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# Design of primers for evaluation of some gram-negative bacteria isolated from intensive care unit patients in Wasit province, Iraq

Rana Essa Muslem and Rana H. Raheema *

*Department of Medical Microbiology, Faculty of Medicine, University of Wasit, Iraq.*

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## Abstract

Intensive care unit (ICU) patients frequently have consequences from healthcare-associated infections (HAIs), which include bacteremia, pneumonia, urinary tract, skin, or soft tissue infections. . In this study, a total of 100 clinical specimens (urine, sputum and pus) were collected from patients admitted in the ICU. Results showed eighty two were positive growth culture and admitted to the intensive care unit distributed 1 (1.2%) *Klebsiella oxytoca,* 5(6.25%)*Proteus mirabilis,* 2 (2.4%) *Acinetobacter baumannii* , 1 (1.2%) *Serratia marcescens* and 1 (1.2%) *Burkholderia cepacia group* bacteria . The results of this study showed that the diagnostic test for *16S rRNA* bacteria (*Proteus mirabilis*), which numbered 5 (100%), bacteria (*Acinetobacter baumannii*), which numbered 2 (100%), bacteria (*Burkholderia cepacia* ), which numbered 1 (100%), bacteria (*Serratia marcescens* ), which numbered 1 (100%), and Bacteria (*klebsiella oxytoca* ), which numbered 1 (100%) after completing the phenotypic and biochemical diagnosis diagnostic test (*16S rRNA*).

**Keywords:** 16S rRNA; *Acinetobacter baumannii*; *Proteus mirabilis*; *Serratia marcescens*; *Klebsiella oxytoca*; *Burkholderia cepacia* complex; Intensive care unit

## 1. Introduction

Intensive care unit (ICU) patients frequently have consequences from healthcare-associated infections (HAIs), which include bacteremia, pneumonia, urinary tract, skin, or soft tissue infections [1]. The ICU personnel and doctors may act as conduits for the transfer of germs from other inpatient units to ICUs [2]. Therefore, HCWs and ICU staff must maintain the highest levels of hand cleanliness. The ICU environment's contamination also has a significant impact on how patients and HCWs pick up nosocomial bacteria [3]. The gram-negative bacteria that recorded in the intensive care unit are Enterobacteriaceae, including *Acinetobacter baumannii, Proteus mirabilis, Serratia marcescens, klebsiella oxytoca,* and *Burkholderia cepacia complex* [4]. Determining the spread of gram negative bacteria from intensive care unit patients in Kut City, Wasit Province, Iraq and characterizing it at the molecular level through analyses of *16S rRNA* genes.

## 2. Material and methods

A cross-sectional study was done in the intensive care unit (ICU) of the Alzahraa , Alkarama hospitals from the 3rd October 2021 to 20th February 2022 . A total of 100 clinical samples, including: urine, sputum and pus culture media such as mannitol salt agar, MacConkey agar, blood agar, and chocolate agar. The growth showed different bacterial colonies whose morphological and biochemical characteristics were tested. Then DNA was extracted; purity and concentration were confirmed with Nanodrop. The purity of gram negative bacteria (1.8-2), and the concentration was between 50-360 ng/µl. Primers were mentioned as the procedure by [5].

* Corresponding author: Rana H. Raheema
Department of Medical Microbiology, Faculty of Medicine, University of Wasit, Iraq.

