The Review of DIABETIC STUDIES

RESEARCH

Molecular Detection of Some Tetracycline Resistance Genes Among Pseudomonas Aeruginosa Isolated from Diabetic Patients

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■ Abstract

Background: *Pseudomonas aeruginosa* is frequently responsible for nosocomial infections, including pneumonia, immunocompromised hosts, and infections in people with structural lung diseases such as cystic fibrosis. **Methods:** One hundred specimens from burns, wounds, diabetic foot, throat swabs, and urine were collected from diabetic patients between the ages of (20-60) years. They were then cultivated using brain-heart infusion, MacConkey agar, and Cetrimide agar. 30 out of 100 (30%) specimens gave positive results for *P. aeurogingosa* isolates, including 12 out of 30 (40%) male and 18 out of 30 (60%) female specimens. Biochemical tests and the Vitek 2 system were used to identify and diagnose the *P. aeruginosa* isolates alongside *Tet C* detection via PCR. After that, an antibiotic sensitivity test was then conducted. **Results:** results showed that there was 27/30 (90%), and 26/30 (86.7%) of *P. aeruginosa* isolates gave highly resistant results for the drugs Trimethoprim-sulfamethoxazole and Ceftazidime, respectively, in contrast to 27/30 (90%), 25/30 (83.3%), and 18/30 (60%) of *P. aeruginosa* isolates that gave high sensitive results for the Polymyxin B, Minocycline, and Meropenem respectively. 16/30 (53.3%) of *P. aeruginosa* isolates showed sensitive results for the tetracycline, whereas 14/30 (46.7%) showed resistant results for the Tetracycline drug. **Conclusion:** The molecular results of the current study showed 16/30 (53.3%) isolates of *P. aeruginosa* which carried the *tet C gene*.

Keywords: *P. aeruginosa, tet C* gene, Tetracycline, Diabetes mellitus, Bacterial resistance.

1. Introduction

The most harmful species in the Pseudomonadaceae family is Pseudomonas aeruginosa. *P. aeruginosa*, is a gram-negative, non-spore-forming rod that is straight or slightly curved and is 1 to 3 um in length and 0.5 to 1.0 um in width, has a polar flagellum and many cell surface fimbriae or pili which provide motility [1]. One of the most prevalent and opportunistic nosocomial (hospital-acquired) pathogens, *P. aeruginosa* is responsible for numerous serious and frequently lethal infections [2]. In a multicentral study conducted in San Antonio City (USA), the outcome revealed that the prevalence of *P. aeruginosa* is 4.2% among studied sample infections[1]. In a systematic review conducted in China, the outcome revealed that the prevalence rate was 19.4% among studied sample infections[2]. Similarly, in unicentre multisport study conducted in Duhok (Iraq), the rate seems to be relatively high (29.32%). The intrinsic resistance to multiple drugs (MDR) and the significant risk of treatment-induced tolerance make *P. aeruginosa* infections difficult to treat [3]. In a clinical retrospective study conducted over 5 years in Ethiopia, the resistance rate of *P. aeruginosa* were relatively high (39.4%) [4].

Nosocomial infections, which include pneumonia, urinary tract infections, surgical site infections, and bacteremia, are frequently caused by *P. aeruginosa*. The frequency of *P. aeruginosa* among all illnesses linked to healthcare is estimated to be between 7.1% and 7.3% [5]. An even greater proportion of healthcare-associated infections are caused by *P. aeruginosa*. The most prevalent site of *P. aeruginosa* infection is the respiratory system, accounting for 16.2% of patient infections and 23% of all ICU-acquired infections, according to a large international observational point-prevalence study of infections in ICU patients [5]. Most of which were reported to be multidrug resistant (MDR) [6].

Diabetes mellitus associated with immunopathy resulting in enhanced virulence factors of any infectious diseases including *P. aeruginosa* coupled with vasculopathy and immunopathy making endogenous handling immune reaction against pathogens highly difficult especially in critical cases, such as, diabetic foot [7]. Taking into consideration that up to 25.8% of lower limb amputation in diabetics was reported to be

associated with antibiotic resistance *P. aeruginosa* [8]. Evidence are supporting findings of studies who have claimed that diabetes increases expression of virulence factors of *P. aeruginosa* with increasing chances of resistance [9,10].

