Genotypic characterization and novel multilocus sequence types of *exoU+ Pseudomonas aeruginosa* from different clinical infections and environments

Caracterización genotípica y nuevos tipos de secuencia multilocus de *exoU+* de *Pseudomonas aeruginosa* presente en diferentes infecciones clínicas y entornos

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ABSTRACT

Introducción: The *exoU* gene, a marker for highly virulent strains of *Pseudomonas aeruginosa*, is the major contributor to a wide variety of healthcare-associated infections. **Methods:** In this study, the antibiotic susceptibility profile, prevalence and genotyping of *exoU+ P. aeruginosa* were demonstrated. A total of 101 isolates of *P. aeruginosa* were analysed from different clinical and environmental sources. **Results:** The antibiotic susceptibility profile classified these isolates as extensively drug resistant (35.6%), multidrug resistant (40.5%) and non-multidrug resistant (23.7%). The prevalence of *exoU* gene was screened by PCR and 23 *exoU+* genotypes were detected which all were clinical isolates. Multilocus sequence typing (MLST) analysis of seven loci assigned these *exoU+* genotypes to 21 sequence types (STs) from which 16 new STs were identified. The prevalent STs were ST-308 and ST-235. Phylogenetic analysis using the concatenated nucleotide sequences of the seven housekeeping genes, exoU and the ITS region differentiated these *exoU+* strains into five main groups. However, distinct evolutionary origins for some new sequence types were also indicated. **Conclusions:** The studied isolates showed the coexistence of *exoU-* and *exoU+* genotypes of clinical *P. aeruginosa* in Kurdistan with a majority of MDR and XDR pattern. The prevalent STs found in other hospitals worldwide and at the international level.

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INTRODUCTION

The Gram-negative bacterium *Pseudomonas aeruginosa* is ubiquitous and widespread microorganism in a variety of environments such as soil, water and on hosts[1]. In spite of its wide spread in nature and the potential to cause community-acquired infections, this opportunistic pathogen is predominantly associated with hospital-acquired infections, including respiratory infections, bacteremia, skin and soft tissue infections, osteomyelitis, urinary tract infections, gastroenteritis, otitis and eye infections[2, 3, 4]. The severity of *P. aeruginosa* infections is further complicated in immunocompromised patients or patients who are requiring mechanical ventilation[5]. It has also been proven that *P. aeruginosa* has an extraordinary capacity to easily develop powerful resistance to almost all commercially available antibiotics either by the acquisition of horizontally transferred resistance genes or by mutation in chromosomally encoded genes[6, 7]. These make infections caused by this pathogenoften difficult to treat and frequently life threatening due to its multidrug-resistant (MDR) phenotypes[8, 6].

The pathogenesis of this bacterium is multifactorial; multiple and diverse determinants of virulence contribute to the wide range of diseases caused[9]. *P. aeruginosa* possesses type III secretion system (T3SS) as a complex macromolecular machinery to inject and intoxicate host cells with up to four effector proteins; ExoS, ExoT, ExoY and *ExoU*[10]. The *exoU*gene encodes the highly cytotoxic T3SS effector *ExoU*[11]. This toxin has a phospholipase A2 activity that is highly cytotoxic to a range of eukaryotic cells; ExoU disrupts cell membranes following its delivery into the cytoplasm and causes rapid celldeath[12]. It has been reported that *exoU* gene is present in cytotoxic strains of *P. aeruginosa*, while non-cytotoxic strains lack this particular gene[13], making the presence of *exoU*a marker to identify highly virulent strains of this bacterium[14]. The production of ExoUis associated with increased mortality and disease severity, and has been recognized as a major detrimental virulence factor in acute illnesses[13].

