cultures and Biochemical test	*P. mirabilis*
Blood agar	Swarming phenomena
MacConkey agar	Pale isolates (lactose non-ferment)
Eosin Methylene blue	Growth with colorless (not metallic sheen)
Indol	-
Methyl red	+
Vogas Proskauer	-
Citrate utilization	-
TSI	K/A^{-+}
Urease	+
Oxidase	-
Catalase	+
Gelatin Liquefaction	+
Motility	+
Lactose fermenter	-
Maltose fermenter	-

K/A-+ mean alkaline/acid or ferments glucose with negative CO2 production and H2S gas production , (-) a negative result, (+) a positive result

In Table 4.2 showed several biochemical tests which done *Proteus* isolates. All the 23 isolates of *P. mirabilis* showed positive results to the biochemical tests, methyl red, urease, catalase, gelatin liquefaction and motility but, indole, Vogas Proskauer, oxidase, citrate utilization test, lactose and maltose fermenter were negative.

This bacteria were first identified as related to *Proteus spp.* by swarming phenomena on blood agar, showed pale colonies on MacConkey agar, and when examined under a microscope, the bacteria appeared as straight rods and gram negative when stained with gram stain. The samples were identified as *P. mirabilis* using the automated system VITEK 2 to confirm and complete identification of the biochemical results and antibiotics sensitivity tests.

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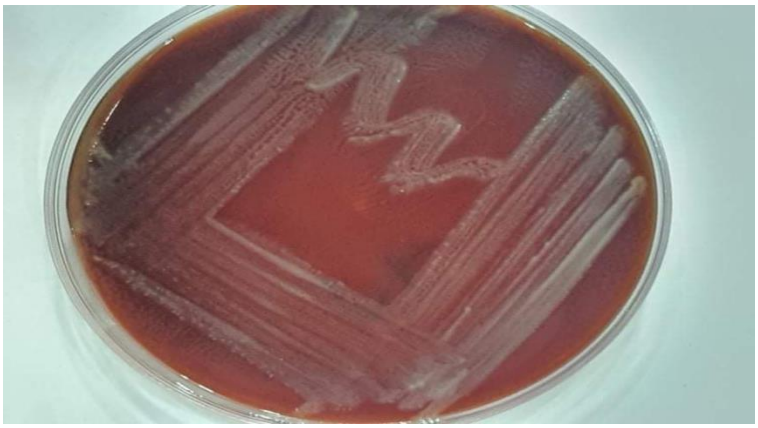

Figure 4.4 Swarming phenomenon of *P. mirabilis* isolation from sample in the current study on blood agar

Depict the swarming phenomenon generated by *P. mirabilis* on blood agar in Figure 4.4, which was isolated in the current study (Williams 1973). The two species' distinctive zonal growth *P. mirabilis* and *Proteus vulgaris*, also known as swarming. The creation of concentric zones of *P. mirabilis* growth capable of covering the entire surface of solid culture medium is described as swarming (Smith 1972).
P. mirabilis was not the predominant isolate (23% only) in UTIs.

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