

4-7-2022

Antibiotic Susceptibility Of Proteus Mirabilis That Isolates Of Diabetic Foot Ulcers In Al- Diwaniyah Hospital

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Recommended Citation

Jaber, Aseel Hussein and Almiyah, Suaad A. Fazaa (2022) "Antibiotic Susceptibility Of Proteus Mirabilis That Isolates Of Diabetic Foot Ulcers In Al- Diwaniyah Hospital," *Al-Qadisiyah Journal of Pure Science*: Vol. 27: No. 1, Article 14.

DOI: 10.29350/qjps.2022.27.1.1528

Available at: <https://qjps.researchcommons.org/home/vol27/iss1/14>

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Antibiotic susceptibility of *Proteus Mirabilis* that isolates of Diabetic foot ulcers in Al- Diwaniyah Hospital

<p>Authors Names</p> <p>a.Aseel Hussein Jaber b.Suaad A. Fazaa Almiyah</p> <p>Article History</p> <p>Received on: 10/4/2022 Revised on: 20/5/2022 Accepted on: 5/6/2022</p> <p>Keywords: Proteus mirabilis, diabetes foot ulcers, VITEK2, Antibiotics</p> <p>DOI:https://doi.org/10.29350/jops.2022.27.1.1528</p>	<p>ABSTRACT</p> <p>The current study have been included 250 samples diabetic foot ulcer patients attending in Al-Diwaniyah Teaching Hospital and private clinics at a period of study from beginning October 2021 to February 2022, Isolates were identified by morphological form on blood agar and macConkey agar, traditional biochemical tests and then confirmed by Vitek 2 system.</p> <p>The result of bacterial culture have been recorded that only 80 samples had positive result to <i>P. mirabilis</i> formed (32%) of this result. Prevalence of <i>P. mirabilis</i> isolates in male more than female (34.78%) and (28.57%) respectively, after then were determination of antibiotics susceptibility pattern of recovered isolates. The isolates showed that (100%) were resistant to penicillin G, (85%) Cephalexin , (75%) Cefotaxime, (100%) Ampicillin, (56.25%) Gentamycin, (35%) Amikacin,(100%) Amoxicillin / Clavulanic Acid, (58.75%) Chloramphenicol, (20%) Meropenem, (100%) Tetracycline, (22.5%) Imipenem, (83%) Streptomycin. Keywords: <i>Proteus mirabilis</i>, diabetes foot ulcers, VITEK2, Antibiotics</p>
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Introduction

Diabetes mellitus (DM) is a category of metabolic illnesses characterized by issues with blood glucose management (Altieri et al.,2022). Despite the fact that type 1 and type 2 diabetes have distinct causes, both are linked to a slew of problems that damage the heart, kidneys, eyes, and nerves. Damage to the macrovasculature is the primary cause of cardiovascular diseases, heart failure, atherosclerosis, and cerebrovascular events. The other primary sequelae, known as 'microvascular' damage, emerge as a diabetic triopathy, which includes diabetic kidney disease, diabetic retinopathy, and diabetic neuropathy (Eid *et al.*,2019). The number of chronic and acute illnesses in the general population will rise as diabetes prevalence rises. Many of the burdens associated with diabetes are caused by macrovascular complications such as coronary heart disease, stroke, and peripheral vascular disease, as well as microvascular complications such as end-stage renal disease (ESRD), retinopathy, and neuropathy, as well as lower-extremity amputations (LEA). Cancers, age-related consequences (e.g. dementia), infections, and liver disease are among the many causally associated illnesses that are becoming more well recognized (Harding *et al.*, 2019).

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Diabetes foot ulcer (DFU) infections are the leading cause of diabetic hospitalization (Davani *et al.*,2021). DFU is the most devastating consequence of diabetes mellitus and is linked with significant morbidity, death, and poor quality of life (Khodair and Al-Asady,2021). The most prevalent consequence of diabetes is diabetic foot ulceration. Its development is influenced by a number of risk factors (Ahmad *et al.*,2017). Amputations in patients with diabetes account for at least half of all amputations, with the most prevalent cause being an infected diabetic foot ulcer. Reduced lower-extremity amputation risk requires a full understanding of the causes and management of diabetic foot ulcers Diabetes-related foot disorders are prevalent and costly, and diabetics account for over half of all amputation hospital admissions (Boulton *et al.*, 2018).

