Molecular and Pathological Study of Proteus SPP. Isolated from UTI Infected Diabetic patient in Salah Aldeen Province in Iraq

Mayyadah Ali Ahmed AL-Gburi

Dr. Fatih Karayürek

Dr. Bashar Sadeq Noomi AL-Hadeethi

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. 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 vitek results, only 23 isolates (23%) were identified as Proteus mirabilis. DNA was extracted from bacterial isolates to detect 16s RNA, hmpA, and ureC genes. PCR technique was used for detection these genes via specific primers. The results of the current work recorded (100%) for 16sRNA, hmpA, and ureC genes. On the other hand, the highest rate of antibiotics sensitivity for all isolates were amikacin and ciprofloxacin (73.91%). In addition, the highest rate of antibiotics resistance were Ampicillin and Norfloxacin (95.65%). The result of the sequencing test for 16s RNA showed the genetic affinity and divergence between Proteus mirabilis isolates.

Keywords: *UTI, Proteus mirabilis, Diabetic type 2, ureC gene, hpmA gene.*

INTRODUCTION

Bacterial urinary tract infections (UTIs) are one of the most widespread human infections worldwide. Appropriate knowledge in identifying associated factors with UTIs can allow for simple and timely disease control interventions (Al-Gasha et al. 2020).

UTIs are the second most common bacterial infection in the community, after respiratory infections in humans and they affect both men and women of all ages. It is unknown how primary community-acquired disease occurs or how it is transmitted, although most cases are thought to be sporadic. UTIs can occur in the hospital or in the community. Most UTIs are thought to develop and be acquired in the community (Najar, et al. 2009).

UTI can produce a short-term disease with symptoms such as dysuria, lower back pain, and fever, as well as long-term kidney scarring (Hoberman et al. 2003, Camacho et al. 2004).

Increased of urinary frequency, dysuria, cloudy of urine and in some cases hematuria are common symptoms of UTIs (Dason et al. 2011).

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).

Type-2 diabetes has been associated not only with community acquired UTIs but with catheter-associated UTIs, health care-UTI and recurrent urinary tract infection (rUTI) after

kidney transplantation (Datta et al. 2014, Lim et al. 2013).

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 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).

The significant pathogenicity of this bacterium, as well as its antibiotic resistance, is due to the ureC virulent gene (Tolulope et al. 2021).

Aim of study

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

2. Detection of some virulence genes that contribute to bacterial pathogenicity

Materials and Methods

Study Groups

Ethical statement

All of the participants agreed to give the investigator urine samples. All participants gave their informed consent in accordance with the Declaration of Helsinki.

Patient group

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. The facilitate the task of working in the hospital according to approval No. 1104 on 14-09-2021 in Appendix 1.

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.(Pernille et al. 2019)

Isolation and identification of bacteria

De Cueto

Antibiotic Sensitivity Test (vitek)

Twelve antibiotic disks (Cefepime, ampicillin, trimethoprim /sulphamethazol, gentamicin, ciprofloxacin, ceftazidime, pipracillin, cefotaxime, nalidixic acid azteronam, imipenem and amikacin, Fosfomycin, Meropenem, Nitrofurantion, Norfloxacin, Ceftazidime) were used to detect the sensitivity of 20 isolates of P. mirabilis according to method described earlier [De Cueto et al. 2004] .

Genotyping assays

Chromosomal DNA extraction

The Chelex®100 kit/USA Kit was used to extract DNA from an activated pure culture of P.mirabilis bacteria. Loopful was used to remove a tiny amount of pure bacterial colonies from each sample and transfer them to a 0.6 mL tube containing 200 of Chelex®100 and 100 of TE, which was then immersed in a water bath at 95 °C. Samples were removed after 10 min and centrifuged for 10 min at 13,000 rpm. The samples should then be carefully removed from the top aqueous layer containing DNA and placed in 0.2 mL containers. Then, at -4°C, put it in the refrigerator.

DNA amplification (PCR)

To create a large number of copies of a gene, a PCR (Polymerase Chain Reaction) was utilized. As a result, the amount of DNA available for other testing methods is insufficient. The steps was cleared in table 1.

The PCR used to amplification and detection of hpmA and ureC genes and 16S rRNA gene amplicon and identification. Agarose gel prepared for DNA electrophoresis to obtain gene bands .(Al-Hamdani, et al. 2020).