The precise characterization of species in clinical settings may have consequences in diagnosis, antimicrobial therapy, and infection control policies[15]. To assess the genetic micro- and macro-variations used in epidemiological investigations, the genetic relatedness among pathogenic isolates must be established by phylogenetic- and population-based analysis[16]. Multilocus sequence typing (MLST), as a genotyping method based on the nucleotide sequence analysis and the allelic differences among seven house-keeping genes (*acsA, aroE, guaA, mutL, nuoD, ppsA,* and *trpE*), has been widely used for genetic diversity and epidemiological investigation of *P. aeruginosa*[17, 18]. Although it relies only on the evaluation of the core genome diversity, it is a robust, standard and portable technique that has been proven to be a valuable tool for analyzing the epidemiology of bacterial infection, as it is highly discriminating and provides insight into genetic structure of infected populations [19, 20, 21]. It also provides a comprehensive database that allows researchers to compare the results obtained from different sample types in different locations[22].

It has been previously reported that *P. aeruginosa* accounted for 26.1% of the total nosocomial infections in Kurdistan[23]. A higher prevalence has been reported by studies elsewhere in Iraq, such as 46.6% in Najaf[24] and 67.7% in Basrah[25]. However, to our knowledge, there is no comprehensive investigation or previous report from Kurdistan investigating the prevalence and genotype properties of*exoU*+ *P. aeru-*

ginosa isolates in this region. Therefore, in this present study we performed a molecular identification and genotyping analysis of *P. aeruginosa* isolates obtained from soil and a range of clinical infections at Duhok, Erbil and Sulaymaniyah hospitals to study the antibiotic resistant pattern, prevalence and genotype characterization of highly virulent strains of *P. aeruginosa* in Kurdistan.

MATERIALS AND METHOD

Bacterial strains

A total of 101 strains of *P. aeruginosa* were collected from both clinical and environmental sources. Most of these were clinical isolates (n=87) which were obtained from hospitalized patients (both sexes and from different ages) in different wards at three major public hospitals in Kurdistan namely; Azadi-Hospital in Duhok, Rizgari Hospital in Erbil and General Medicine Hospital in Sulaymaniyah. The clinical isolates were collected from variousinfection sources; including burn, middle ear, wound, respiratory tract (sputum and bronchial alveolar lavage) as well as urine samples. In contrast, the environmental isolates (n=14) were collected from soil samples of some animal farms at the above districts.

Isolation and identification of P. aeruginosa strains

The isolates of *P. aeruginosa* were initially identified using selective agar culture media according to the protocol described by Khan *et al.*,[26] with some modifications. Samples were cultured in brain heart infusion broth at 37°C for 24 hours. Then, each sample was streaked on MacConkey agar and incubated for 24 hours at 37°C. A single suspected colony that showed the characteristic appearance was selected and plated onto the Cetrimide agar plate, incubated overnight at 42°C. Isolates were primarily identified as *P. aeruginosa* if they showed growth at 42°C. Colonies were purified and transferred onto Pseudomonas Isolation Agar and incubated for 24 hours at 37°C. A single purified colony was then inoculated into nutrient broth and incubated for 24 hours at 37°C and then kept in 50% glycerol and stored at -20°C until used.

Antibiotic sensitivity testing

Screening for antibiotic resistance was performed by the Kirby Bauer disc diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines[27]. Fourteen of commercially available antibiotic discs (Oxoid, Basingstoke,UK); including Ciprofloxacine (5µg), Piperacillin (100µg), Imipenem (10µg),Amikacin (30µg), Ceftazidime (30µg), Chloramphenicol (30µg), Doxycycline (30µg), Gentamicin (10µg), Erythromycin (15µg), Cefotaxime (30µg), Amoxacillin/Clavulanic Acid (30/10µg), Ticarcillin (75µg), Cefixime (5µg) and Tetrcyclin (30µg) were used against all isolates. Isolates that produced resistant to at least three antibiotics from different antibiotic classes were defined as multidrug-resistant, whereas isolates remain susceptible to only one or two antibiotics from all antimicrobial categories were identified as extensively drug-resistant[28].

