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Research Article

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Prevalence β-Lactamase Broad-Spectrum Type TEM between Isolates of Proteus Mirabilis in AL-Diwaniya City

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ABSTRACT

The samples of the study were collected from different clinical sources 185 isolates of hospitals, the Diwaniyah city during the period from October 2015 to April and divided the samples, according to sources collected into five groups (46 swab ear, 42 swab burns, 37 a stool sample, 59 urine sample and 1 blood sample), as results showed that cultural and biochemical tests 69 isolated belong to bacteria Proteus mirabilis, diagnosis was confirmed by api 20E and the use of polymerase chain reaction.In the study showed that there are (60.86%) isolates were resistant these isolates vowed multi-resistant of antibiotics (multidrug resistance) the highest proportion among the three types of resistance, and the isolates of the overall resistance (extensive drug resistance) ratio of resistance (34.78 %) , while the third type of resistance the resistance ratio was increased by two (4.34 %) and represents the resistance of each species of antibiotics in the current study (pand drug resistance) . Tested 24 isolates P.mirabilis to examine the capability of production of enzymes β- lactam TEM type using PCR, was bla_{TEM} -177 the most frequently identified gene among isolates of P.mirabilis percentage (87.5%), while the bla_{TEM} -160 (59.38%), and bla_{TEM} -72 (56.25%), bla_{TEM} -1(56.25%), bla_{TEM} -156 (53.13%), bla_{TEM} -89 (31.25%), while bla_{TEM} -3 counted the lowest percentage among the studied genes in P.mirabilis ratio (6.25%). Analyzed Phylogenetic tree genetic tree analysis bla_{TEM} by Mega6 program, were used genetic tree analysis of the type UPGMAtree Test yields 14 sample of isolates P.mirabilis. compared 2 isolates of P.mirabilis multi-resistant of antibiotics and producing resistance gene type TEM β-lactamase to analyze the genetic tree. The results of the analysis, there is a clear Identify of isolates of P.mirabilis bla_{TEM} local isolates with worldwide origin P.mirabilis compared with the rest of the other species that appear in the genetic tree analysis.

Keywords: Salicylic acid; Schiff bases; Tetrazole; Azo compound

INTRODUCTION

P. mirabilis and one of the most important bacterial species negative bacteria belong to the family of intestinal and these bacteria of great significance in the spread of hospital-acquired infections Nosocomial infections [1,2] and is one of the most common pathogens that cause infections acquired community community acquired infections, and for being opportunistic bacteria opportunistic therefore causing many injuries pathological when its presence is in their natural habitat [2,3].The resistant of *P.mirabilis* especially multi-drug antibiotic resistance become developing with time as well as the production of this type of enzymes β-lactamase, most of the genes multiple drug resistance antibiotics is transmitted by plasmids [4]. β-lactamase broad spectrum enzymes produced by negative bacteria It gives the bacteria produced an increase in resistance to antibiotics commonly used [5]. So is the study of β-lactamase enzymes is very important and necessary to decrease the production and spread of β-lactamase [6]. These enzymes cause failed treatments to control the various bacterial infections, for resistance of antibiotics interested researchers [7] these enzymes become increasing gradually with time and is developing rapidly [8]

MATERIALS AND METHODS

Collection of samples

During the period from October 2015 to April 2016, a total of 185 nonduplicate clinical samples in Al-Diwaniya city. Samples which were collected by sterile swabs and containers had been cultured on blood agar and macConkey agar, to get pure colonies subculture done on macConkey agar, incunated for overnight at 37C°.

Polymerase Chain Reaction Amplification (PCR)

Polymerase Chain Reaction Amplification (PCR) was used to screen for the occurrence the genes bla_{TEM} in *P.mirabilis* that risestance to antibiotic β-lactam : , bla_{TEM} -177, bla_{TEM}-89, bla_{TEM} -156 , bla_{TEM} -160 bla_{TEM}-72 , bla_{TEM} -3, bla_{TEM} -1 . the Primers were designed from sequences deposited in the GenBank database Table (1). The complete template DNA for the PCR amplification was extracted from the supernatant of a combination of *P.mirabilis* cells produced by salting out method [9]. PCR amplification was using 5 µl of the template DNA, 2 µl of each primer, 10 µl master mix, and 1 µl of Taq DNA polymerase in a total volume of 20 µl. A thermocycler (Mastercycler gradient; Eppendorf, Hamburg, Germany) was programmed with the suitable conditions [9]. Then, 5µl of each PCR product was examined by electrophoresis on 1% (w/v) TAE agarose gel having 0.1 µl/mL ethidium bromide [10]. The amplicon were then visualized on a UV transilluminator and photographed (BioDoc-Analyse; Biometra, Goettingen, Germany).