Table 1: PCR conditions cycles

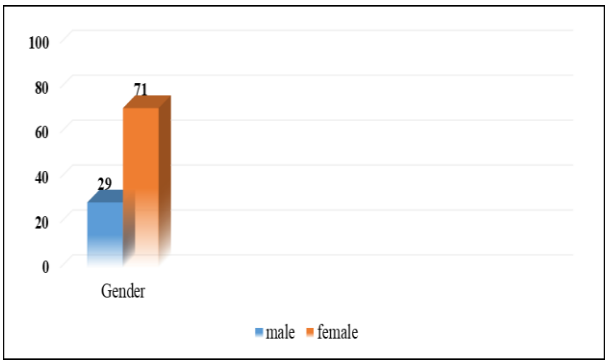
PCR steps	Time	Temperature	Cycle
Pre denaturation	5 min	95°C	1
Denaturation	30 sec	95°C	35
Annealing	1 min	58	
Extension	30 sec	72°C	
Final extension	5 min	72°C	1
Hold	∞	4°C	

Results and Discussion

Study Groups Distributions

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 1.

Figure 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). Furthermore, noted that the prevalence of UTIs in diabetic patients was higher in women (76.2%) than in men (23.8%). When diabetic women are on oral medication or receive insulin injections, their risk of UTI increases by up to fourfold (Khan et al. 2022, Boyko et al. 2002).

Table 2: 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

In Table 2: 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 2.

Bacterial Isolation and Identification

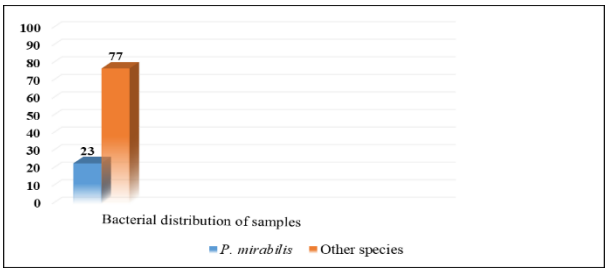
In the current study, the bacterial isolates were identified using selective and differential media, microscopic examination, biochemical tests, and VITEK automated system. The initial identification, which included morphological features on culture media were done at the hospital in situ. All isolates 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 3.

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 2).

In Figure 2 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 2 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).

Table 3: 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 3 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.