Genomic DNA isolation and PCR amplifications

Genomic DNA was extracted from all 101 strains using High yield DNA Purification Kit as described in the supplier's manual (Cinnagen-Iran). *P. aerugino-*

sa isolates were identified at both genus and species level by duplex PCR[29] with minor modifications. The complete *exoU* gene was amplified, and sequenced (sequence data not included), using primers *exoU*-F (5'-ATGCATATCCAATCGTTGG-'3) and *exoU*-R (5'-CTAGCAATGGCACTAATCG-'3)[30]. PCR amplifications were carried out using 200 µL PCR tube with a reaction mixture of 25 µL. Each of the reaction mixtures contained 10X PCR buffer, 2.5mM each of dATP, dCTP, dGTP and dTTP (Fermentas), 1.5 mMolL^{-1} of MgCl2, 1 U of *Taq* polymerase (Fermentas), 10pmol µL-1 of each primers, 25-50ng of bacterial DNA and milli-Q water up to 25µl. PCR withthe following cycling parameters was performed:Initial denaturation at 94°C for 2min; 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min and extension at 72°C for 2min; and a final extension at 72°C for 7min. The internally transcribed spacer (ITS) region,located between the 16S and 23S rRNA genes, was amplified using primers PA1 5'-GCCCGTCACACCATGGGAG-'3 and PA2 5'-TCGCCTSTGRRGCCAAGGC-'3 as previously described[31] and amplification reaction mixture described above. PCR program was as follow:initial denaturation of 2 min at 94°C followed by 30 cycles of 1 min at 94°C, 45s at 68°C, and 1 min at 72°C, and a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis on a 1.2% (W/V) agarose gels, stained with ethidium bromide and visualized by UV transilluminator.

Cloning and sequencing of ITS region

The PCR products were purified using QIAquick PCR purification Kit (Qiagen). The Purified PCR products then cloned into pGEM-T Easy Vector System I according to the manufacture's manual (Promega) and the ligation mixture added to DH5α competent cells. Colony PCR was performed to confirm the presence of target insert. The positive clones were subjected to plasmid extraction and sequencing.

Selection, amplification and sequencing of the loci

The housekeeping genes *acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*(Table: 2) have been selected according to the MLST scheme for *P. aeruginosa*(http://pubmlst.org/paeruginosa/). MLST was performed according to the protocol published by Curran *et al*[22] with minor modifications. The housekeeping genes were amplified using the amplification reaction mixture described above with adding PWO *Taq* polymerase. The reaction conditions were: denaturation at 94°C for 1 min, annealing at 58-60°C for 1 min, and extension at 72°C for 1 min for 35 cycles with a final extension at 72°C for 5min. The amplified PCR products were purified with MinElute PCR Purification Kit (50) (Qiagen), according to the manufacture's protocol. Sequencing was carried out with internal nested primers as described at the http://pubmlst.org/paeruginosa/info/primers.shtml website. The purified PCR products were sequenced using a 48 capillary ABI 3730 Genetic analyser for Sanger sequencing in DNA Sequencing facility at-The University of Manchester-UK.

Sequence data analysis

The forward and reverse sequences were imported, assembled, edited, trimmed and verified in Geneious, version R8.1[32] and then saved in Fasta format. Positions in which gaps were present in any of the aligned sequences were excluded from the analysis. All sequences were confirmed by individual BLAST

searches to determine whether the corresponding data matched well with the appropriate gene of *P. aeruginosa*. The individual genes were estimated under best-fit models of nucleotide substitution selected with jModel Test[33].The phylogenetic analyses performed by using GARLI web service hosted at molecularevolution.org[34] using GARLI 2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). The partitioned model was selected, which allowed partitioning of the data into six subsets, namely *acsA-trpE*, *aroE-guaA*, *mutL-exoU*, *nuoD*, *ppsA* and *ITS*, each of which were assigned separate evolutionary models, namely TPM1uf+I, HKY+I, TrN+I, HKY+G, HKY, and TPM1+I, respectively.Confidence intervals for bootstrap probabilities based on 1000 replicates. The correlation between genetic distances with geographical distances and infection sites were investigated using Mantel test in R (mantel.test). The significance threshold was set at 0.05.

Allele and sequence type assignment

The *Pseudomonas aeruginosa* MLST database at (http://pubmlst.org/paeruginosa) was used to assign numbers to each distinct allele within a locus. Thus, each individual isolate was given seven numbers known as allele profile that yielded to its sequence type (ST). Any allele that did not match with existing alleles in the database was designated as a "new" allele. Any sequence type that did not match with the existing database allele combination was numbered as a "new" sequence type. Isolates that share five or more identical alleles (single-or double-locus variants) were considered part of the same clonal complex[26][3].

