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ORIGINAL ARTICLE

The Phylogenetic Analysis of Biofilm Producing, Multi-Drug Resistant P.Aeruginosa Isolated from Different Clinical Cases

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ABSTRACT

To perform this study, out of 32 Pseudomonas aeruginosa isolates, about 20 multi-drug resistant isolates were selected, diagnosed firstly depending on their cultural, microscopic, and biochemical characteristics, then the diagnoses had been confirmed through 16s rRNA gene.

The Kirby-Bauer method of antibiotic sensitivity test were performed, by using an eleven common antibiotics, and the results were as the following, the resistance ratios of Ampicillin and Amoxicillin were 100%, which is mean that all isolates were resistant to these two antibiotics, the resistance ratio reached 90% for the Pipracillin, the resistance ratio of Cefepime and Ceftazidime was 70%, the resistance ratio of Ciprofloxacin was 65%, the resistance ratios of Amikacin and Tobramycin were 45%, 40%, respectively, the resistance ratio of Aztreonam was 35%, the resistance ratio of meropenem was 20%, finally, the resistance ratio of Imipenem was 10%, that represent the lowest resistance ratio, so in this study Imipenem was the most efficient antibiotic.

The phylogenetic tree analysis showed the following results, for the gene 16S rRNA, there is a great similarity between our two local isolates and other global isolates, the phylogenetic tree analysis of the gene algD showed a low similarity when compared with the global isolates, while the phylogenetic tree analysis of the gene pelF showed both high and low similarities, whereas the degree of similarity between our two local isolates and the global isolates for the gene pslD ranged between 79% and 99%.

INTRODUCTION

P.aeruginosa is a widely distributed bacteria that can be isolated from different environments like soil, water, air, animals, and humans (1). These bacteria can live at minimal nutritional environments such as the dry surfaces in the operating rooms, medical equipment, drainage basins, and distilled waters (2). P.aeruginosa is an opportunistic pathogen that is rarely affects healthy people (3), it is usually causes infections in an immunocompromised people, such as patients with AIDS, malignant tumors, and diabetes mellitus (4). The risk of the infections with this bacteria came from their high resistant to conventional antibiotics (5). It has a natural resistance against many different antibiotics, and the number of its multi-drug resistant strains is continually increasing all over the word (6), this is mainly due to a low permeability of their outer membrane (7), and the expression of different kinds efflux pumps (8) in addition to it's high ability to develop a chromosomal resistance mutations against wide variety of antibiotics, especially when there is a high concentration of those antibiotics (9).

The high virulence, metabolic flexibilities, physiologic adaptation abilities, as well as a multi-drug resistance characteristics, all of these features makes the eradication of this bacteria became so difficult (10), this is forced World Health Organization (WHO) to put it on the top of the pathogens that need to discover and develop a new treatment methods (11). And because of the large variety of antibiotics used to treat the infections of P.aeruginosa, this study came to find out the most effective antibiotics, as well as to determine the prevalence of these bacterial strains by comparing them with the global isolates.

MATERIALS AND METHODS

Isolation and diagnosis of bacterial isolates: About 100 clinical sample were collected, 32 bacterial isolates were P.aeruginosa, out of these 20 isolate were selected for this study, which were chosen because of their multidrug resistance nature. They were diagnosed basing on their cultural, microscopic, and biochemical characteristics according to Forbes et al (12), then the diagnosis confirmed by 16S rRNA.

Antibiotic sensitivity test: The Twenty isolates of P.aeruginosa were subjected to antibiotic sensitivity test of disc diffusion method (Kirby-Bauer), and the following antibiotics had been used (Ampicillin, Amoxicillin, Pipracillin, Cefepime, Ceftazidime, Aztreonam, Meropenem, Imipenem, Tobramycin, Amikacin, and Ciprofloxacin) which are the most commonly used drugs. The inhibition zones were measured and compared with CLSI standard values (13).