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

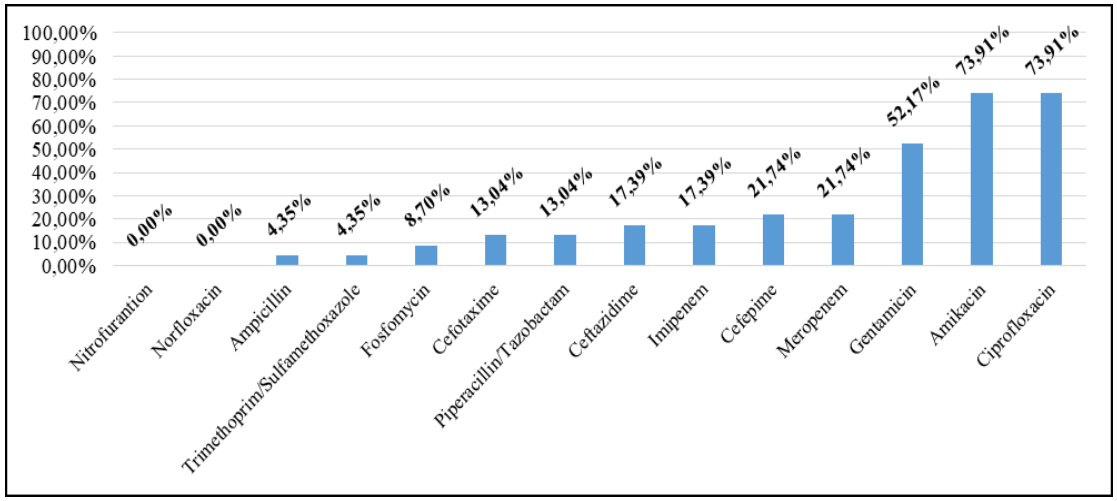
Bacterial Sensitivity to Antibiotics

Table 4: Antibiotic list for P. mirabilis in vitek automated system

No.	Antibiotics	Resistance	susceptible	Intermediate
1.	Amikacin	0 %	73.91%	26.09%
2.	Ampicillin	95.65%	4.35%	0 %
3.	Cefepime	78.26%	21.74%	0 %
4.	Cefotaxime	86.96%	13.04%	0 %
5.	Ceftazidime	82.61%	17.39%	0 %
6.	Ciprofloxacin	17.39%	73.91%	8.70%
7.	Fosfomycin	86.96%	8.70%	4.35%
8.	Gentamicin	47.83%	52.17%	0 %
9.	Imipenem	78.26%	17.39%	4.35%
10.	Meropenem	73.91%	21.74%	4.35%
11.	Nitrofurantion	91.30%	0 %	8.70%
12.	Norfloxacin	95.65%	0 %	4.35%
13.	Piperacillin/Tazobactam	82.61%	13.04%	4.35%
14.	Trimethoprim/Sulfamethoxazole	91.30%	4.35%	4.35%

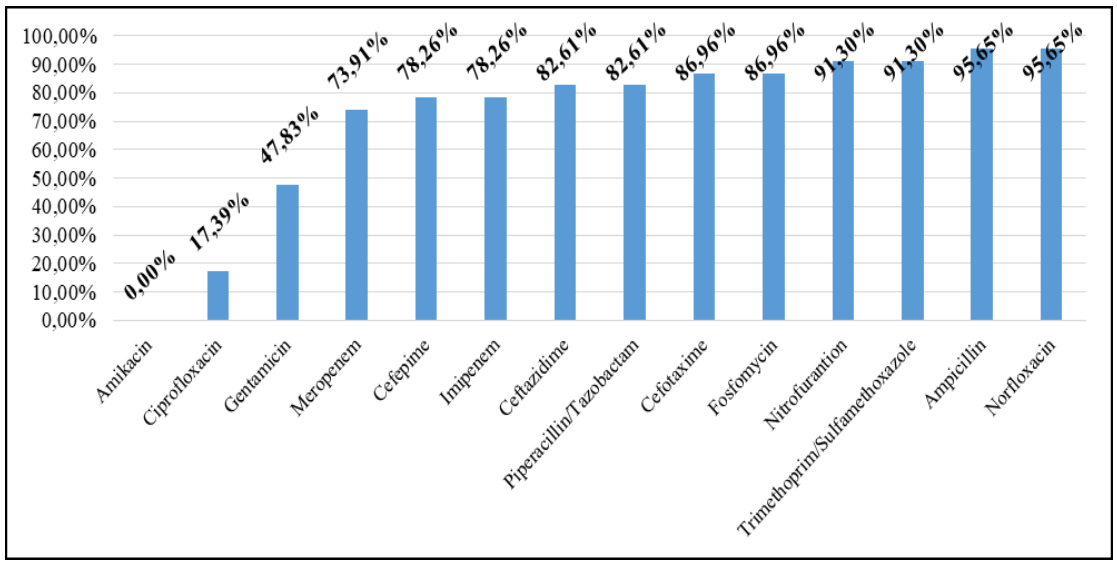
In Table 4 shows the results of the antibiotics against P. mirabilis, which was isolated from the patients under study, where the effects of these antibiotics were measured automatically via the Vitek automated system, and the results were as mentioned in Table 4.