**Table 1** Primers' sequence of 16S rRNA for gram negative

| Bacteria | Primers | Primers (Sequence (5' – 3') | Product size(bp) | References |
|---|---|---|---|---|
| *Acinentobacter baumanni* | 16S rRNA | F:CCTACCMGGCGACGATCTG<br>R:GATTMCGCTCGCACCCTCT | 291 | The current study |
| *Burkholderia Cepacia* | 16S rRNA | F:AGGCAGCAGTGGGMTTTT<br>R:ACCMTGCAGTTCCCAGGTT | 292 | |
| *Klebsiella Oxytoca* | 16S rRNA | F:CTGGMCTGAGACGGTCC<br>R:GTCAGTCTTGTCCAGGGG | 439 | |
| *Serratia Mareescens* | 16S rRNA | F:TGCCTGATGATGGAGGGGGAT<br>R:GGAGTTAGCCGGTGCTTCTT | 382 | |
| *Proteus mirabilis* | 16S rRNA | F:CTGCCCGATAGAGGGGGATA<br>R:GGAGTTAGCCGGTGCTTCTT | 383 | [6] |

Several trials of thermal cycles were done using Mastermix Gold Multiplex 20x to optimize the PCR reactions to obtain annealing temperature for the (*16S rRNA*) primer. For those annealing temperatures we gradient (51 ℃, 53 ℃, 55 ℃, 57 ℃, 59 ℃, 61 ℃, 63 ℃) [7].

**Table 2** Components of monoplex PCR's master mix and amplification procedures to detect *16S rRNA* genes final volume 20 µl

| Components | Reaction volume (µl) |
|---|---|
| master mix Bioneer | Lyophilized powder |
| Forward primer | 1 |
| Reverse primer | 1 |
| DNA Template | 5 |
| Free water DDW | 13 |
| Total volume reaction | 20 |

**Table 3** Thermal cycling program for monoplex *16S rRNA* for *Klebsiella oxytoca*

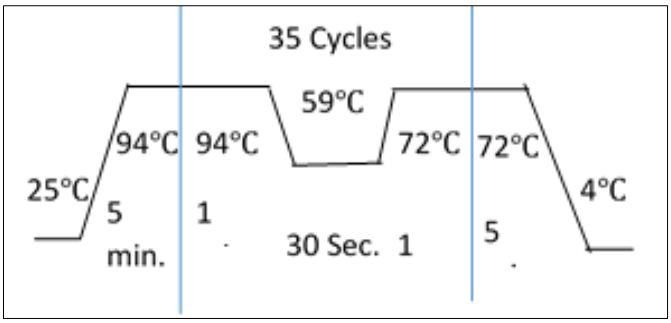
| Genes | PCR cycling profile | Products size |
|---|---|---|
| 16S rRNA |  | 438bp |

**Table 4** Thermal cycling program for monoplex *16S rRNA for Proteus mirabilis*

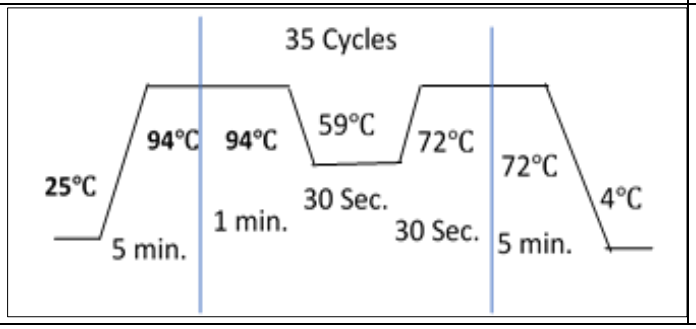
| Genes | PCR cycling profile | Products size |
|---|---|---|
| 16S rRNA |   | 383bp |