A phylogenetic tree analysis for some isolates: The PCR products of the genes 16S rRNA, algD, pslD, and pelF for some isolates were sent to Macrogen company in South Korea, and a phylogenetic trees for these isolates were designed.

RESULTS AND DISCUSSION

The genetic diagnosis of P.aeruginosa isolates based on the gene 16S rRNA: After electrophoresis, the results of 16**S** rRNA gene detection showed that, this gene is present in all P.aeruginosa isolates (100% percent), with 1500 base pairs (bp) size, figure (1). These results are in agreement with the results of Abdullah and Mahdi (14). The detection of 16S rRNA gene is one of the most accurate diagnostic methods, because it is one a housekeeping genes that distinguish each bacterial species from others, and it does not change over time. The 16S rRNA sequences also used in studying the evolutionary relationships between different strains of a same species, and it is a perfect criterion in bacterial classification (15).

Figure 1: electrophoresis results of 16S rRNA. using 1.5% concentration of agarose, and 70 volt for an hour. The column M represent DNA ladder, while the columns (1-20) are the bacterial isolates.

Multi- drug resistance patterns of P.aeruginosa isolates: All isolates included in the current study showed a multiple resistance pattern, meaning that they were a multi-drug resistant by the ratio of 100%, this is close related to the results of Kashfi et al (16), since their percentage of Multi-drug resistance was 94%. The ratios of multiple resistance was as the following, the resistance of four antibiotics was 10%, the resistance of five antibiotics was 5%, the resistance of six antibiotics was 35%, the resistance of seven antibiotics was 30%, and the resistance of eight antibiotics was 20%. Table (1).

Table 1: the numbers and ratios of multi-drug resistant P.aeruginosa

Numbers of the isolates	Numbers of antibiotics resisted by the bacteria	Resistance ratios
2	4	10%
1	5	5%
7	6	35%
6	7	30%
4	8	20%

As for antibiotics, all isolates included in this study were resistant to Ampicillin (AMP) (Ampicillin belongs to amino penicillin antibiotics group), meaning that the resistance percentage to Ampicillin was 100%, this is completely identical to (17). The resistance percentage to Amoxicillin (AMC) was 100% also, this is completely matched the result of (18).

The resistance percentage to Pipracillin (PRL), (which belongs to Ureido-Penicillins group), was 90%, this result agreed with (19), where she recorded 88.46% resistance of this antibiotic in burn patients, and 100% resistance of the environment surrounding these patients.

The resistance percentage to Ceftazidime (CAZ), (which belongs to the cephalosporins third generation), was 70%, this is close related to (17), as she recorded 69% percent.

The resistance percentage to Cefepime (FEP), (which belongs to the cephalosporins fourth generation), was 70%, this is close related to the Indian study (20), where a resistance rate of 63%. had been recorded.

The resistance percentage of our P.aeruginosa isolates to Aztreonam, (which belongs to the Monobactams group), was 35%, this is agreed with (21) where a resistance rate of 32.2%. had been recorded.

The resistance percentage to meropenem (MER), (which belongs to the Carbapenems group), was 20%, this is exactly resemble to (22) in Turkey, where he obtained a resistance rate of 20.1%.

There are only two isolates showed a resistance activity against Imipenem (IMI) antibiotic (which belongs to the Carbapenems group also), so the resistance rate was 10%, this is agreed with (23) who recorded 6.2% percent.

P.aeruginosa isolates in our study showed 40% resistance to Tobramycin (TOB), (it belongs to aminoglycosidic antibiotics), our result agreed with (24), who recorded 42.4% resistance to this antibiotic in his study.

The resistant percentage to Amikacin (AMK), in our study reached 45%, this is close related to (25), where a resistance rate of 47.3% had been recorded.

The resistant percentage to Ciprofloxacin (CIP) was 65%, this is identical to (26) in a study conducted in Uganda, where the resistance rate to this antibiotic was 64%.

