RESEARCH ARTICLE

Molecular characterization of *Pseudomonas aeruginosa* isolates from Sudanese patients: A cross-sectional study

[version 1; peer review: 1 approved with reservations, 1 not approved]

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V1 First published: 25 Jul 2018, 7:1135
<https://doi.org/10.12688/f1000research.15316.1>
 Latest published: 25 Jul 2018, 7:1135
<https://doi.org/10.12688/f1000research.15316.1>

Abstract

Background: 16S rRNA gene sequence analysis is a robust tool for characterization of new pathogens in clinical specimens with suspected bacterial disease. The aim of this study was to characterize *Pseudomonas aeruginosa* isolated from clinical specimens by sequencing the 16S rRNA gene.

Methods: Forty bacterial isolates were obtained from different clinical specimens (wound, urine and sputum) using enrichment selective media and biochemical tests to characterize and identify the bacteria as *P. aeruginosa*. DNA was extracted from *P. aeruginosa* using the Chelex method. A universal primer was used to amplify 16S rRNA genes by a conventional PCR technique. The amplified PCR products were sequenced, and the sequences were viewed by Finch TV program version 1.4.0. The identity and similarity of the nucleotide sequence of the isolated strains was detected by comparing them with published sequences using BLASTn. Phylogenetic trees were constructed using Phylogeny.fr software.

Results: Sequence analysis by BLASTn displayed high similarity and identity with *P. aeruginosa* from China KX461910, Australia JN609194 and with other *P. aeruginosa* isolates from the GenBank database.

Conclusions: Our observation of isolates from different origin sites, further show the utility of 16s rRNA PCR amplification. This reveals the high specificity of the primers and accuracy of the PCR. Thus, 16S rRNA sequencing can be used to identify genetically atypical *P. aeruginosa*

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Approval Status ? X

	1	2
version 1 25 Jul 2018	? view	X view

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isolates from different origins.

Keywords

Pseudomonas aeruginosa, 16S rRNA, Alignment, PCR, phylogenetic tree, Blast, Sudan.

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Author roles: **Amoon RH:** Investigation, Methodology, Writing – Original Draft Preparation; **Abdallha AH:** Investigation, Methodology; **Sharif AO:** Investigation, Methodology; **Moglad EH:** Formal Analysis, Supervision, Writing – Original Draft Preparation; **Altyb HN:** Conceptualization, Supervision, Writing – Review & Editing; **Elzaki SG:** Supervision; **Salih MA:** Project Administration

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Amoon RH, Abdallha AH, Sharif AO *et al.* **Molecular characterization of *Pseudomonas aeruginosa* isolates from Sudanese patients: A cross-sectional study [version 1; peer review: 1 approved with reservations, 1 not approved]** F1000Research 2018, 7:1135 <https://doi.org/10.12688/f1000research.15316.1>

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Introduction

Pseudomonas aeruginosa is a gram-negative bacterium that is found widely in the environment and engages in various forms of interactions with eukaryotic host organisms. It is an opportunistic pathogen that is widely spread in humans, giving rise to a broad spectrum of infections in community and healthcare facilities^{1,2}. Due to the extended spread of *P. aeruginosa* habitat, the control of the organism in a hospital setting is very difficult, and makes it practically impossible to prevent contamination³. The major threat is the infection of patients who are immunocompromised or those in burns, neonatal and cancer wards^{4,5}. Infection of *P. aeruginosa* is still one of the main causes of death among the critically ill and patients with impaired immune systems in spite of the development of newer and stronger antibiotics.

Sequencing of 16S rRNA worldwide has provided interesting and useful information⁶⁻⁹. For instance, with the use of 16S rDNA sequencing, 215 novel bacterial species, 29 of which belong to novel genera, have been discovered from human specimens in the past 7 years of the 21st century (2001–2007). One hundred of the 215 novel species, 15 belonging to novel genera, have been found in four or more subjects¹⁰. In Sudan, there is deficient data on sequencing about bacteria; therefore it is important to investigate what kinds of bacteria affect Sudanese people. Consequently, the objective of this study was to isolate and characterize *P. aeruginosa* from samples obtained from Sudanese patients by sequencing the 16SrRNA gene.