Table 1: DNA Primer which purchased from Bioneer (Korea) company

Primer	Sequence		Amplicon
16S rRNA	F	TCTTGTGAGAGCGGGGGATA	725bp
	R	AGTTGCAGACTCCAATCCGG	
TEM-3	F	TGCATCTTTGAGCGCTCTGA	318bp
	R	CGTTTTCTGAGACGACCCCA	
TEM-72	F	TCCTTGAGAGTTTTCGCCCC	581bp
	R	CAGTGCTGCAATGATACCGC	
TEM-89	F	GGGAACCGGAGCTGAATGAA	254bp
	R	CAGTGCTGCAATGATACCGC	
TEM-160	F	CTCTAGCTTCCCGGCAACAA	149bp
	R	CAGTGCTGCAATGATACCGC	
TEM-177	F	TGATAACACTGCGGCCAACT	358bp
	R	CAGTGCTGCAATGATACCGC	
TEM-1	F	TCCTTGAGAGTTTTCGCCCC	452bp
	R	TTGTTGCCGGGAAGCTAGAG	
TEM-156	F	AGATCAGTTGGGTGCACGAG	637bp
	R	CAGTGCTGCAATGATACCGC	

RESULTS

A total of 85clinical samples have been *Proteus* ssp collected, 69 belong to *P. mirabilis* That isolate from different sourace .the highest isolation rate of *P. mirabilis* was the stool samples 19(12.02%), ear swabs in second place in isolation 24(9.18%). either burns samples and urine have equal proportions 16 (8.64%) from each other,while blood samples proportion 0.54% just one of isolation. The other species belonging to the genus Proteus isolation rate 16 (8.64%).as results showed that cultural and biochemical tests and gene 16rRNA PCR in the figure 1 belong 69 isolated to *P mirabilis* .

Table2: Incidence of the isolated *Proteus* ssp in different clinical sample sites

No.(%)	Source of samples					*Proteus* ssp
	Blood	Burn	Ear	Urine	Stool	
(%37.29)69	(0.54) 1	(8.64) 16	(9.18) 17	(8.64) 16	(12.02) 19	*P. mirabilis*
(%8.64)16	0(0)	(1.08)2	(2.16)4	(2.16)4	(3.24)6	*Other Proteus ssp*

X^2= 36.129 *found morale differentiation in the Table2

According to the gender and age , 40(57.97%) samples were collected from female while 29(42.02%) samples were collected from male. the highest rate of *P.mirabilis* in the age 30-21 age increased by 34.78%, followed by the age

group 31-40 years, which recorded a rate of 13.4%, as shown in the table (3). While researcher didn't agree with him (14) for the class (29-20) increased by 21.78%, and (39-30) increased by 17.82%.

Table 3: Distribution *P. mirabilis* according to the gender and age

Gender/Age	Female	Male	NO%
10-Jan	3 (4.34)	(7.24)5	8 (11.59%)
20-Nov	5 (7.24)	(4.34)3	8 (11.59%)
30-21	9 (13.4)	15 (21.73)	24 (34.78%)
40-31	7 (10.14)	2 (2.89)	9 (13.04%)
50-41	5 (7.24)	2 (2.89)	7 (%10.14)
>51	11 (15.94)	2 (2.89)	13 (18.84%)
NO%	40(%57.97)	29(%42.02)	69(100%)

X^2=26.41 found morale differentiation in the Table3.