The Phylogenetic tree analysis of the gene 16S rRNA: After their sequence investigation, the following codes were given to our six isolates of P.aeruginosa; (ON413673), (ON413674), (ON413675), (ON413676), (ON413677), and (ON413678), these isolates had been recorded in GenBank. And after caring out a phylogenetic tree analysis of the gene 16S rRNA for these six isolates, the results in fig (2), table (2), showed high affinity-ratios between our local isolates and a global isolates, where the similarity ratio between the isolate (ON413673) and both of Indian (HM439972) and Pakistani (KM017986)) isolates was 99.64%, which represent the highest percentage, while the lowest ratio was 97.41%, between our local isolate (ON413675) and each of Indian (LC487882), Indonesian (MA598019), and a two Chinese (MK607451), (MN911373) isolates. The remaining ratios were in between.

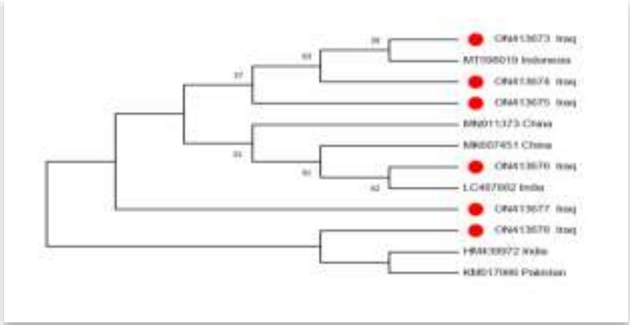

Figure 2: The phylogenetic tree analysis of the gene 16s rRNA, for six P.aeruginosa isolates

Table 2: DNA sequencing and similarities ratios of the gene 16S rRNA

Global isolates		Indonesia	China	China	India	India	Pakistan
Local isolates		MA598019	MN911373	MK607451	LC487882	HM439972	KM017986
1	ON413673	99.287	99.287	99.287	99.287	99.6438	99.6435
2	ON413674	98.8393	98.8393	98.8393	98.8393	98.3929	98.3036
3	ON413675	97.4153	97.4153	97.4153	97.4153	97.8629	97.861
4	ON413676	98.0392	98.0392	98.0392	98.0392	98.3957	98.3957
5	ON413677	98.9305	98.9305	98.9305	98.9305	99.3767	99.4652
6	ON413678	97.9483	97.9483	97.9483	97.9483	98.4835	98.4835

The Phylogenetic tree analysis of the gene algD, which encodes for the alginate Exopolysaccharides: The results in the table (3) showed that, the similarity between our (ON715703), (ON715702) and the global isolates was low in most cases, where the similarity ratio between the isolate (ON715703) and each of Belgian (CP051547.1), American (CP042269.1), and German (CP050148.1) isolates was 24.6% , while similarity ratio between the isolate (ON715702) and each of Belgian (CP051547.1), Korean (CP030910.1), Chinese (CP061376.1), and German (CP050148.1) isolates was 27.9%, but there is a perfect matching between the American isolate (CP089067.2) with the tow local isolates (ON715702) and (ON715703) in ratios of 100% and 99.8% respectively, fig (3).

The Phylogenetic tree analysis of the gene pelF which encodes for the pellicle Exopolysaccharides: The results shown in the table (4) showed that, the highest affinity-ratio was 96.7%, between two Chines isolates (CP077988.1), (CP045552.1) and our local isolate (ON715704), while the lowest similarity ratio 28.24%, was between our isolate (ON715705) and each of Russian (CP051770.1), German (CP093013.1), Colombian (CP095774.1), and Australian (CP053117.1) isolates, fig (4).