Methods

Clinical isolates

This was cross sectional laboratory based study, conducted in Khartoum state in the period from January to April 2016. The study was approved by the Ethical and Scientific Committee of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center of Research, Khartoum, Sudan (approval number 03-16) which ensures that all ethical considerations for conducting the research in a way that protects patient's confidentiality and privacy are followed. Informed consent was obtained from the hospital laboratories (laboratory manager) after providing them with the ethical approval to collect samples during routine procedures from the microbiology laboratories. Participants' privacy and confidentiality was protected for all samples; personal information was not of great value in the current study and was thus not taken. Consequently, the Ethical and Scientific Committee waived the need for patient consent.

A total of 40 isolates of *P. aeruginosa* (all samples available) were obtained from three hospitals in Khartoum State (Al Ribat Hospital, Bahri Hospital and Souba Hospital).

The bacterial isolates were collected from sputum culture, urine culture and wound infection because these types of cultures were dominant. Standard biochemical tests⁷ were performed on all samples for the isolation and identification of the bacterial isolates and were performed at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI),

Department of Microbiology. *P. aeruginosa* presence was confirmed in all 40 samples. All media required for biochemical tests were obtained from LAB M, UK and Laboratories Flow Media, Sudan.

DNA extraction

Bacterial DNA was isolated by the Chelex-based protocol⁷ for all samples without deviation from the methodology.

Conventional polymerase chain reaction (PCR)

All bacterial genomic DNA were used as templates for PCR amplification of the 16S rRNA gene. The two primers used were 27F (5' AGAGTTTGATCCTGGCTCAG-3') and 1495R (5' CTACGCTACCTTGTTACGA- 3') for forward primer and reverse primer, respectively (Macrogen, South Korea). The 25µL PCR reaction mixture (Intro Biotechnology, South Korea) contained 1µL DNA, 1x reaction buffer (10x) with 3mM MgCl₂, 2.5U i-Taq™ DNA polymerase (5 U/µL), 2.5mM dNTPs, 1µL of 10 pmol of each primer, and 1x of gel loading buffer, followed by completing the volume to 25µL with nuclease free water. Thermal cycling conditions were as follows: 94°C for 5 min; 30 cycles of denaturation (94°C for 1 min), annealing (58°C for 1 min), extension (72°C for 2 min); final extension at 72°C for 10 min. PCR was performed on a Bio-Rad (DNA engine/Dyad Peltier) automatic thermal cycler. Duplicate PCR of every sample were carried out for confirmation^{11,12}. PCR products (5µl) were analyzed by gel electrophoresis in 1.0% agarose stained with ethidium bromide. The results were photographed under ultraviolet light machine (Transilluminator; Uvite, UK) to detect the specific amplified product by comparing it with 100 base pairs standard DNA ladder (Figure 1) and the remains from PCR products were store at -20°C until sequencing.

Sequencing of 16S rRNA gene

Isolates were packaged according to the International Air Transport Association guidelines and shipped with authorized permission to Macrogen Company (Seoul, South Korea). Purification and standard forward sequencing of 16S rRNA were done by ABI Genetic Analyzer (Applied Biosystems). We randomly selected 20 isolates only for DNA sequencing due to the limitation of resources.

Figure 1. Agarose gel electrophoresis of PCR products after amplification of the 16S rRNA gene. Lanes: 1-8 positives 16SrRNA gene molecular weight marker (O'Range Ruler 100 DNA Ladder, SM1143-Fermentas).

Bioinformatics analysis

The chromatogram sequences were visualized using Finch TV program version 1.4.0¹³. The nucleotide sequences of the 16S rRNA gene were searched for sequences similarity using online BLASTn¹⁴. Highly similar sequences (accession numbers KX108935.1, KT943978.1, JN609194.1, KX214108.1, KU672378.1, KU764451.1 and FJ648815.1) were retrieved from NCBI GenBank and subjected to multiple sequence alignment using BioEdit software version 7.2.5. Newick format was withdrawn from ClustalW¹², to create phylogenetic trees in Phylogeny.fr software¹⁵. MEGA6 software version 0.06 was used for confirmation of phylogenetic trees¹⁶.

Results

A total of 40 clinical isolates were identified as *P. aeruginosa* by conventional methods, including growth characteristics, colony morphology, and biochemical tests. The results revealed that *P. aeruginosa* were dominant in wound infection cultures

(42.5%), followed by (32.5%) from urine cultures and (25%) from sputum cultures.