Figure 1: Ethidium bromide-stained agarose gel PCR of genes examination of *P. mirabilis* have diagnosis gene 16S rRNA. Where an DNA molecular size marker M: Marker ladder 2000-100bp Lanes No. (1--24) isolates were positive for the gene(16rRNA) gene length of 305bp. The electrophoresis performed at 60 volt for 2 hr

In The present study results showed a higher proportion of the presence gene bla_{TEM} -177, has been in isolation for 21 bacteria *P.mirablis* by 87.50%, followed by gene bla_{TEM} -160 as Seen in isolation for 19 *P.mirablis* and bacteria by 59.3%, as shown in the figure 2, with the proportion of the presence of Jane blaTEM -160 bacterium P.mirablis record 0.85%. The resistance gene bla_{TEM} -1 has a presence in 18 *P.mirablis* isolated by (56.25%).

The proportion of gene blaTEM-1 64% shown in the figure 3. While the proportion of the presence of gene blaTEM-3 for *P.mirablis* reached the lowest rate of the enzyme-resistant type bla_{TEM} and by 6.25% shown in the figure 3. But gene blaTEM-89 has a presence in 10 isolates only *P.mirabilis* and increased by 31.25% shown in the figure 4. the results of the current study, the proportion of the presence of resistance gene blaTEM-72 in 18 isolated and by 56.25% shown in the figure 4, as the results of the present study was that the percentage of gene bla_{TEM}-159 in 17 isolated and by 53.13% shown in the figure 5.

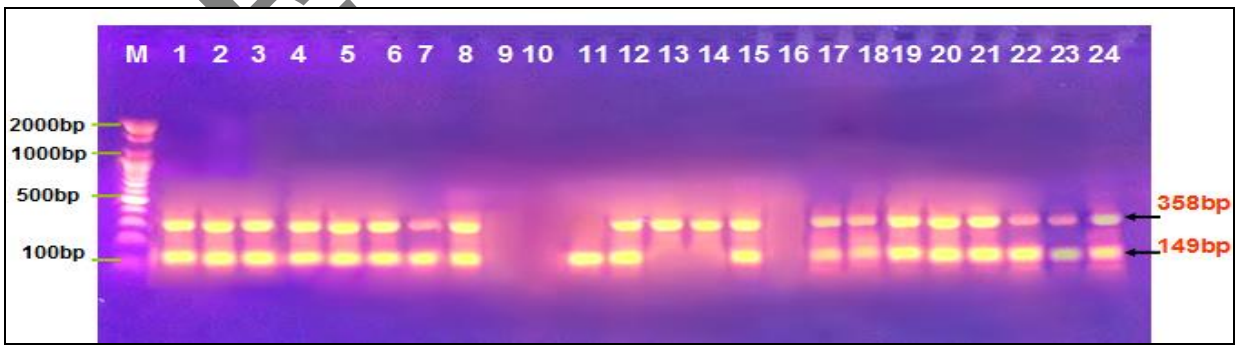

Figure 2: Ethidium bromide-stained agarose gel mPCR of genes examination of antibiotics Group Extended spectrum beta-lactamase genes in isolates of *P. mirabilis*. Where an DNA molecular size marker M: Marker ladder 2000-100bp Lanes No. (1-8 12-15 and 17-24) isolates were positive for the gene beta-lactamase (bla_{TEM}-177) gene length of 358bp. Lanes No. (1-8 11, 12, 15 and 17-24) isolates were positive for the gene (bla_{TEM} -160) gene length of 149bp. The electrophoresis performed at 60 volt for 2 hr