Proteus species are gram-negative bacteria that are found in wounds, particularly diabetic wounds (Hegazy, 2016). Proteobacteria species are Gram-negative bacteria found in wounds, especially diabetic wounds (Hegazy, 2016). The world is facing a serious diabetes epidemic and available reports indicate that all of these patients are at risk of developing diabetic foot ulcers. Neuropathy-specific DFUs represent about 50-60% of all DFUs. Signs or symptoms of vascular dysfunction are observed in 40 to 50 percent of all patients with the vast majority of ischemic nerve ulcers, and only a minority of patients with purely ischemic ulcers. Diabetic foot infections are usually polymicrobial in nature, and include both aerobic and anaerobic, which can cause caries to any part of the body especially the distal part of the lower leg (Ruke, 2019).

Bacterial resistance to antimicrobial agents is a serious worldwide problem with regard to the treatment of infectious diseases. Understanding the molecular basis of how resistance genes are acquired and transferred may contribute to the creation of new antimicrobial strategies. The spread of antibiotic resistance is usually associated either with clonal spread of epidemic strains or through independent acquisition of resistance genes on plasmids, transposons or integrators (Hegazy., 2016).

Research aims

Isolation and identification of *Proteus mirabilis* bacteria from diabetic foot ulcers patients and assessment of antibiotic sensitivity.

Material and Methods

1. Collection of Samples:

From 250 samples, 80 positive samples for *Proteus Mirabilis* were collected, where collected by sterile cotton swabs from diabetic foot ulcer patients attending in Al-Diwaniyah Teaching Hospital and private clinics at a period of study from beginning October 2021 to February 2022.

2. Bacterial diagnosis:

Collected samples by sterile cotton swabs from diabetic foot ulcer patients, after their transferred to the laboratory by Sterile cotton swabs with transport medium. Then diagnostic according to morphology on culture media, biochemical test and Vitek 2 System.

A. Culture of samples:

All swab samples cultured directly on MacConky agar and blood agar medium, incubated at 37° C for 24 hr. Isolates purified several times until pure isolates were obtained, were identified depending on the morphology, general characteristics of colony and biochemical tests.

B. Biochemical diagnosis:

The biochemical tests were done to identification the bacterial isolates, biochemical test, namely oxidase, indole, citrate utilization, catalase, urease production, H₂S formation, lactose fermented, Voges- proskauer reaction, Methyl red, Triple sugar iron test (TSI) and Simmon citrate (Hawkey, 2006; Forbes *et al.*, 2016; Jacobsen *et al.*, 2008).

C. Vitek 2 System:

The Vitek 2 System was used to identify the bacterial isolates. The Vitek 2 system detects bacteria and other microorganisms Based on the examination of substrate consumption patterns, a microbe has been discovered. The cards to be used were chosen based on the situation (Pincus, 2010).

3. Antimicrobial susceptibility test:

Antimicrobial susceptibility testing was performed on MHA medium using the Kirby Bauer disc diffusion technique, as per NCCLS guidelines.

Results

1. Isolation of *Proteus mirabilis*

Table(1) : number and rates to *Proteus Mirabilis* isolates from Diabetic Foot Ulcers

Isolates	Gender	Number of isolates	Number of <i>Proteus Mirabilis</i>	%
Diabetic Foot Ulcers	Male	138	48	34.78
	female	112	32	28.57
	Total	250	80	32
X ²			0.109	
P value			0.295*	

* No significant difference at P<0.05

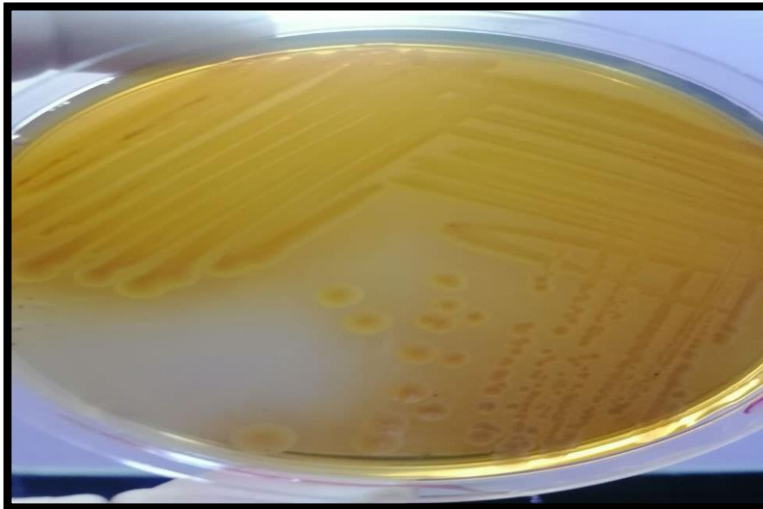
1. Bacterial diagnosis:

D. Culture of samples:

The isolate colonies appeared on blood agar ripple movement or swarming and the smell of bacterial growth which is similar to smell of fish rotting as in (Figure 1), and isolates are looked light yellow colonies, are medium in size and the edges smooth and lactose non fermenting on MacConkey agar as (Figure 2).