Even during treatment, *P. aeruginosa* readily develops resistance tactics to many classes of antibiotics. Various resistance mechanisms frequently coexist, resulting in combined resistance [11]. As the most effective β-lactams, carbapenems are now commonly employed as the standard and empirical treatment for severe infections brought on by MDR *P. aeruginosa* [12]. Effective penetration, colonization, and persistence within the host organism are made possible by the pathogen's capacity to anticipate virulence factors stimuli. P.aeruginosa type IV pili is crucial for pathogenicity and adhesion to a variety of cell types [13]. Limitations of the availability of effective antibiotic for eradication of *P. aeruginosa* due to high resistance rate, the defective immunity of patients with diabetes mellitus, and the high rate of diabetes in general population, these reasons have been responsible to commence the present study which has focused on the determination the effectiveness of tetracycline in eradication of resistance *P. aeruginosa*.

2. Materials and Methods

2.1. Specimen Collection

One hundred specimens were collected from diabetic patients aged between (20-60) years from different clinical sources including burns, wounds, diabetic foot, throat swap and urine specimens for the period from 15 August to 25 November 2024 from the Medical City Hospital in Baghdad - Iraq.

2.2. Isolation and Identification Of P. Aeruginosa

All specimens were cultured on MacConkey agar, Cetrimide agar, a selective medium for *P. aeruginosa*, and brain heart infusion. They were then incubated for 24 hours at 37°C. The presence of 30/100(30%) positive growths for *P. aeruginosa* was detected, the positive specimens were examined with biochemical testing, and the Vitek 2 system was used to identify and diagnose *P. aeruginosa* isolates.

2.3. Antibiotic Susceptibility Testing

Using the Kirby-Bauer method, all of the positive isolates of *P. aeruginosa* were tested for bacterial susceptibility with the class of antibiotics, including Minocycline, Meropenem, Trimethoprim Sulfamethoxazole, Ceftazidime, and Polymyxin B on Muller-Hinton agar.

2.4. Molecular Detection of the Tet C gene among P. Aeruginosa

Using the Genomic DNA Mini Kit, gram-negative bacteria including *P. aeruginosa* were extracted in accordance with the manufacturer's instructions (Favorgen) and the extracted DNA was then utilized to amplify genes involving tetracycline (*Tet C*).

3. Results

One hundred specimens were collected from patients between the ages (20-60) years from various clinical sources including burns, wounds, diabetic feet, throat swabs, and urine specimens. Thirty out of one hundred (30%) specimens gave positive for *P. aeruginosa* isolates including 12/30(40%) males and 18/30 (60%) females shown in (Table 1) and (Table 2).

Table 1: Distribution of Bacterial Growth with Gender.

Gender	Number (%)	*Growth of P. Aeruginosa (%)*	Growth of other Bacteria (%)
Male	50 (50%)	12/30(40 %)	30/70(42.86%)
Female	50(50%)	18/30 (60 %)	40/70(57.14%)
Total	100(100%)	30(100%)	70(100%)

Table 2: Distribution of Bacterial Growth with Age.

Ages	Growth of P. *Aeruginosa* (%)		Growth of other Bacteria (%)	
	Male	Female	Male	Female
(20-35) years	3/12(25%)	6/18(33.3%)	12/30(40%)	14/40(35%)
(35-50) years	5/12(41.7%)	8/18(44.4%)	10/30(33.3%)	16/40(40%)
(50-60) years	4/12(33.3%)	4/18(22.2%)	8/30(26.7%)	10/40(25%)
Total	12(100%)	12 (100%)	30(100%)	40(100%)

Table 3: Identification Results and some Biochemical tests for *P. Aeruginosa* Isolates.

Test	Result
Gram stain	Gram-negative rods
Cetrimide agar	Green colony
Catalase	Positive
Urease	Negative
Oxidase	Positive
Motility	Positive
Voges-Proskauer	Negative
Indole production	Negative
Methyl red	Negative
Citrate Utilization	Positive
TSI	K/K

The results of the present study showed all *P. aeruginosa* isolates gave gram-negative rods, positive for the urease, catalase, oxidase tests and motility. Table 3 shows details of biochemical tests for the *P. aeruginosa* isolates.