**Table 5** Thermal cycling program for monoplex *16S rRNA for Serratia marcescens*

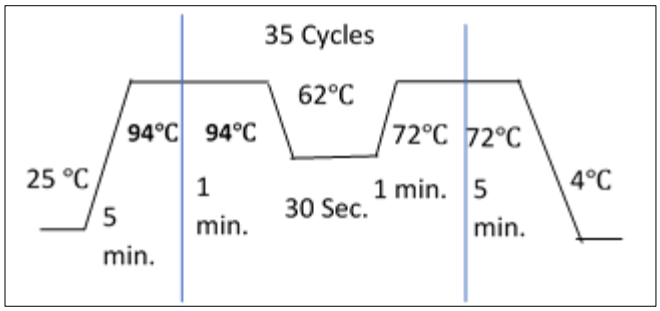
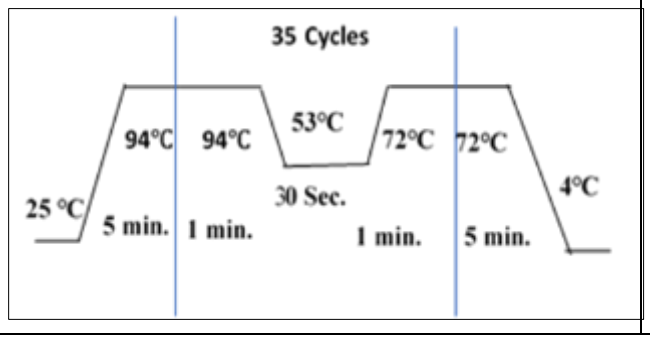
| Genes | PCR cycling profile | Products size |
|---|---|---|
| 16S rRNA |   | 382bp |

**Table 6** Thermal cycling program for monoplex *16S rRNA* for *Acinentobacter baumannii*, *Burkholderia cepacia* complex:

| Genes | PCR cycling profile | Product s size |
|---|---|---|
| 16S rRNA |   | 291bp<br>292bp |

According to who adapted this protocol as a reliable and efficient tool for the identification via multiplex PCR assays of the most common encoding of the beta-lactamase genes for gram negative bacteria. Gel Electrophoresis and Documentation [6].

## 3. Sequencing of PCR amplicons of *Acinentobacter baumannii*

The resolved PCR amplicons were sequenced from using the same forward and reverse PCR primers using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea (Macrogen Inc. Geumchen, Seoul, South Korea). BioEdit and MEGA 11 software were used to analyze the obtained ABI sequence files by clearing any unwanted nucleotides sequences. In order to identify the obtained nucleotide sequences, BLAST tool of the NCBI website was employed.

### 3.1. Interpretation of sequencing data

The sequencing results of the PCR products were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. The exact target locations were retrieved using the NCBI – BLAST tool and sequence matches were obtained for each sample.

### 3.2. Comprehensive phylogenetic tree construction

A specific comprehensive tree was constructed in this study according to the cladogram construction described by [8]. The observed variant was compared with their neighbor homologous reference sequences using the NCBI-BLAST server [9]. Based on the Clustal omega suit [10], multiple sequence alignments were made for the retrieved nucleic acid sequences. Subsequently, an inclusive tree was built by the neighbor-joining method and visualized using BioEdit and MEGA 11 software. The observed variants as well as its corresponding reference sequences were incorporated in the constructed comprehensive cladogram. The obtained phylogenetic tree was optimized to be properly presented. Finally the data's statistical analysis has been carried out with the use of SAS(Statistical Analysis System - version 9.1) [11].

## 4. Results and discussion

The current study was conducted on a total of one hundred clinical specimen urine, sputum and pus ) which were collected from patients admitted in the ICU. Eighty two were positive growth culture distributed according to the patient's age, the highest incidence was among 20-29 age groups with (25.6 %). While the lowest incidence was among (50-59) and (70-79) age group (3.6%), (3.6%) respectively, the mean ±SD of age was 15.556 ranging from 1 years to 90 years .Eighty two pure positive culture, 30(36.5%) were female of patients 52(63.5%) were male and admitted to the intensive care unit distributed 1 (1.2%) *Klebsiella oxytoca,* 5(6.25%) *Proteus mirabilis,* 2 (2.4%) *Acinetobacter baumannii* , 1 (1.2%) *Serratia marcescens* and 1 (1.2%) *Burkholderia cepacia group* bacteria . As shown in Table (7).