(Figure 1): *P. mirabilis* on blood agar



(Figure 1): *P. mirabilis* on blood agar

B. Biochemical diagnosis:

Biochemical tests of the *P. mirabilis* isolates are summarized in Table(1).

Table (1): Biochemical tests of *Proteus mirabilis*

Biochemical	Species	<i>Proteus mirabilis</i>
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Oxidase	-
Urease production	+
Catalase	+
H ₂ S formation	+
Citrate utilization	+
Voges- proskauer reaction	-
Methyl red	+
Indol	-
Simmon citrate test	-
Lactose fermented	-
Tribble sugar iron test (TSI)	Alk/A H ₂ S with gas

+ Positive , - Negative , K = alkaline, A = acidic.

2. Antimicrobial susceptibility:

Table (2) : Rates of sensitivity , Intermedite and resistance to antibiotics by *Proteus Mirabillis*

No.	Antibiotic Name	R	I	S
1.	Amikacin	28 (35.0 %)	20 (25.0 %)	32 (40.0 %)
2.	Gentamicine	45 (56.25 %)	10 (12.5 %)	25 (31.25 %)
3.	Streptomycin	67 (83.75 %)	3 (3.75 %)	10 (12.5 %)
4.	Amoxicillin - Clavulanic Acid	80 (100.0 %)	0 (0.0 %)	0 (0.0 %)
5.	Meropenem	16 (20.0 %)	10 (12.5 %)	54 (67.5 %)
6.	Imipenem	18 (22.5 %)	12 (15.0 %)	50 (62.5 %)
7.	Cefotaxime	60 (75.0 %)	10 (12.5 %)	10 (12.5 %)
8.	Cephalexin	68 (85.0 %)	5 (6.25 %)	7 (8.75 %)
9.	Chloramphenicol	47 (58.75 %)	14 (17.5 %)	19 (23.75 %)
10.	Ampicillin	80 (100.0 %)	0 (0.0 %)	0 (0.0 %)
11.	Pencillin G	80 (100.0 %)	0 (0.0 %)	0 (0.0 %)

12.	Tetracycline	80 (100.0 %)	0 (0.0 %)	0 (0.0 %)
	X ²	425.99		1
	P value	0*		2

* Significant difference at P<0.05

R= Resistance , I= Intermedite , S= Sensitive

Discussion

This study revealed the prevalence of *P. mirabilis* 80(32%) of 250 swabs in diabetic foot ulcers patients, Identification of this bacteria by using the conventional methods include culture and biochemical tests . On MacConkey agar, colonies of *P. mirabilis* looked pale, yellow, and lactose non fermenters, similar to (Forbes *et al.*, 2016). Also, The results of *P. mirabilis* culture on blood agar plates and exhibit the swarming phenomenon as well as the fish odor, mucoid and non-hemolytic colonies which agrees with (Al-Aabideen ,2005).

Biochemical assays were utilized to further identify *P. mirabilis* isolates, which revealed positive production. Catalase, Urease, Citrate Utilization, Methyl Red, and H₂S Formation were all positive, whereas Oxidase, Indole, Lactose Fermentation, and Voges-Proskauer were all negative (VP), The present research's biochemical tests accord with those of a previous study (Hawkey, 2006; Forbes *et al.*, 2016; Jacobsen *et al.*, 2008) Furthermore, The results of *P. mirabilis* identification using the Vitek2 system revealed that all isolates were *P. mirabilis*, with a percentage of identification ranging from (95-99%). This percentage was consistent with (Sung *et al.*, 2000), who reported that identification of *Proteus mirabilis* by the Vitek 2 system was (97%). manual biochemical assays are commonly employed for bacterial identification. The advantages of traditional procedures were that they were inexpensive, but the downsides were that they took time and were prone to contamination present, false positive result and require a large amount of sample, while the automated biochemical tests such as VITEK 2 system. the VITEK 2 system used in many previous studies was detected bacteria faster, efficient and away from the contamination that may prevent detection of the pathogen. In the present investigation, biochemical testing revealed that all of the isolates were also from the *P. mirabilis* species, which is the same as in the previous study (Drzewiecka *et al.*, 2016).