All suspected isolates that grew on cetrimide agar (selective medium), produced positive results that were identical to the biochemical tests for *P. aeruginosa* isolates. After all of the positive specimens were sent to the Vitek2 compact system, which confirmed that every suspected isolate was *P. aeruginosa*, shown in (Table 4).

Table 4: Identification of *P. Aeuroginosa* Isolates by Vitek 2 Compact System.

APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	+	dMNE	+	BXYL	-	BAlap	+
ProA	+	LIP	+	PLE	-	TyrA	-	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	+	GIT	+	MNT	+	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	-	BGUR	-
O129R	+	GGAA	-	IMLTa	+	ELLM	-	ILATa	-		

According to the results of the current study, 27/30 (90%), and 26/30 (86.7%) of *P. aeruginosa* isolates gave highly resistant results for the drugs Trimethoprim-sulfamethoxazole and Ceftazidime, respectively, compared with 27/30 (90%), 25/30 (83.3%), and 18/30 (60%) of *P. aeruginosa* isolates gave high sensitive results for the polymyxin B, Minocycline, and Meropenem respectively. Additionally, 16/30 (53.3%) of *P. aeruginosa* isolates demonstrated sensitive results for the tetracycline drug, while 14/30 (46.7%) of these isolates gave resistant results for the tetracycline drug (Table 5).

Table 5: Distribution of Antibiotics Resistance among *P. Aeruginosa* Isolates.

Antibiotics	Sensitive	Resistance	Total
Trimethoprim-Sulfamethoxazole	3/30 (10%)	27/30 (90%)	100%
Ceftazidime	4/30 (13.3%)	26/30 (86.7%)	
Polymyxin B	27/30 (90%)	3/30 (10%)	
Minocycline	25/30 (83.3%)	5/30 (16.7%)	
Meropenem	18/30 (60%)	12/30 (40%)	
Tetracycline	16/30(53.3%)	14/30(46.7%)	

3.1. Molecular detection of Tetracycline C gene among P. aeruginosa isolates

In the current study, molecular results showed 16/30 (53.3%) isolates of *P. aeruginosa* which appeared in positive bands at the correct product size (418 bp) for *tet C* gene, as in the (Figure 1).

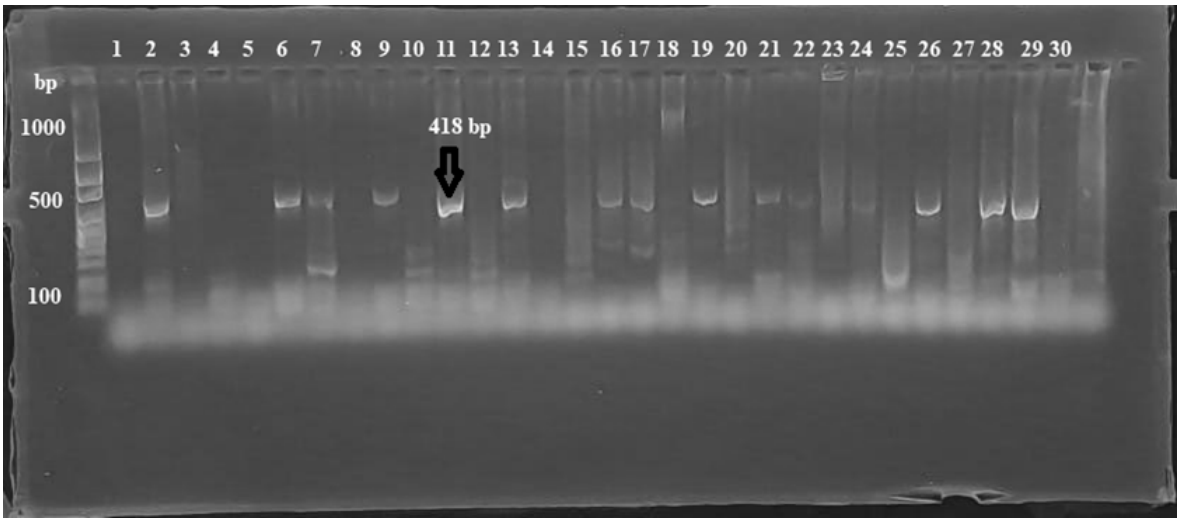

Figure 3: PCR Amplification Products Stained with Ethidium Bromide Stain dye for the *Tet C Gene* of *P. Aeruginosa* Isolates Using 90 Minutes and 80 Volts and Agarose Gel (1) %, with Ladder (L) Used that has a DNA Molecular Size Marker (100-bp), and all of the Isolates showed Positive bands for *tet C Gene* Amplification Except for no. 1, 3, 4, 5, 8, 10, 12, 14, 15, 18, 20, 25, 27, and 30, which were Negative Bands.