**Table 7** Bacteria distribution according to specimens

| Culture results | Urine of specimens (Valid Percent) | Sputum of specimens (Valid Percent) | Pus of specimens (Valid Percent) |
|---|---|---|---|
| *Klebsiella oxytoca* | 0(0%) | 1 (1.2%) | 0 (0%) |
| *Proteus mirabilis* | 0 (0%) | 0(0%) | 5(6.25%) |
| *Acinetobacter baumannii* | 0 (0%) | 2 (2.4%) | 0 (0%) |
| *Serratia marcescens* | 1 (1.2%) | 0 (0%) | 0 (0%) |
| *Burkholderia cepacia* group | 0 (0%) | 1 (1.2%) | 0 (0%) |

Results of identification *Klebsiella oxytoca* showed pink lactose fermenter colonies on MacConkey agar. Positive test for urease, indole, voges-proskauer, Simmon citrate but negative for oxidase, methyl red, motility test, similar results was recorded by [12].

Results of *Acinetobacter baumannii* showed nonmotile and give negative result to oxidase, indole, methel red, voges proskauer and avirable to urea. All isolates were positive to catalase, simmons citrate and triple-sugur-iron test was alkaline / no change, these results are identical with those obtained by [13, 14].

The results of *Proteus mirabilis* appear as bacilli, rapidly motile by flagella. Swarming phenomena on blood agar plate. It is non-lactose fermenter so it gives a pale colony on macConky agar. Negative test for indole and voges-proskauer but positive test for urease, simmon, s citrate, $H_2S$ is produced, nitrate reduction motility and methyl red. Similar finding was recorded by [6, 14, 15].

The results of *Serratia. marcescens*, showed that isolate had given result for indole , urease test , citrate assimilation , motility and catalase positive . Methyl red, voges proskauer and oxidase negative and TSI alkaline / acidic or acidic / acidic with no production of $H_2S$ and gas. Similar finding was recorded by [16].

The results of *Burkholderia cepacia complex* showed that isolate had given a result at negative for indole .But positive for methyl red , voges proskauer , urease test , citrate assimilation , motility ,catalase and oxidase ,TSI alkaline / acidic with no production of $H_2S$ and gas. Similar finding were recorded by [17]. The percentage of all other bacterial growth, which was isolated in this results are consistent with findings from other Iraqi studies [18,19]. Also similar result was recorded by [20,21, 22].

### 4.1. Molecular detecting of 16S rRNA gene

It is a specialized assay that has high sensitivity and is based on the diagnosis of many bacterial genera on the *16S rRNA* gene. This gene is used for differential studies between bacteria types. It is also used as a differential gene for bacterial species..The agarose gel of PCR products was shown in Figure (1 ) , (2), (3).

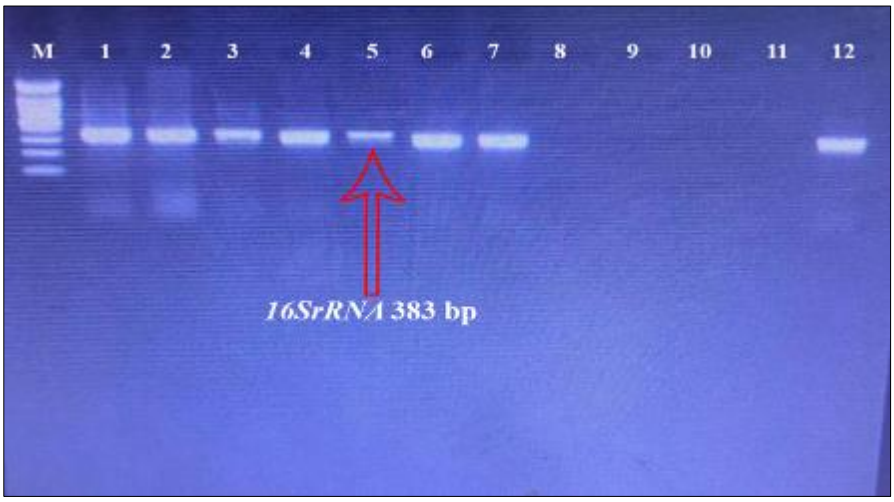



**Figure 1** Gel electrophoresis of amplified 16S rRNA gene from Proteus mirabilis using conventional PCR. Agarose 1.8 %, 100 V/cm for 35 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 2000 bp DNA ladder. Lane (1- 12 ) : , 16S rRNA 383 bp