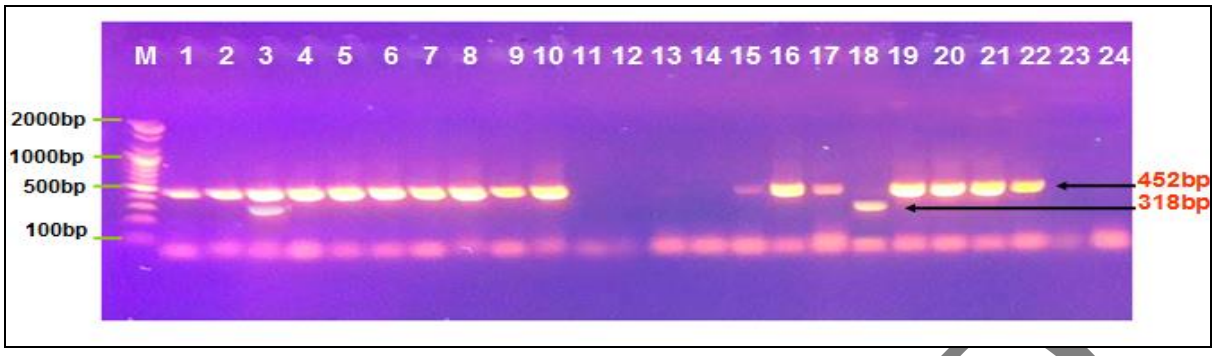

Figure 3: Ethidium bromide-stained agarose gel mPCR of genes examination of antibiotics Group Extended spectrum beta-lactamase genes in isolates of *P. mirabilis*. Where an DNA molecular size marker M: Marker ladder 2000-100bp Lanes No. (1-10 15-17 and 19-22) isolates were positive for the gene beta-lactamase (bla_{TEM}-1) gene length of 452bp. Lanes No. (3 and 18) isolates were positive for the gene (bla_{TEM} -3) gene length of 318bp. The electrophoresis performed at 60 volt for 2 hr

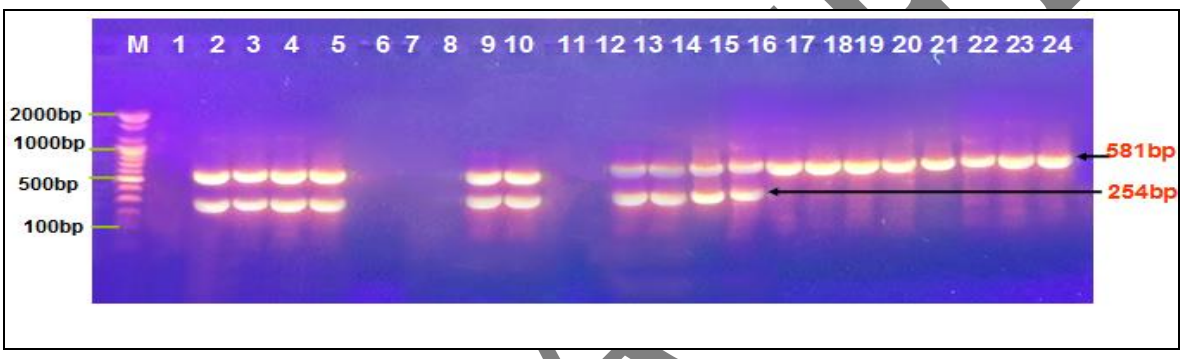

Figure 4: Ethidium bromide-stained agarose gel mPCR of genes examination of antibiotics Group Extended spectrum beta-lactamase genes in isolates of *P. mirabilis*. Where an DNA molecular size marker M: Marker ladder 2000-100bp Lanes No. (2-5 ,9,10, and 13-24) isolates were positive for the gene beta-lactamase (bla_{TEM}-72) gene length of 581bp. Lanes No. (2-5,9,10 and 13-17) isolates were positive for the gene (bla_{TEM} -89) gene length of 254bp. The electrophoresis performed at 60 volt for 2 hr

Figure 5: Ethidium bromide-stained agarose gel PCR of genes examination of antibiotics Group Extended spectrum beta-lactamase genes in isolates of *P. mirabilis*. Where an DNA molecular size marker M: Marker ladder 2000-100bp Lanes No. (1-8 11-13 and 17-15 ,21 and 22) isolates were positive for the gene beta-lactamase (bla_{TEM}-156) gene length of 637bp.The electrophoresis performed at 60 volt for 2 hr

Phylogenetic tree analysis to bla_{TEM} gene (DNA Sequencing)

Test yields 14 samples of isolates *P.mirabilis*. They took two isolation each gene of bla_{TEM} studied a bla_{TEM}-1, bla_{TEM}-3, bla_{TEM}-72, bla_{TEM}-89, bla_{TEM}-156 bla_{TEM}-160, bla_{TEM}-177 compared isolates of *P.mirabilis* resistant of β-lactamas antibiotics with worldwide origin *P.mirabilis*.