Figure 3: The bacterial susceptible against antibiotics under study



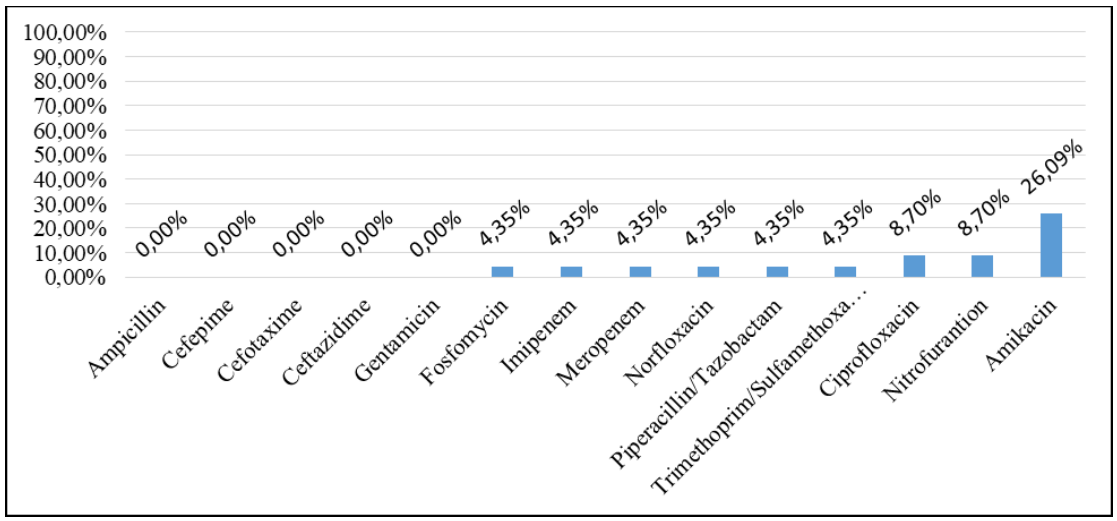
In Figure 3 shows the ratio of susceptible strains for antibiotics under study, which were only 17 (73.91%) for Amikacin and Ciprofloxacin, 12 (52.17%) for Gentamicin, 5 (21.73%) for Cefepime and Meropenem, 4 (17.39%) for Ceftazidime and Imipenem, 3 (13.04%) for Cefotaxime and Piperacillin/Tazobactam, and 2 (8.69%), 1(4.34%), 1(4.34%) for Fosfomycin Trimethoprim/Sulfamethoxazole and Ampicillin, respectively.

Figure 4: The bacterial resistance against antibiotics under study



On the other hand, the antibiotic resistance ratios in Figure 4 were as follows: Ampicillin and Norfloxacin 22 (95.65%), Nitrofurantion and Trimethoprim/Sulfamethoxazole 21 (91.30%), Cefotaxime and Fosfomycin 20 (86.95%), Ceftazidime and Piperacillin/Tazobactam 19 (82.60%), Cefepime and Imipenem 18 (78.26%), Meropenem 17 (73.91%), Gentamicin 11 (47.82%) and Ciprofloxacin 4 (17.39%).

Figure 5: The intermediate test against antibiotics under study



In Figure 5 illustrate the intermediate ratio of antibiotics and the results were 6 (26.08%) for Amikacin, 2 (8.69%) for Ciprofloxacin and Nitrofurantion, and 1 (4.34%) for Fosfomycin, Imipenem, Meropenem, Norfloxacin, Piperacillin/Tazobactam, and Trimethoprim/Sulfamethoxazole, respectively.

Our results are in contrast to those who found P. mirabilis to be susceptible to ciprofloxacin and ceftazidime in 36% and 52% of cases, respectively. Furthermore, the results of the current study was disagree to some extent who have explain that the P. mirabilis was intermediate resistance for cefepime, cefotaxime, ceftazidime,

ciprofloxacin (53.2%, 51.1%, 44.7%, 38.3% respectively) as well as, amikacin , imipenem and meropenem (25.5%, 8.5%, and 6.4% respectively) (Kadhim et al. 2017). The same study showed agreement in the result with gentamicin (42.6%).

Moreover, results were in agreement with current study results, which noted that the resistant strains of P. mirabilis to ciprofloxacin reached 16.2% (Hernández et al. 2000). Only 13.6% of P. mirabilis strains were resistant to that antibiotic (Ko et al. 2008).

4.4 Molecular Detection Genes

Table 5: Investigation genes for P. mirabilis in the current study

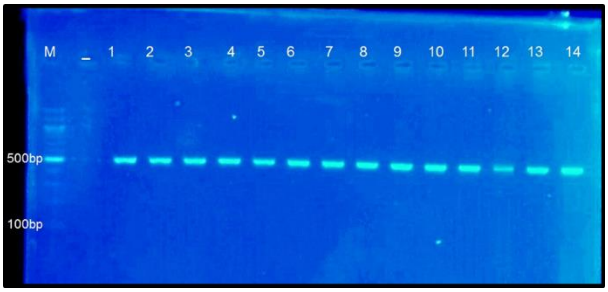
No.	No. of bacterial isolates	Detection genes	Percentage
.	23	*16s RNA*	100%
.	23	*hpmA*	100%
.	23	*ureC*	100%

The results in Table 5 illustrate the genes detected in the bacterial isolates under study. A PCR method was used for the detection of 16s RNA as well as genes that encode for the virulence factors, including hpmA and ureC which encode for hemolycin and urease production, respectively, in P. mirablis.