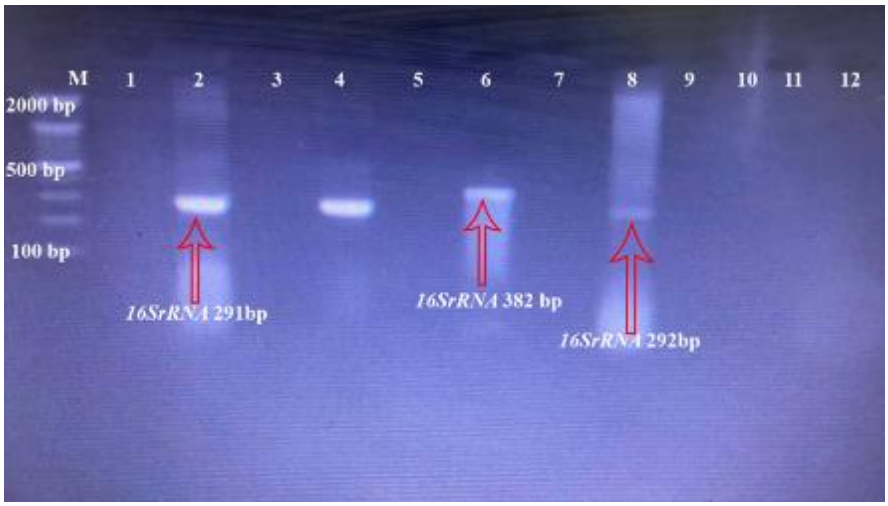



**Figure 2** Gel electrophoresis of amplified *16S rRNA* gene from *Acinetobacter baumannii*, *Burkholderia cepacia* and *Serratia marcescens* using conventional PCR. Agarose 1.8%, 100 V/cm for 35 min. 2000 bp DNA ladder, Lane (M). Lane (1- 12 ) : , *16S rRNA* 291 bp , *16S rRNA* 292 bp and*16S rRNA* 382 bp

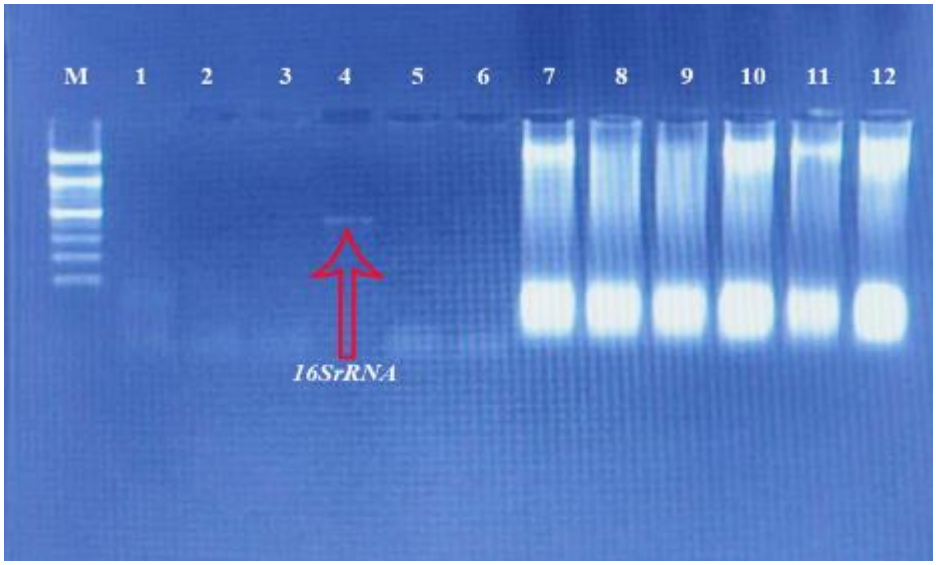



**Figure 3** Using conventional PCR, the *16S rRNA* gene from *Klebsiella oxytoca* was amplified and then electrophoresed. Agarose 1.8%, 100 V/cm for 35 min., 2000 bp DNA ladder, Lane (M). *16S rRNA*, 439 bp, in lanes 1 through 12