Table 4: Relationship between genes bla_{TEM} and sourace of *P.mirablis*

Source/Gene	Ear	Stool	Uirne	Burn	Blood
bla_{TEM}-177	6(5.7%)	6(5.7%)	5(4.7%)	3(2.8%)	1(0.9%)
bla_{TEM} -160	5(4.7%)	6(5.7%)	5(4.7%)	2(1.9%)	1(0.9%)
bla_{TEM} -72	7(6.6%)	3(2.8%)	4(3.8%)	3(2.8%)	1(0.9%)
bla_{TEM} -1	7(6.6%)	5(4.7%)	4(3.8%)	2(1.9%)	1(0.9%)
bla_{TEM} -156	7(6.6%)	4(3.8%)	4(3.8%)	1(0.9%)	1(0.9%)
bla_{TEM} -89	4(3.8%)	3(2.8%)	1(0.9%)	1(0.9%)	1(0.9%)
bla_{TEM} -3	1(0.9%)	1(0.9%)	0(0%)	0(0%)	0(0%)
Sum	37(35.2)	28(26.6%)	23(21.9%)	12(11.4%)	6(5.7%)

X^2=71.321 *found morale differentiation in the Table4

Table 5: Relationship between genes bla_{TEM} and the age

Gene/Age	blaTEM -177	blaTEM -160	blaTEM -1	blaTEM -72	blaTEM -156	blaTEM -89	blaTEM -3	SUM
10-Jan	4(3.80)	3(2.85)	2(1.90)	4(3.80)	2(1.90)	2(1.90)	--	17(70.83%)AC
20-Nov	5(4.76)	5(4.76)	2(1.90)	2(1.90)	4(3.80)	3(2.85)	1(0.95)	22(91.66%)AB
30-21	5(4.76)	4(3.80)	4(3.80)	5(4.76)	3(2.85)	2(1.90)	--	23(95.83%)B
40-31	5(4.76)	2(1.90)	1(0.95)	2(1.90)	1(0.95)	1(0.95)	--	12(50%)C
50-41	3(2.85)	3(2.85)	3(2.85)	2(1.90)	6(5.71)	2(1.90)	--	19(79.16%)ABC
>51	2(1.90)	2(1.90)	6(5.71)	3(2.85)	1(0.95)	1(0.95)	1(0.95)	16(66.66%)C

X^2=18.996 *found morale differentiation in the Table 5

Table 6: Relationship between genes bla_{TEM} and the gender

Gender/Gene	Female	Male	Sum
blaTEM-177	12(11.42)	9(8.57)	21(20)
blaTEM -160	6(5.71)	13(12.38)	19(18.09)
blaTEM -72	8(7.61)	10(9.52)	18(17.14)
blaTEM -1	13(12.38)	5(4.76)	18(17.14)
blaTEM -156	11(10.47)	6(5.71)	17(16.19)
blaTEM -89	7(6.66)	3(2.85)	10(9.52)
blaTEM -3	1(0.95)	1(0.95)	2(1.90)
Sum	58(55.23%)	47(44.76%)	105 (100%)