Nucleotide sequence accession numbers

The nucleotide sequences determined in the current study were submitted to GenBank. The accession numbers for the ITS region, *exoU* gene, *acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*-genes are KX641417- KX641439, KX641440 - KX641462, KX773949 - KX773971, KX773972 - KX773994,KX773995 - KX774017, KX774018 - KX774040,KX821688-KX821710, KX774041 - KX774063 and KX774064 - KX774086, respectively.

RESULTS AND DISCUSSIONS

In this study, the bacteriological results revealed that a total of 101 isolates of *P. aeruginosa* were identified out of 523 specimens tested, representing 19.3% of the total samples. According to their sources, 87 were clinical and 14 were environmental isolates. All *P. aeruginosa* isolates that were identified by selective agar culture media, biochemical and microscopic tests, were confirmed at the genus and species level by 16S rDNA based PCR assay. Among the two sets of primers used in a duplex PCR, the genus-specific primer pair (PA-GS) amplified an amplicon of 618 bp corresponding with special band of *Pseudomonas* genus and the species-specific primer pair (PA-SS) yielded an amplicon of 956 bp, corresponding with a special band of *P. aeruginosa* in all the isolates tested. Thus, all the isolates were confirmed at the molecular level as *P. aeruginosa* by PCR.

The results of antibiotic susceptibility for all isolates were as follows: 18.81% (imipenem), 26.73% (ceftazidime), 39.60% (amikacin), 42.57% (ciprofloxacin), 44.55% (cefotaxime), 49.50% (chloramphenicol), 58.41% (gentamicin), 64.35% (cefixime),

69.30% (piperacillin), 71.28% (ticarcillin), 73.26% (amoxacillin/clavulanic acid), 82.17% (erythromycin), 84.15% (tetracycline) and 95.04% (doxycycline). Furthermore, 23.76% were found to be non-drug resistant isolates, 35.64% were extensively drug resistant (XDR) isolates and 40.59% were found to be multidrug-resistant (MDR) strains.These results indicate that patients infected by *P. aeruginosa* in this region are in high antibiotic resistance risk. Such high resistance rate could be explained by prior exposure to different types of antibiotics and in empirical combination therapy. Also, the exceedingly using of these antibiotics, spread of resistance strains, influx of low quality and/or not always WHO prequalified antibiotics into the markets as well as self-medications might be the main reasons behind such high antibiotic resistant rates.

The results of the prevalence of *exoU* gene revealed that 23 out of 87 clinical isolates were *exoU*+ genotypes representing 26.4% of the total clinical isolates (Figure: 1). This result was similar to previous survey (25.4%) carried out byGarey *et al.*,[14] in USA and slightly higher than that reported by Bradbury *et al.*,[35] in Tasmania which was18.0%. However, in our analysis, this rate was markedly lower than other studies conducted in Iran by Jabalameli *et al.*,[36], Yousefi-Avarvand *et al.*,[37] and Azimi *et al.*, (38) who showed 64.5%, 65.4% and 52.0% of *exoU* positive genotypes, respectively. This lower prevalence rate of horizontally transferred*exoU*virulence gene[39] in our studymight be due to the less clonal diversity of the studied isolates in Kurdistan. Furthermore, it was observed that all *exoU*+ *P. aeruginosa* were exclusively from clinical sources and not in any of the environmental isolates (Figure 1). However, this result is in contrast with the studies conducted by Bradbury *et al.*,[35] and Streeter *et al.*,[40] who reported the presence of *exoU*gene in both environmental and clinical strains. The reason for this difference is unclear; it may reflect the genuine differences between *P. aeruginosa* populations isolated from different geographical sites. However one must be cautious since false negatives can be generated through a PCR based analysis [30]. What is clear is that the presence of*exoU* gene is a variable trait in *P. aeruginosa*[38].