Detection of 16sRNA gene

In Figure 6 shows the specific PCR amplification of fragments of the 23 isolates' 16S rRNA genes, which revealed bands of about 500 bp.

Figure 6: The results of P. mirabilis 16sRNA gene amplification (500 bp in size)



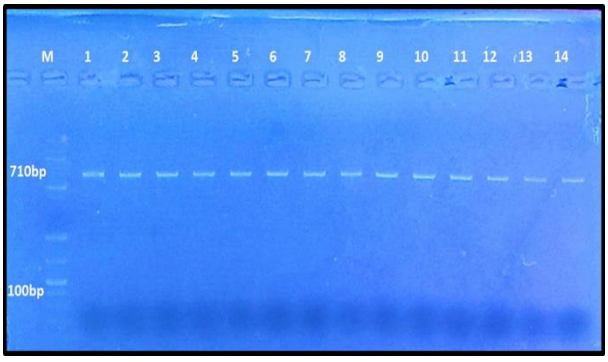
For 80 minutes at 120 V, the PCR products were electrophoresed in 1.5 percent agarose gels containing 0.5 mg/mL red safe stain. The bands were highlighted with UV light. M lane represents a 100 bp molecular weight marker (DNA marker). Lanes 1-14 are dedicated to the 16sRNA gene. These results were similar to results, who obtained a 100% identical sequence of the 16S rRNA gene for P. mirabilis.

Detection of hpmA gene

In Figure 7 shows the specific band of hpmA gene at (710 bp). These results represent the specific gene in P. mirabilis.

The results showed that the hpmA gene was present in all of the bacterial isolates studied (100%). Demonstrated that the hpmA hemolysin gene is more common in Proteus isolates (98%)with correlated hemolytic activity, and the hpmA gene was found in each isolate tested using the Southern blot method (Swihart and Welch 1990). Recorded the same results in a survey of 211 isolates, and all of them showed that they encode a hpmA gene.

Figure 7 The results of P. mirabilis hpmA gene amplification (710 bp in size).



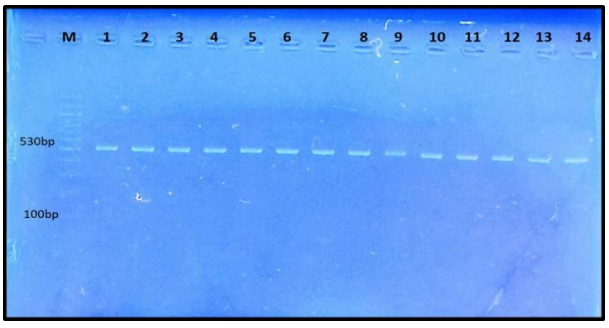
The PCR products were electrophoresed in 1.5 percent agarose gels containing 0.5 mg/mL red safe stain for 80 minutes at 120 V. UV light was used to highlight the bands. M lane represents a molecular weight marker (DNA marker) of 100 bp. Lanes 1-14 are for the hpmA gene.

Similarly, the results of are similar with the results of the current work, who noted have shown that hpmA gene was present in all isolates at rate (100%) (Lazm et al. 2018). These findings are in accordance with findings, which claimed that the rate of this gene in P. mirablis isolates is 100 %, including isolates from UTI patients (Ali and Yousif 2015). The existence of the hpmA gene in the isolates corresponds to findings that HpmB must cleave the HpmA protein's N-terminal peptide in order to trigger and transport the hemolytic HpmA protein outside the cell (Uphoff and Welch 1990).

Detection of ureC gene

In Figure 8 depicts the specific band of the ureC gene at (530bp). These findings represent the specific ureC gene in P. mirabilis. The findings of the current study revealed that the ureC gene was found in all of the bacterial isolates tested (100 %). The results of the current work are in agreement with the results presented by Muneeralatrash, who explain the ability of P. mirabilis to produce urease reaching 100% through its ability to analyse urea with water.