4. Discussion

P. aeruginosa was the most prevalent, tolerant, and dangerous organism in wound and burn infections. The virulence of bacteria is attributed to their ability to produce a variety of virulence factors, including extracellular and cell-correlated virulence factors like elastases, Type III protein secretion, pyocyanin, and alginate [14]. *P. aeruginosa* is one of the main causes of severe hospital-acquired and community-onset bacterial infections in humans, particularly because of its remarkable capacity to acquire resistance genes [15,16].

The production of beta-lactamase enzymes by *P.*

aeruginosa such as Penicillinase, which attacks the beta-lactam ring in the nucleus of both penicillins and cephalosporins rendering them ineffective antigens as well as the production of broad-spectrum enzymes known as ESBLs, which have efflux pumps and alter outer membrane orifices, are among the primary causes of *P. aeruginosa* resistance to beta-lactams [17].

In present study showed 27/30 (90%) and 26/30 (86.7%) of *P. aeruginosa* isolates gave highly resistant results for the Trimethoprim-Sulfamethoxazole and Ceftazidime drugs respectively, compared with 27/30 (90%), 25/30 (83.3%) and 18/30 (60%) of *P. aeruginosa* isolates gave high sensitive results for the Polymyxin B, Minocyclin, and Meropenem, respectively. A total of 16/30(53.3%) of *P. aeruginosa* isolates gave sensitive results for the Tetracycline drug compared with 14/30(46.7%) of these isolates gave resistant results for the Tetracycline drug [18].

The results of one study obtained by Akrami et al ., (2024) where 250 of *P. aeruginosa* isolates they found resistant to ceftazidime (86%) [19]. According to additional results by Ratajczak et al. (2021), 73 (61.6%) of the *P. aeruginosa* isolates were meropenem-resistant [20]. An additional study conducted by Al-Salihe in 2022 revealed that 34/60 (56.66%) isolates of *P. aeruginosa* had the highest rate of ceftazidime resistance, compared to 1/60 (1.66%) and 2/60 (3.33%) isolates of *P. aeruginosa* that appeared to be resistant to trimethoprim-sulfamethoxazole and colistin, respectively [21].

In the current study, molecular results showed 16/30 (53.3%) isolates of *P. aeruginosa* which appeared in positive bands at the correct product size (418 bp) for the *tet C* gene. Gram-negative bacterial isolates, particularly those from E. coli, showed the highest rate of resistance to Trimethoprim-sulphamethoxazole a ratio (89%), followed by tetracycline (87%) and colistin sulfate (21%) [22].

In another study, out of 60 screened isolates of E.coli, 25/60 (41.67%) of specimens gave positive results for the TetA gene, while 7/60 (11.67%) showed the carriage of the tet B gene [23]. Finally, in a different study conducted in Iran by Jomehzadeh et al. (2023), 73 specimens from Shigella species appeared with results 45/73 (61.6%) and 21/73 (28.7%) that carried positive results for tetA and tetB genes respectively [24]. Special care is required in diabetic patient due to immune deficits leading to increased exposure and limited handling of infection [25, 26] and the virulence of infection could be harder [27-29].

5. Conclusion

Pseudomonas aeruginosa is one of the common infectious diseases for diabetic patients and have shown response to the therapy of tetracycline drug. In diabetic middle-age female, *Pseudomonas aeruginosa* is more prevalent compared to male or other age group. Sensitivity to tetracycline and minocycline was more than 50% and this is considered as an effective against *Pseudomonas aeruginosa* expressing *tet C* gene. We recommend to apply tetracycline for area of infections in diabetic patients to treat lower urinary tract infections, upper respiratory infections, diabetic foot, and wounds. recommendation also involved testing *Pseudomonas aeruginosa* for other types of antibiotics

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