The distribution of *exoU*+ isolates according to their geographical locations showed that the highest number (n=14 out of 23) of *exoU*+ isolates were detected in Sulaymaniyah, whereas, the lowest number (n=1 out of 23) were from Erbil (Figure 1), and there was a statistically significant difference between the locations and the prevalence of the *exoU* virulence gene carriage (χ2d.f.=2, N=101, p = 0.0054). Furthermore, according to their infection sources, results revealed that*exoU*+ isolates were identified from nearly all infection sites (Figure 1). However, no statistically significant differences were observed between the infection sites and the prevalence of *exoU* virulence gene (χ2d.f.=4, N=101, p = 0.878). This is mostly might be due to the fact that *exoU*+ strains have the ability to cause acute infections in almost all types of human infections, as ExoU has been shown to acquire cytolytic activity to different cell types such as epithelial cells, macrophages and fibroblasts[4].

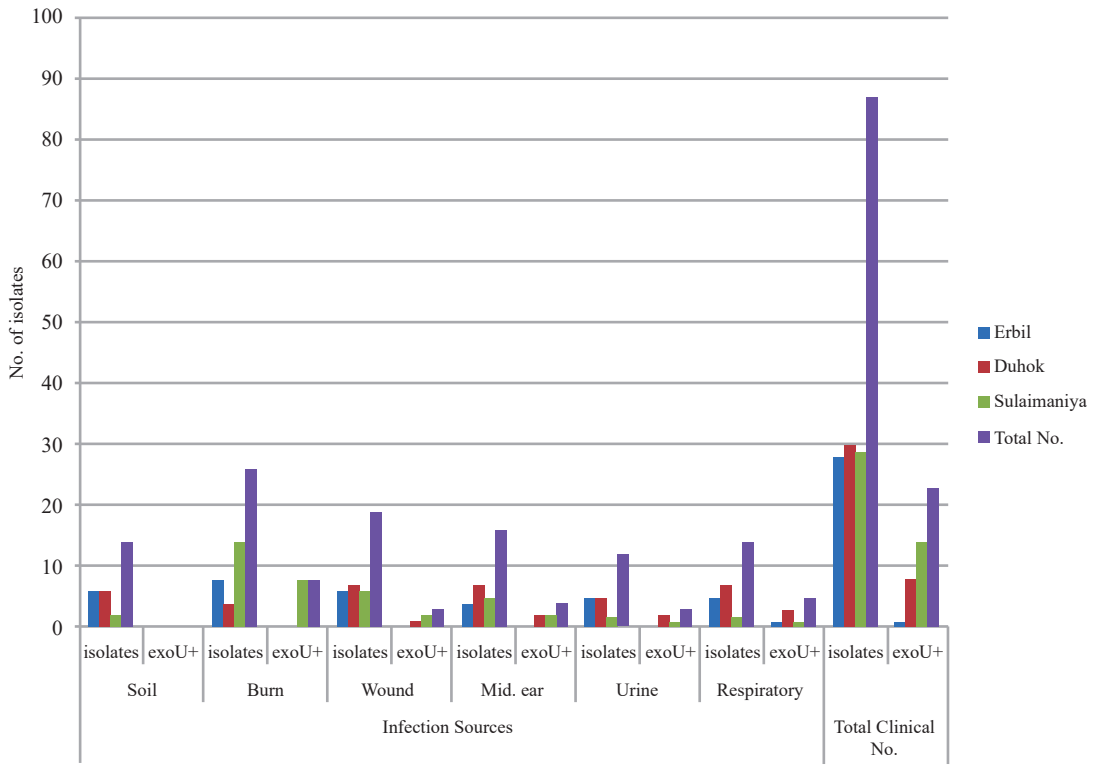

Figure 1. Distribution of the *exoU+ P. aeruginosa* isolates according to their locations and source of infections.