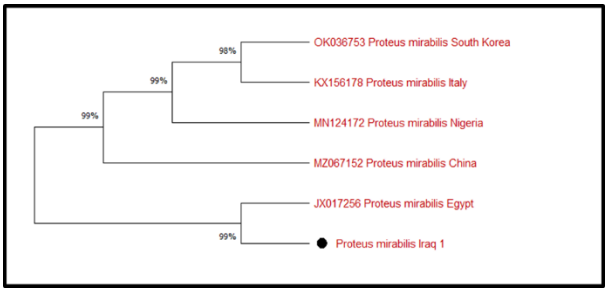
Figure 8: The results of P. mirabilis ureC gene amplification (530 bp in size)



The PCR products were electrophoresed in 1.5 percent agarose gels containing 0.5 mg/mL red safe stain for 80 minutes at 120 V. UV light was used to highlight the bands. M lane represents a molecular weight marker (DNA marker) of 100 bp. Lanes 1-14 are for the ureC gene. The detection of P. mirabilis genes by PCR technique for positive patients were recorded in ureC gene (33.3 %) in urine while in wound were recorded ureC gene (50%). As a result, described that the ureC gene was existing in 91.7% of all identified Proteus spp. isolates (Pathirana et al. 2018). UreC was expressed by 96.66% of human P. mirabilis isolates collected from the urinary tract (Ali and Yousif 2015). In contrast, observed that 90.91% of P. mirabilis were positive for ureC (Zhang et al. 2015). Furthermore, several studies, including, reported a wide distribution of the ureC gene in P. mirabilis (Lu et al. 2000)

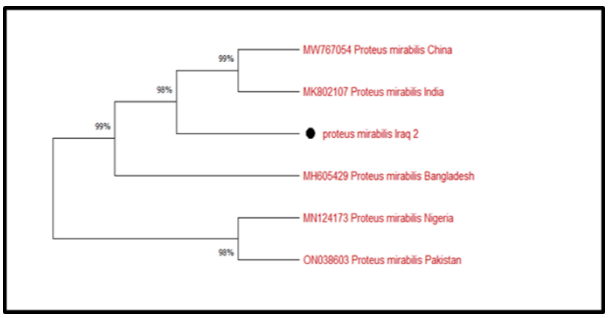
Result Sequencing Tests

Figure 9: Phylogenic tree for sample 1



Sample 1: After looking at the genetic affinity tree, the results showed that there is a very large convergence between isolate No. 1 with the Egyptian isolate (Figure 9), as the transverse lines indicate convergence and in a lesser percentage with the Chinese isolate, but the divergence with the Nigerian, Italian and Korean isolates was the same.

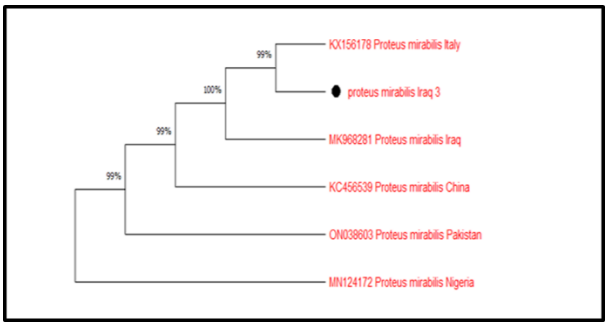
Figure 10: Phylogenic tree for sample 2



Sample 2: After looking at the genetic affinity tree, the results showed that there is no significant affinity between isolate No. 2 with global isolate, but there was a slight affinity with Indian and Chinese isolates, and to a lesser extent with Bangladeshi isolate, but the divergence with Nigerian and Polish isolates was to the same degree Figure 10.

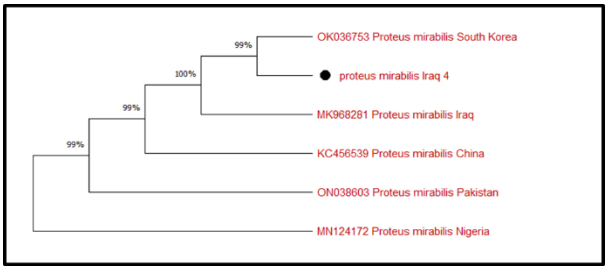
Sample 3: After looking at the Tree of Warthian Convergence, the results showed that there was a convergence between isolation No. 3 with the Italian isolation, and there was a slight convergence with Iraqi isolation and, to a lesser extent, with Chinese isolation, but the divergence with Nigerian and Polish isolations was to varying degrees Figure 11.