The 16SrRNA in the isolates was amplified by PCR. In total, 20 were sent for characterization by sequencing of PCR products, 5 out of these 20 had a clear chromatogram, leading to further sequence analysis. Sequence analysis by BLASTn revealed 100% identity with *P. aeruginosa* from Iran (KX108935.1), China (KT943978.1), Australia (JN609194.1), Austria (KX214108.1), India (KU672378.1) and South Africa (KU764451.1), and slightly different from a sample from Australia (FJ648815): there was one substitution of A to G in position 148 (Figure 2). The phylogenetic tree of the 16S rRNA gene and other 16S rRNA genes obtained from the database revealed that the tree is classified into two branches. All isolates have a common ancestor. The isolate 120 is at the upper branch, isolate 113 and isolate 114 are sister groups, as shown in Figure 3.

Figure 2. BioEdit multiple sequence alignment of the five isolates and other selected strains. The isolates had 100% identity with samples from Iran, China, Austria, India, and South Africa. There was one substitution of A to G in position 148 from a sample from Australia (FJ648815).

Figure 3. Phylogenetic tree of the 16S rRNA gene and other 16S rRNA genes. The tree is classified into two branches. All isolates have a common ancestor. The isolate 120 is at the upper branch, isolate 113 and isolate 114 are sister groups.

Discussion

P. aeruginosa can cause various clinical infections. In the present study, 42.5% of isolates were identified from wound infection. Beside the highly preserved conserved primer binding sites, 16S rRNA gene sequences include hypervariable regions with high conservation that have the ability to characterize species-specific signature sequences beneficial to the characterization of bacteria^{17,18}. The present results agree with Didelot *et al.*, who reported that 16S rRNA gene sequencing is now common in medical microbiology as a quick and inexpensive alternative to phenotypic approaches of bacterial identification¹⁹. We investigated the phylogenetic affiliation of the pseudomonads and reexamined the 16S rRNA sequence data available in public databases. According to an alignment of 16S rRNA sequences, we identified *P. aeruginosa*-specific signature sequences.

16S rRNA sequencing can be used to identify genetically atypical *P. aeruginosa* isolates from different origins. Our observation of 40 isolates from 40 samples, further showed the utility of 16S rRNA PCR amplification. This reveals the high specificity of the primers and accuracy of the PCR. Sequencing of the 16S (rRNA) gene was used as an effective tool for determining phylogenetic relationships between bacteria. The features of this molecular target make it valuable and useful for bacterial detection and identification in the clinical laboratory.

Conclusions

In conclusion, 16S rRNA-based PCR assay and sequencing is highly specific, sensitive and reliable for identification of

P. aeruginosa and its differentiation from other genotypic closely related *Pseudomonas* species. DNA sequencing of the 16S rRNA gene has been used as an effective tool to study bacterial phylogeny and taxonomy relationships between bacteria and for bacterial detection.

Data availability

The results of the nucleotide sequences of the 16S rRNA gene were submitted in the GenBank database. Accession numbers: [KX650648](#), [KX650649](#), [KX650650](#), [KX650651](#) and [KX650652](#).

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgments

The authors are grateful to Africa City of Technology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Microbiology and Parasitology department, National Center for Research, Khartoum, Sudan and department Epidemiology, Molecular Epidemiology Laboratory Biology, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan for their kind support.