Genotyping analysis was performed for all the *exoU+* isolates using MLST. The whole-gene sequence data for the seven loci obtained from PCR amplification primers, MLST fragment lengths data obtained from MLST nested primers and their variable sites are given in Table 1. The allelic sequences obtained have been searched for in the *P. aeruginosa* MLST database at (http://pubmlst.org/paeruginosa). All allele sequences were found in the database and none of the seven genes exhibited new alleles. The number of alleles per each loci were varied and ranged from six (*ppsA*) to 12 (*aroE*). The number of polymorphic sites obtained from MLST fragment lengths was generally low and ranged between five (*ppsA*) to 19 (*aroE*) in all studied loci (Table 1). This result is comparable with that obtained by[20] who reported an overall low number of polymorphic sites and low values of the nucleotide diversity per site. The ratio of non-synonymous to synonymous nucleotide changes (dN/dS) was also calculated for all 7 loci (Table 1) and found to be between 0.054% (*trpE*) and 2.69% (*aroE*). A ratio of dN/dS<1 suggests that genes are predominantly evolving by purifying selection[20].

Table 1. Analysis of the sevenloci of the *exoU+ P. aeruginosa* isolates.

	alleles	Length (bp)	No. of variable sites	Length (bp)	No. of variable sites	% Variable	
acsA	10	810	41	390	17	4.35	0.094
aroE	12	852	32	495	19	4.24	2.69
guaA	7	843	33	372	7	1.88	1.83
mutL	9	702	60	441	11	2.49	0.123
nuoD	9	798	64	366	15	4.09	0.343
ppsA	6	880	32	369	5	1.62	0.864
trpE	8	774	24	441	16	3.62	0.054
ITS	-	659	9	-	-	-	-

In addition, if a strain possessed a new allelic combination profile, at least one allele different among the seven examined genes, was classified as a new sequence type. The 23 *exoU+* strains were assigned to 21 STs from which 16 new STs were identified. The allele combinations of all new sequence types obtained were submitted and deposited in the *P. aeruginosa* MLST database at (http://pubmlst.org/paeruginosa) and ST numbers were given by the database. The allelic profiles for each genes analysed and their corresponding ST numbers are given in Table 2. To our knowledge, this is a first report investigating *P. aeruginosa* sequence types in Kurdistan.

Table 2. Infection source, location, MLST allelic profiles and sequence types of *exoU+ P. aeruginosa* isolates.

Isolate	Source	Location	Allele Profile							ST	Note
			acs	*aro*	*gua*	*mut*	*nuo*	*pps*	*trp*		
Pa-11	Urine	Duhok	11	8	9	3	1	6	9	2195	New
Pa-12	Respiratory	Duhok	13	4	5	5	12	7	15	308	
Pa-14	Burn	Sulaymaniyah	38	11	3	13	1	2	193	2196	New
Pa-16	Middle Ear	Sulaymaniyah	13	4	5	5	12	7	15	308	
Pa-17	Urine	Sulaymaniyah	38	11	3	13	1	2	4	235	
Pa-18	Respiratory	Sulaymaniyah	38	11	3	13	1	2	4	235	
Pa-19	Wound	Duhok	9	13	3	6	64	33	42	1632	
Pa-20	Burn	Sulaymaniyah	11	5	1	65	4	4	10	1516	
Pa-21	Burn	Sulaymaniyah	38	11	3	65	13	2	10	2197	New
Pa-22	Wound	Sulaymaniyah	32	52	5	85	13	6	3	2198	New
Pa-23	Burn	Sulaymaniyah	38	11	3	13	22	2	3	2199	New
Pa-24	Burn	Sulaymaniyah	5	61	79	11	25	7	124	2200	New
Pa-25	Burn	Sulaymaniyah	38	11	3	13	91	2	3	2201	New
Pa-26	Respiratory	Duhok	13	75	5	5	12	7	15	481	
Pa-27	Burn	Sulaymaniyah	38	11	3	13	1	2	3	2202	New
Pa-28	Middle Ear	Duhok	87	4	114	13	53	38	3	2203	New
Pa-29	Respiratory	Erbil	17	32	5	13	4	4	3	2204	New
Pa-30	Burn	Sulaymaniyah	38	176	3	13	13	2	3	2205	New
Pa-31	Wound	Sulaymaniyah	38	150	3	11	13	2	3	2206	New
Pa-32	Respiratory	Duhok	82	75	5	133	13	7	3	2207	New
Pa-33	Middle Ear	Sulaymaniyah	38	150	3	11	12	2	3	2208	New
Pa-34	Middle Ear	Duhok	6	196	11	11	4	4	193	2209	New
Pa-35	Urine	Duhok	13	52	9	138	13	6	3	2210	New