Figure 11: Phylogenic tree for sample 3



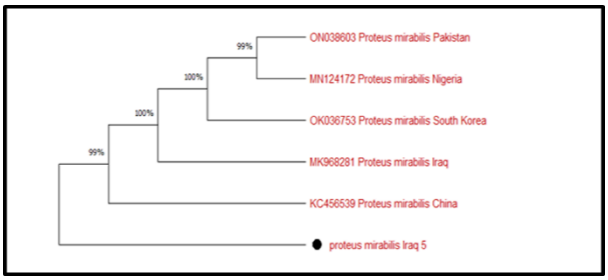
Sample 4: After looking at the Tree of Warthian Convergence, the results showed that there was a convergence between isolation No. 4 with Korean isolation, and there was a slight convergence with Iraqi isolation and, to a lesser extent, with Chinese isolation, but the divergence with Pakistani and Nigerian isolations to varying degrees Figure 12.

Figure 12: Phylogenic tree for sample 4



Sample 5: After looking at the genetic affinity tree, the results showed that there is no affinity between isolate No. 5 with global isolates, but there was a slight affinity with Chinese isolate, and at a lower rate with Iraqi isolate, but divergence was with Korean isolate, and with a greater percentage with Nigerian and Polish isolates Figure 13.

Figure 13: Phylogenic tree for sample 5



CONCLUSON AND RECOMMENDATION

1- Concerning the virulence genes ureC and hmpA, all clinical isolates were encoded for these genes, which contributed to their pathogenicity.

2- P. mirabilis was not the predominant isolate (23% only) in UTIs.

3- Clinical isolates showed the highest antibiotic sensitivity (73.91%) for amikacin and ciprofloxacin. On the other hand, P. mirabilis recorded the highest antibiotic resistance (95.65%) for ampicillin and norfloxacin.

4- The sequencing test result was interpreted on the basis of genetic affinity and divergence between bacteria Proteus mirabilis

Reference

Al-Gasha, F.A.S., Al-Baker, S.M. and Alrobiai, F.A. 2020. Prevalence of Urinary Tract Infections and Associated Risk Factors Among Patients Attending Medical City Hospital in Baghdad City, Iraq. American Journal of Infectious Diseases., 16 (2): 77-84.

Larramendy, S., Deglaire, V., Dusollier, P., Fournier, J. P., Caillon, J., Beaudeau, F., & Moret, L. (2020). Risk factors of extended-spectrum beta-lactamases-producing Escherichia coli community acquired urinary tract infections: a systematic review. Infection and Drug Resistance, 3945-3955.

Hoberman, A., Charron, M., Hickey, R.W., Baskin, M., Kearney, D.H. and Wald, E.R. 2003. Imaging studies after a first febrile urinary tract infection in young children. The New England journal of medicine., 348(3), 195–202.

Camacho, V., Estorch, M., Fraga, G., Mena, E., Fuertes, J., Hernández, M.A., Flotats, A. and Carrió, I. 2004. DMSA study performed during febrile urinary tract infection: a predictor of patient outcome?. European journal of nuclear medicine and molecular imaging., 31(6), 862–866.

Dason, S., Dason, J.T. and Kapoor, A. 2011. Guidelines for the diagnosis and management of recurrent urinary tract infection in women. Canadian Urological Association journal = Journal de l'Association des urologues du Canada., 5(5), 316–322.

Boyko, E.J., Fihn, S.D., Scholes, D., Abraham, L. and Monsey, B. 2005. Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic postmenopausal women. American journal of epidemiology., 161(6), 557–564.

Datta, P., Rani, H., Chauhan, R., Gombar, S. and Chander, J. 2014. Health-care-associated infections: Risk factors and epidemiology from an intensive care unit in Northern India. Indian journal of anaesthesia., 58(1), 30–35.

Lim, J.H., Cho, J.H. and Lee, J.H. 2013. Risk factors for recurrent urinary tract infection in kidney transplant recipients. Transplant Process., 45(4), 1584–1589.

Adeolu, M., Alnajar, S., Naushad, S.S. and Gupta, R. 2016. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International journal of systematic and evolutionary microbiology., 66(12), 5575–5599.