X^2=22.271 *found morale differentiation in the Table 6

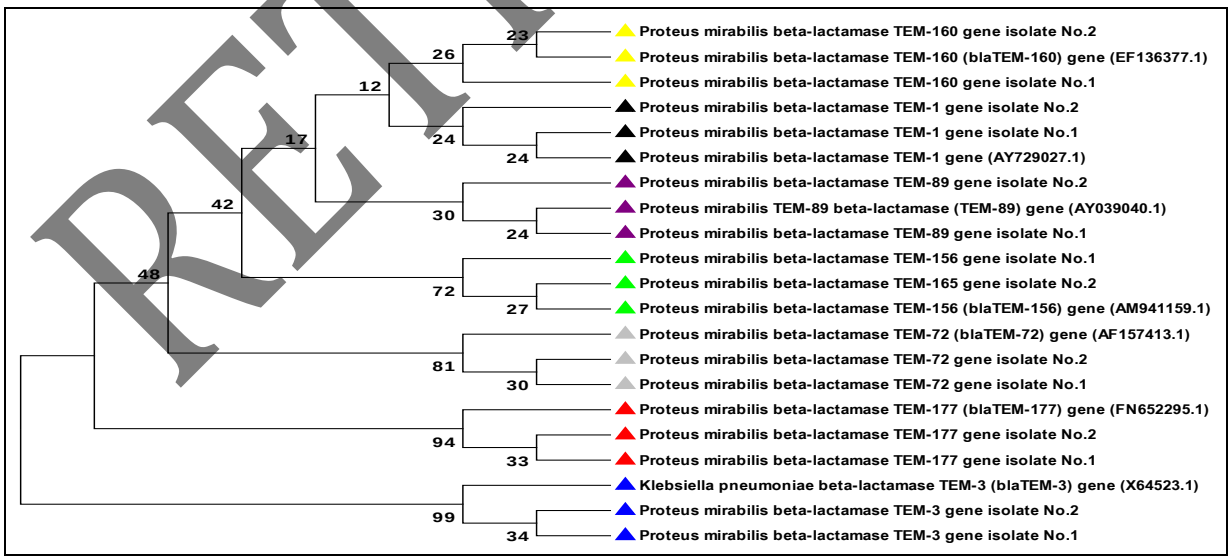

Figure 6: Analyzed Phylogenetic tree genetic tree analysis by Mega6 program, were used genetic tree analysis of the type (Unweighted Pair Group Method) UPGMAtree Showed

In the Figure 7 nor differences between the sequences gene bla_{TEM} local isolates *P.mirabilis* and bla_{TEM} global isolates *P.mirabilis* But there are differences between the variants of the gene TEM, example when comparing bla_{TEM}-89 record in the beginning of the Figure 7 referred to three number with a different bla_{TEM}- 72, But when comparing bla_{TEM}-89 and blaTEM-3 non-existent bacteria *P.mirabilis* so compared with *Klebsiella pneumona* because it's nearst sequence and that bacteria intestinal contain the same sequences of enzymes resistance to it from the method to acquire through plasmids passed on between the same family. So during the study recorded for the first time gene bla_{TEM}-3 in bacteria *P.mirabilis* at the local level in the city of Diwaniyah, in the global level in the bacteri. And do not record this gene in the bacteria in GenBank . blaTEM-3 presence P.mirabilis this gene in two isolation only, one the source ear infection while the second isolation from a patient have 18 year old person with a case of bacteremia due to bacteria *P.mirabilis* in the city of Diwaniyah. different this gene for blaTEM-89 and by 2.88 the first isolation and by 2.91 second in isolation either standard isolation by 7.69. This shows that the absence of a new and dangerous types of the community in the city of Diwaniyah and bacteria *P.mirabilis* despite being seen everywhere in hospitals in Diwaniya and although resistant multiple to antibiotics and but do not exposed to mutations in the genes of resistance to antibiotics treatment and reducing infection.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. Proteus mirabilis beta-lactamase TEM-89 gene isolate No.1																					
2. Proteus mirabilis beta-lactamase TEM-89 gene isolate No.2	0.00																				
3. Proteus mirabilis TEM-89 beta-lactamase (TEM-89) gene (AY039040.1)	0.00	0.00																			
4. Proteus mirabilis beta-lactamase TEM-72 gene isolate No.1	0.01	0.01	0.01																		
5. Proteus mirabilis beta-lactamase TEM-72 gene isolate No.2	0.01	0.01	0.01	0.00																	
6. Proteus mirabilis beta-lactamase TEM-72 (blaTEM-72) gene (AF157413.1)	0.01	0.01	0.01	0.00	0.00																
7. Proteus mirabilis beta-lactamase TEM-3 gene isolate No.1	2.42	2.42	2.88	2.82	2.82	2.82															
8. Proteus mirabilis beta-lactamase TEM-3 gene isolate No.2	2.47	2.47	2.91	2.85	2.85	2.85	0.00														
9. Klebsiella pneumoniae beta-lactamase TEM-3 (blaTEM-3) gene (X64523.1)	3.56	3.53	7.69	4.78	4.77	7.70	0.00	0.00													
10. Proteus mirabilis beta-lactamase TEM-177 gene isolate No.1	0.02	0.02	0.02	0.01	0.01	0.01	2.69	2.72	3.45												
11. Proteus mirabilis beta-lactamase TEM-177 gene isolate No.2	0.02	0.02	0.02	0.01	0.01	0.01	2.70	2.73	3.49	0.00											
12. Proteus mirabilis beta-lactamase TEM-177 (blaTEM-177) gene (FN652295.1)	0.02	0.02	0.01	0.01	0.01	0.01	2.79	2.83	7.04	0.00	0.00										
13. Proteus mirabilis beta-lactamase TEM-160 gene isolate No.1	0.01	0.01	0.01	0.01	0.01	0.01	2.79	2.94	4.93	0.02	0.02	0.02									
14. Proteus mirabilis beta-lactamase TEM-160 gene isolate No.2	0.01	0.01	0.01	0.01	0.01	0.01	2.80	2.95	4.77	0.02	0.02	0.02	0.00								
15. Proteus mirabilis beta-lactamase TEM-160 (blaTEM-160) gene (EF136377.1)	0.00	0.00	0.00	0.01	0.01	0.00	2.79	2.83	6.92	0.01	0.01	0.01	0.00	0.00							
16. Proteus mirabilis beta-lactamase TEM-156 gene isolate No.1	0.01	0.01	0.01	0.01	0.01	0.01	2.85	2.89	5.43	0.02	0.02	0.02	0.01	0.01	0.01						
17. Proteus mirabilis beta-lactamase TEM-156 gene isolate No.2	0.01	0.01	0.01	0.01	0.01	0.01	2.86	2.89	5.38	0.02	0.02	0.02	0.01	0.01	0.01	0.00					
18. Proteus mirabilis beta-lactamase TEM-156 (blaTEM-156) gene (AM941159.1)	0.01	0.01	0.01	0.01	0.01	0.01	2.86	2.89	8.29	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00				
19. Proteus mirabilis beta-lactamase TEM-1 gene isolate No.1	0.00	0.00	0.01	0.01	0.01	0.01	2.93	2.93	4.65	0.02	0.02	0.01	0.00	0.00	0.01	0.01	0.01	0.01			
20. Proteus mirabilis beta-lactamase TEM-1 gene isolate No.2	0.00	0.00	0.01	0.01	0.01	0.01	2.93	2.93	4.65	0.02	0.02	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00		
21. Proteus mirabilis beta-lactamase TEM-1 gene (AY729027.1)	0.01	0.01	0.01	0.01	0.01	0.01	2.88	2.91	8.28	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.00	