All new STs except three were found to exhibit either MDR or XDR pattern. Furthermore, ST235 (which

is globally known as a high risk clone[41]) was detected among two isolates typed in Sulaymaniyah. Also, ST308 (which is associated with different acquired β-lactamases, particularly IMP and VIM MBLs[42]) was identified in Duhok and Sulaymaniyah. The ST235 and ST308 in our hospitals were found to be highly drug-resistant and *exoU*+ genotypes. This might further confirm and provide evidence for the existence of multidrug-resistant global clones, denominated high-risk clones, circulating in hospitals worldwide. Different studies have been shown that ST235 (belongs to CC235) are commonly associated with the dissemination of particular resistance genes especially those conferring resistance to clinically im

portant β-lactam antibiotics[43, 3]. Detection of these STs that exhibited XDR and highly virulent properties in our hospitals clearly represent one of the most challenging pathogenic strains of *P. aeruginosa* since ST235 strains are not only highly virulent and XDR which make them difficult to treat, but also potent vectors for rapidly spreading and evolution of complex resistance loci. Therefore, monitoring of MDR- and *exoU* gene-carrying isolates has epidemiological significance in the identification of drug-resistant and highly virulent *P.aeruginosa* isolates, especially in high-risk patients.

The internal transcribed spacer (ITS) located between the 16S and 23S rRNA genes, which is not subjected to the same selective pressure as the rRNA genes and the housekeeping genes, show more variations and consequently have a 10-times-greater evolution rate. As such they have been widely used for phylogenetic analysis, typing and evolutionary studies[44]. Therefore in order to allow better discrimination and greater tree robustness, the sequences of the seven housekeeping genes, *exoU* and the ITS region for each strain were concatenated and used to construct an unrooted phylogenetic tree among these strains.Thegenotypes were differentiated into five main groups in the phylogenetic tree supported by bootstrap values (Figure 2), i.e., Group I (which includes PA29, PA20), Group II (PA24, PA19), Group III (PA18, PA17), Group IV (PA35, PA32, PA34, PA33) Group V (PA16, PA12, PA26). However, 10 isolates, which were all assigned as new STs, were not clustered with any of these groups and found to be unrelated, sharing few if any alleles. This might suggest distinct evolutionary origins for each of these new STs in Kurdistan.