Fonseca, M., Sato, J.L., Lima-Noronha, M.A., Migliorini, L.B., Fernández-Silva, F.S. and Galhardo, R.S. 2018. Increased mutability to fosfomycin resistance in Proteus mirabilis clinical isolates. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases., 58, 27–33.

Tolulope, A., Ewaoche I.S. and Ibemologi, A. 2021. UreC and ZapA virulence genes amplification in clinical specimen of Proteus mirabilis in Bayelsa state, Nigeria Journal Microbiology Experimental., 9(1), 15-20.

Pernille, H., Lars, B., Marjukka, M., Volkert, S., & Anne, H. (2019). Sampling of urine

for diagnosing urinary tract infection in general practice–First-void or mid-stream urine?.Scandinavian Journal of Primary Health Care, 37(1), 113-119.

Harley, J. P. and Prescott, L. M. 2002. Laboratory exercises in Microbiolgy, 5th. ed. McGraw Hill companies, London.

MacFaddin. 2000. Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins, USA.

De Cueto, M., Ceballos, E., Martinez-Martinez, L., Perea, E. J., & Pascual, A. (2004). Use of positive blood cultures for direct identification and susceptibility testing with the Vitek 2 system. Journal of clinical microbiology, 42(8), 3734-3738.

Al-Hamdani, H., & Al-Hashimy, A. (2020). Molecular detection of UREC, HPMA, RSBA AND MRPA genes of Proteus Mirabilis urinary tract infection in patient with rheumatoid arthritis. The Iraqi Journal of Agricultural Science, 51, 245-251.

Sewify, M., Nair, S., Warsame, S., Murad, M., Alhubail, A., Behbehani, K., Al-Refaei, F. and Tiss, A. 2016. Prevalence of Urinary Tract Infection and Antimicrobial Susceptibility among Diabetic Patients with Controlled and Uncontrolled Glycemia in Kuwait. Journal of diabetes research., 6573215.

Khan, S., Hashmi, M.S., Rana, M.A., Zafar, G.M., Asif, S., Farooq, M.T. and Zahoor, S. 2022. Frequency of Urinary Tract Infections in Type 2 Diabetic Patients Taking Dapagliflozin. Cureus., 14(1), e21720.

Otajevwo F.D. 2013. Urinary tract infection among symptomatic outpatients visiting a tertiary hospital based in midwestern Nigeria. Global journal of health science., 5(2), 187–199.

Karlowsky, J.A., Lagacé-Wiens, P.R., Simner, P.J., DeCorby, M.R., Adam, H.J., Walkty, A., Hoban, D.J. and Zhanel, G.G. 2011. Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. Antimicrobial agents and chemotherapy., 55(7), 3169–3175.

Adams-Sapper, S., Sergeevna-Selezneva, J., Tartof ,S., Raphael, E., Diep, B.A., Perdreau-Remington, F. and Riley, L.W. 2012. Globally dispersed mobile drug-resistance genes in gram-negative bacterial isolates from patients with bloodstream infections in a US urban general hospital. Journal Medical Microbiology., 61, 968–974.

Kadhim, A.S. 2017. Antimicrobial Resistance Patterns and Extended Spectrum Beta-lactamases Producing by Proteus mirabilis Isolated from Different Sources. Al-Mustansiriyah Journal of Science., 28(1), 47–54.

Ko, K.S., Lee, M.Y., Song, J.H., Lee, H., Jung, D.S., Jung, S.I., Kim, S.W., Chang, H.H., Yeom, J.S., Kim, Y.S., Ki, H.K., Chung, D.R., Kwon, K.T., Peck, K.R. and Lee, N.Y. 2008. Prevalence and characterization of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated in Korean hospitals. Diagnostic microbiology and infectious disease., 61(4), 453–459.

Swihart, K.G. and Welch, R.A. 1990. The HpmA hemolysin is more common than HlyA among Proteus isolates. Infection and immunity., 58(6), 1853–1860.

Uphoff, T.S. and Welch, R.A. 1990. Nucleotide sequencing of the Proteus mirabilis calcium-independent hemolysin genes (hpmA and hpmB) reveals sequence similarity with the Serratia marcescens hemolysin genes (shlA and

shlB). Journal of bacteriology., 172(3), 1206–1216.

Lu, J.J., Perng, C.L., Lee, S.Y. and Wan, C.C. 2000. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. Journal of clinical microbiology., 38(6), 2076–2080.