isolates show (100%) resistance to penicillin G, Ampicillin, Amoxicillin / Clavulanic Acid and Tetracycline ,The resistance ratio was similar to (Wang *et al.*, 2014); (Mohammed and Hamzah,2021) and (Ali, 2015),this results did not agree with (Young *et al.*, 2006); (Ayoub *et al.*, 2015) and (Shakibaie *et al.*, 2015) reported (48.5%), (9.7%) and (0.0%) , respectively. The variation in relationships might be attributed to the presence of beta-lactamase enzymes in bacterial isolates, The difference is attributable to anatomical and physiological changes, as well as other possible risk factors, as described by (Akram *et al.*,2007); (Manjunath *et al.* , 2011). In the case of aminoglycosides, which included gentamicin antibiotics, the resistance rate in the current study (56.25 %) was similar to that found in previous studies (Ali and Yousif,2017)and (Prakash and Saxena,2013) where resistance was (53.33%) and (57.1%). While (Setteh,2004) and (Al-Bayti *et al.*, 2010); the resistance percentage was (85.20%)

and (83.8 %), According to (Katzung, 2001) Gram-negative bacteria produce gentamicin due to the presence of encoded plasmids in them, enzymes that alter aminoglycosides. In terms of anti-Amikacin resistance, the isolates of *P. mirabilis* bacteria were shown to have a low level of resistance. The proportion of resistance to this antibody was (35%), which was similar to the results obtained by (Kezeer, 2007) and (Bahashwan and Elshafey, 2013), who reported resistance rates of (33.4%) and (38.4%), respectively. While the current study's findings differed from those of (Luzzaro *et al.*, 2001), (Jalooob and Gafil, 2012), and (AL-Bassam and AL-Kazaz, 2013) in that the resistance rates were (1.6 %), (100 %), and (5%), respectively. While (Endimiani *et al.*, 2005) found that all of their isolates of *P. mirabilis* bacteria showed no resistance to the anti-amikacin, as the resistance that these bacteria have against a group of antibiotics to aminoglycosides is caused by a change that occurs under the 30S ribosomal unit to which the antigen is attached, and this change leads to a decrease in the antigen. Because of the quality of their action, availability, and low cost, most patients may opt to employ aminoglycoside antagonists in therapy.

On the other hand, streptomycin was shown (83.75%) resistant and a ratio that was similar to (Gad *et al.*, 2011) (75 %) which was not agreed with (Harada *et al.*, 2014) who reported (15.5%) and (Habibu, 2014) who reported (0.0%) may be due to the etiological agents and their susceptibility/resistance patterns vary according to geographical locations as established by (Cunha *et al.*, 2016).

P. mirabilis (58.75%) looked resistant to chloramphenicol, which was in agreement with (Yah *et al.*, 2007) and approximated to (Mohammed and Hamzah, 2021) where the proportion of resistance was (47.3%), but did not accord with (Ahmed, 2015) who reported (20 %).

For cephalosporin antibiotics represented by cefotaxime, the rate of resistance to this antibiotic was (75%) and was close to a research (Felgo *et al.*, 2010) where the resistance of cefotaxime reached (70%) of isolates of *p. mirabilis*, while the percentage of resistant Cefotaxime (83%) (Abdulghani, 2012). While the percentage of resistance to this antibiotic in our current study was lower than it was in the studies of (Al-Atubi, 2013) and (Kadhun, 2010), where the resistance was (100%) but the resistance was in Our current study is higher than the resistance according to the study of (Mishra *et al.* 2001), (Al-Bassam and Al-Qazzaz 2013) and (Ali and Yousif, 2015), where their percentage reached (30%), (35%), (22.61%). as we mentioned before. The current study of the antibiotic Cephalexin also showed a resistance of (85%) and it is similar to the events of (Yassen and Shareef, 2012), (Luzzaro *et al.*, 2001). As mentioned earlier, their proportions were (100%) and (80%). While the percentage of resistance to this antibiotic in our study was higher than that of (Ali and Yousif, 2015), where their isolates were resistant (42.85%). Cephalosporin resistance may not be limited to the development of beta-lactamase enzymes. Other approaches include altering the permeability of the antigen to the cell membrane, making it difficult for the antigen to pass through it and reach the target point (Spanu *et al.*, 2002).

The resistance rate for carbapenems represented by Imipenem in the current study was (22.5%), which was similar to the results of studies (Kadhim *et al.*, 2014) and (Al-Bassam and Al-Qazzaz, 2013), where the rates of resistance were (25%) and (15%), respectively. In the current study, the proportion of meropenem resistance was (20%), which varies dramatically from (Battikhi and Ammar, 2014), where the percentage of resistance to this antibiotic was (100%). Bacteria have less resistance to carbapenems than to the other antibiotics studied, despite the fact that

carbapenems are one of the most effective antibiotics for treating Gram-negative bacteria infections due to their stability against hydrolysis by beta-lactamase enzymes and a high rate of permeability through the bacteria's external membrane (Hawkey and Munday, 2004).

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