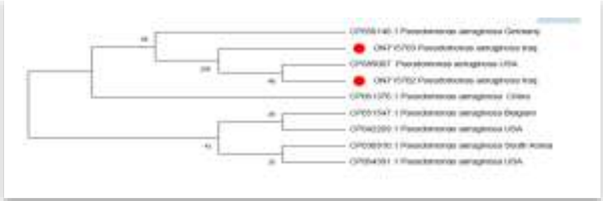

Figure 3: The phylogenetic tree analysis of the gene algD, for tow P.aeruginosa isolates

Table 3: DNA sequencing and similarities ratios of the gene algD

The global Genes of NCBI	The origin	Affinity ratios with ON715702 local isolate	Affinity ratios with ON715703 local isolate
CP051547.1	Belgium	27.9126	24.6206
CP042269.1	USA	27.9126	24.6206
CP030910.1	South Korea	27.9126	24.6206
CP061376.1	China	27.9126	24.6206
CP064391.1	USA	27.4272	24.2833
CP089067.2	USA	100	99.8314
CP050148.1	Germany	27.9126	24.6206

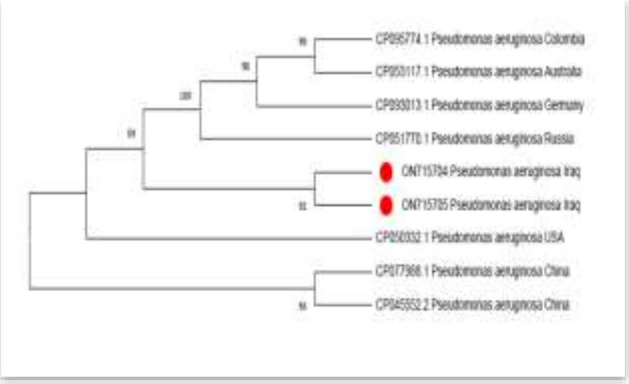

Figure 4: The phylogenetic tree analysis of the gene pelF, for tow P.aeruginosa isolates

Table 4: DNA sequencing and similarities ratios of the gene pelF

The global Genes of NCBI	The origin	Affinity ratios with ON715704 local isolate	Affinity ratios with ON715705 local isolate
CP050332.1	USA	95.6701	95.4639
CP051770.1	Russia	28.4536	28.2474
CP093013.1	Germany	28.4536	28.2474
CP095774.1	Colombia	28.4536	28.2474
CP077988.1	China	96.701	96.4948
CP045552.2	China	96.701	96.4948
CP053117.1	Australia	28.4536	28.2474

The Phylogenetic tree analysis of the gene psID, which encodes for the PSL Exopolysaccharides: The results in table (5) showed that, the highest similarity ratio was 98.87%, between our two isolates (NO715706.1), (NO715707.1) and both of American (CP050322.1) and Colombian (CP041774.1) isolates, while the lowest similarity ratio 79.06%, was between our previously mentioned isolates and the British isolate (LR134300.1), other similarities ratios were in between, fig (4).

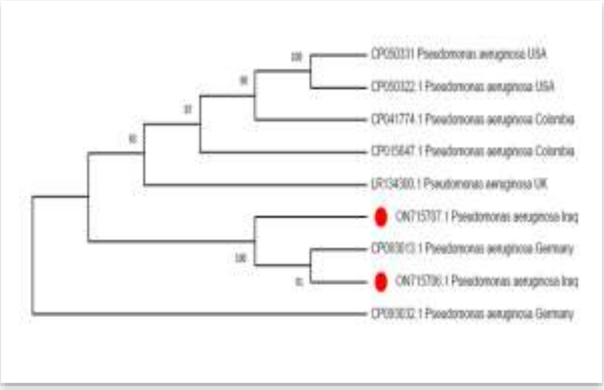

Figure 5: The phylogenetic tree analysis of the gene psID, for tow P.aeruginosa isolates

Table 5: DNA sequencing and similarities ratios of the gene psID

The global Genes of NCBI	The origin	Affinity ratios with NO715706.1 local isolate	Affinity ratios with NO715707.1 local isolate
CP050331	USA	88.877	88.877
CP050322.1	USA	98.877	98.877
CP041774.1	Colombia	98.877	98.877
CP015647.1	Colombia	88.877	88.877
LR134300.1	UK	79.0667	79.0667
CP093013.1	Germany	97.6	94.4
CP093032.1	Germany	88.877	88.877

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