References

- Driscoll JA, Brody SL, Kollef MH: **The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections.** *Drugs*. 2007; **67**(3): 351–368.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Talbot GH, Bradley J, Edwards JE Jr, *et al.*: **Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America.** *Clin Infect Dis*. 2006; **42**(5): 657–668.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Davies J: **Inactivation of antibiotics and the dissemination of resistance genes.** *Science*. 1994; **264**(5157): 375–382.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Khalil MA, Ibrahim Sonbol F, Mohamed AF, *et al.*: **Comparative study of virulence factors among ESBL-producing and nonproducing *Pseudomonas aeruginosa* clinical isolates.** *Turk J Med Sci*. 2015; **45**(1): 60–69.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Empel J, Filczak K, Mrówka A, *et al.*: **Outbreak of *Pseudomonas aeruginosa* infections with PER-1 extended-spectrum beta-lactamase in Warsaw, Poland: further evidence for an international clonal complex.** *J Clin Microbiol*. 2007; **45**(9): 2829–2834.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Poussier S, Vandewalle P, Luisetti J: **Genetic diversity of african and worldwide strains of *Ralstonia solanacearum* as determined by PCR-restriction fragment length polymorphism analysis of the *hrp* gene region.** *Appl Environ Microbiol*. 1999; **65**(5): 2184–2194.
[PubMed Abstract](#) | [Free Full Text](#)
- Lozupone CA, Knight R: **Global patterns in bacterial diversity.** *Proc Natl Acad Sci U S A*. 2007; **104**(27): 11436–11440.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Clindworth A, Pruesse E, Schweer T, *et al.*: **Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies.** *Nucleic Acids Res*. 2013; **41**(1): e1.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hugenholtz P, Pace NR: **Identifying microbial diversity in the natural environment: a molecular phylogenetic approach.** *Trends Biotechnol*. 1996; **14**(6): 190–197.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Woo PC, Lau SK, Teng JL, *et al.*: **Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories.** *Clin Microbiol Infect*. 2008; **14**(10): 908–934.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vilgaly R, Hester M: **Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species.** *J Bacteriol*. 1990; **172**(8): 4238–4246.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cocolin L, Rantsiou K, Iacumin L, *et al.*: **Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods.** *Appl Environ Microbiol*. 2004; **70**(4): 1883–1894.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ozdilek A, Cengel B, Kandemir G, *et al.*: **Molecular phylogeny of relict-endemic *Liquidambar orientalis* Mill based on sequence diversity of the chloroplast-encoded *matK* gene.** *Plant Syst Evol*. 2012; **298**(2): 337–349.
[Publisher Full Text](#)
- Seth-Smith HM, Rosser SJ, Basran A, *et al.*: **Cloning, sequencing, and characterization of the hexahydro-1,3,5-Trinitro-1,3,5-triazine degradation gene cluster from *Rhodococcus rhodochrous*.** *Appl Environ Microbiol*. 2002; **68**(10): 4764–4771.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Boriss R, Chen XH, Rueckert C, *et al.*: **Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM 7^T and FZB42^T: a proposal for *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* subsp. nov. and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on complete genome sequence comparisons.** *Int J Syst Evol Microbiol*. 2011; **61**(Pt 8): 1786–1801.
[PubMed Abstract](#) | [Publisher Full Text](#)

16. Osman NAM, Alrayah IE, Mohamed YM, *et al.*: **Molecular study of Pantone-Valentine Leukocidin genes among *Staphylococcus aureus* clinical isolates in Khartoum State, Sudan.** *Am J Microbiol Res.* 2015; 3(3): 107–111.
[Reference Source](#)
17. Adékambi T, Colson P, Drancourt M: ***rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria.** *J Clin Microbiol.* 2003; 41(12): 5699–5708.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Guasp C, Moore ER, Lalucat J, *et al.*: **Utility of internally transcribed 16S-23S rDNA spacer regions for the definition of *Pseudomonas stutzeri* genomovars and other *Pseudomonas* species.** *Int J Syst Evol Microbiol.* 2000; 50(4): 1629–1639.
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Didelot X, Bowden R, Wilson DJ, *et al.*: **Transforming clinical microbiology with bacterial genome sequencing.** *Nat Rev Genet.* 2012; 13(9): 601–612.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Version 1

Reviewer Report 02 January 2019

<https://doi.org/10.5256/f1000research.16686.r42041>

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Juan Alfonso Ayala Serrano

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Manuscript deals with the characterization of *Pseudomonas* clinical isolates by PCR and 16S rRNA gene sequencing. Only twenty isolates were used for sequence comparison, and actually only five sequences are compared by BLAST and deposited on Genbank DATABASE. Authors claim that sequencing of 16S rRNA gene is useful for *Pseudomonas* identification, but this fact has been shown in hundred of papers. It is not a novelty. Moreover, no other molecular analysis is done with the isolates, as for example, identification of resistance mechanisms. In addition, isolates were already identified as *Pseudomonas aeruginosa* by biochemical methods. A more useful analysis would be to take the full number of isolates and without previous identification, characterize them as *P. aeruginosa*.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Microbiology. Peptidoglycan characterization, Antibiotic resistance, betalactamases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 29 October 2018

<https://doi.org/10.5256/f1000research.16686.r39641>

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Giuseppe Comi

Department of Food Science, University of Udine, Udine, Italy

The paper at the moment is only a description of a group of *Pseudomonas aeruginosa*. The strains come from different areas, the genomic study was made by a Korean lab. What did the authors want to say or to communicate with the paper?

The paper is interesting, and gives additional data about the *P. aeruginosa* species. However :

- Reading the paper I cannot find the aim and the meaning of the work. It would be better to explain the aim of the paper.
- It would be better to explain the aim of the paper.
- Additional literature to explain the aim and the meaning of the work must be included.
- What is the meaning of the results?
- How can the results be used?
- Material and methods must be better written.
- What did the authors do? The collected data and applied a software and no other skills.
- The conclusion must be improved.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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