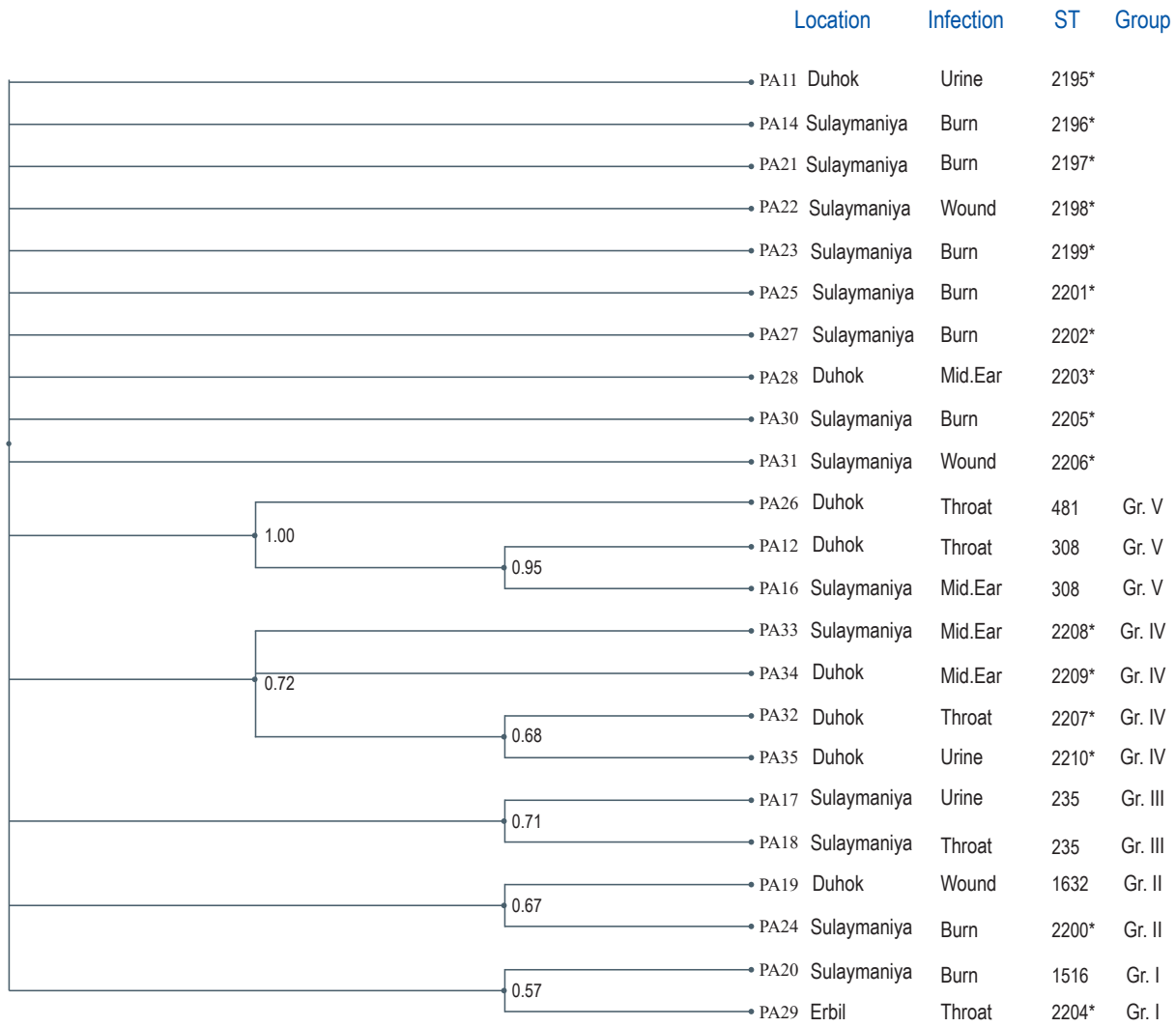

Figure 2. Phylogenetic relationship of the clinical *exoU+ P. aeruginosa* isolates.
Asterisk marks (*) indicate new sequence types described in this study.

The genetic distances of these isolate wereranged from 0.001- 0.019 substitution per site for all the pairwise comparisons made. In regard to compare few kb out of 6Mbp of *P. aeruginosa* genome, this result might indicates a mix of closely related isolates which have a recent common ancestorand some more distantly related strains among the tested strain collection. Furthermore, results of Mantel tests showed significant correlations between isolates and their geographical area (p= 0.027), suggesting that isolates from the same geographical location are closer to each other than isolates from different geographical areas.However, no statistically significant correlations were found between genetic distance and infection sites (p= 0.065). Results suggest that there is no particular correlation between the genetic background of these isolates and their specific infection sites. This result is compatible with *exoU* data discussed before and might further confirm the reliability of MLST as a valuable tool for typing and epidemiological investigations.

CONCLUSIONS

Strains of *P. aeruginosa* isolated from different geographical and infection sites displayed variations in their allelic profiles and STs which may reflect their adaptive ability to different environmental niches

and diversity of infections and diseases caused. Isolates showed the coexistence of *exoU*- and *exoU*+ genotypes of clinical *P. aeruginosa* in Kurdistan. Patients infected by *P. aeruginosa* in this region are in high antibiotic resistance risk due to the prevalence of high risk clone of*P. aeruginosa*. Therefore, precise identifications and characterization of this bacterium in clinical settings and establishing the genetic relatedness among its highly pathogenic strains are crucial for the purposes of long-term epidemiological investigations and may have positive consequences in diagnosis, antimicrobial therapy, and infection control policies.

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