Figure 7: measure the genetic differences in the genetic sequence of bases between the genes of beta-lactamase $_{TEM}$ genes in the local *P.mirabilis* in Diwaniyah city and compared with the beta-lactamase TEM genes in the *P. mirabilis* recorded in the world

DISCUSSION

According to Isolation and Identification of *P mirabilis* in this study agree [11] with a percentage 45%. Also The result of current study didn't agree with [12]. According to the gender that nearst with[13] and didn't agree with [14] belong the infection - to bacterial may be due to the fact that extreme ages [children and seniors] and especially the elderly who that have from chronic diseases and those taking medications immunosuppressive, they are more exposed to infection because of a weakened immune system for these age groups compared to other age groups [15]. The enzymes bla_{TEM} of more enzymes beta-lactamase prevalent that goes back to Class A, which consists of three main groups of enzymes [, bla OXA, blaCTX-M, blaSHV blaTEM], but is blaTEM of the main kinds and these enzymes most likely found in *E.coli* and *K .pneumoniae* and *P.mirabilis* [17]. gene bla_{TEM} -160 in this study are consistent with the researcher[18], with the proportion of the presence of blaTEM -160 bacterium *P.mirablis* record 0.85%. The resistance gene bla_{TEM} -1 has converged these results with the results of with [19] .In another study in the South China conducted by [20] recorded bla_{TEM} -1 at a higher rate than the current study 78%. gene blaTEM-3 for *P.mirablis* record the gene bla_{TEM} -3 in 1987 as the first different type of enzymes the blaTEM where he owns an increase in potency against cephalosporin antibiotics broad-spectrum[21]. But gene bla_{TEM}-89 consistent with the researcher [22], gene blaTEM-72 as the person researcher [23] The results of the DNA Sequencing analysis, there is a clear Identify of isolates of *P.mirabilis* blaTEM local isolates in Diwaniyah city with worldwide origin *P.mirabilis* compared with the rest of the other species that appeared.

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