*4.1.1. Results of sequencing reactions*

Within this locus, one sample was included, which showed approximate length of the ribosomal fragment. By using NCBI BLAST, the sequencing reactions revealed the amplified product's verified identification. The NCBI BLAST engine showed a high sequence similarity between the sequenced sample with *Acinetobacter baumannii* sequences. The NCBI BLAST engine indicated the presence of about 99% of homology with these expected targets that covered the specified portions of the *rRNA* gene sequences.

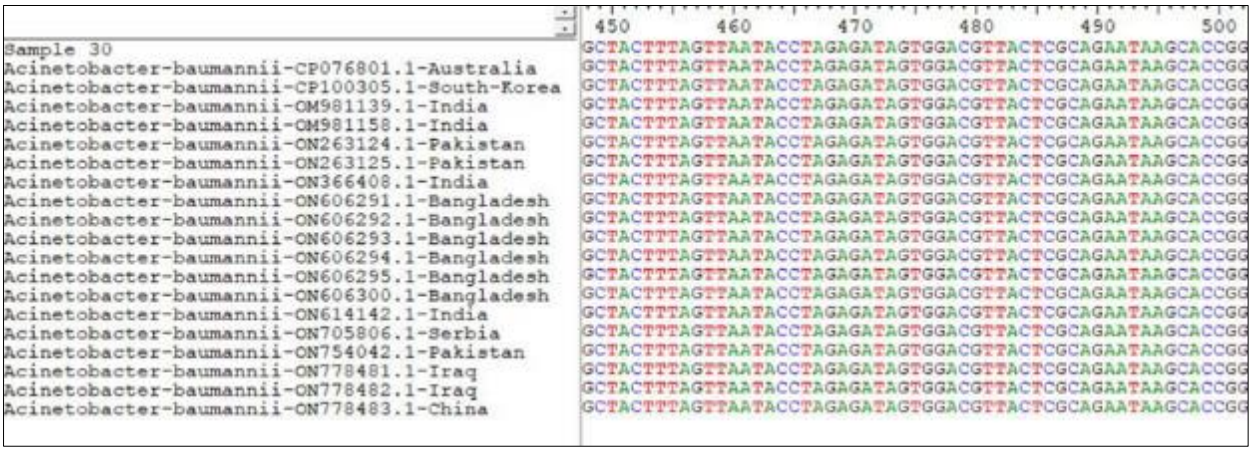



**Figure 4** Nucleic acid sequence alignment of isolated bacterial sample with the most relevant deposited genomic sequence

Based on the examined *16S rRNA* sequences in the examined bacterial samples, a thorough phylogenetic tree was produced. This phylogenetic tree included our screened bacterial sample together with the other deposited DNA sequences, linked with their closely related sequences in a neighbour-joining manner. This complete tree required the presence of a single distinct species, which corresponds to the tree's only included nucleic acid sequence. *Acinetobacter baumannii* was this species. The BLAST program placed this sample close to the Gene Bank accession numbers from several sources of *Acinetobacter baumannii* that had been deposited from various international sources. This sample has similar match with Gene Bank accession numbers of (ON606300.1), which were belonged to Bangladesh strain of the same species. It is important to mention that our isolate alignment gave high similarity (around 94%) with bacterial isolates in NCBI database including *baumannii* and rest belong another *Acinetobacter spp*. When doing dendrogram for the isolate result showed that it has unique clone as compared to all other similar sequences Figure (5).

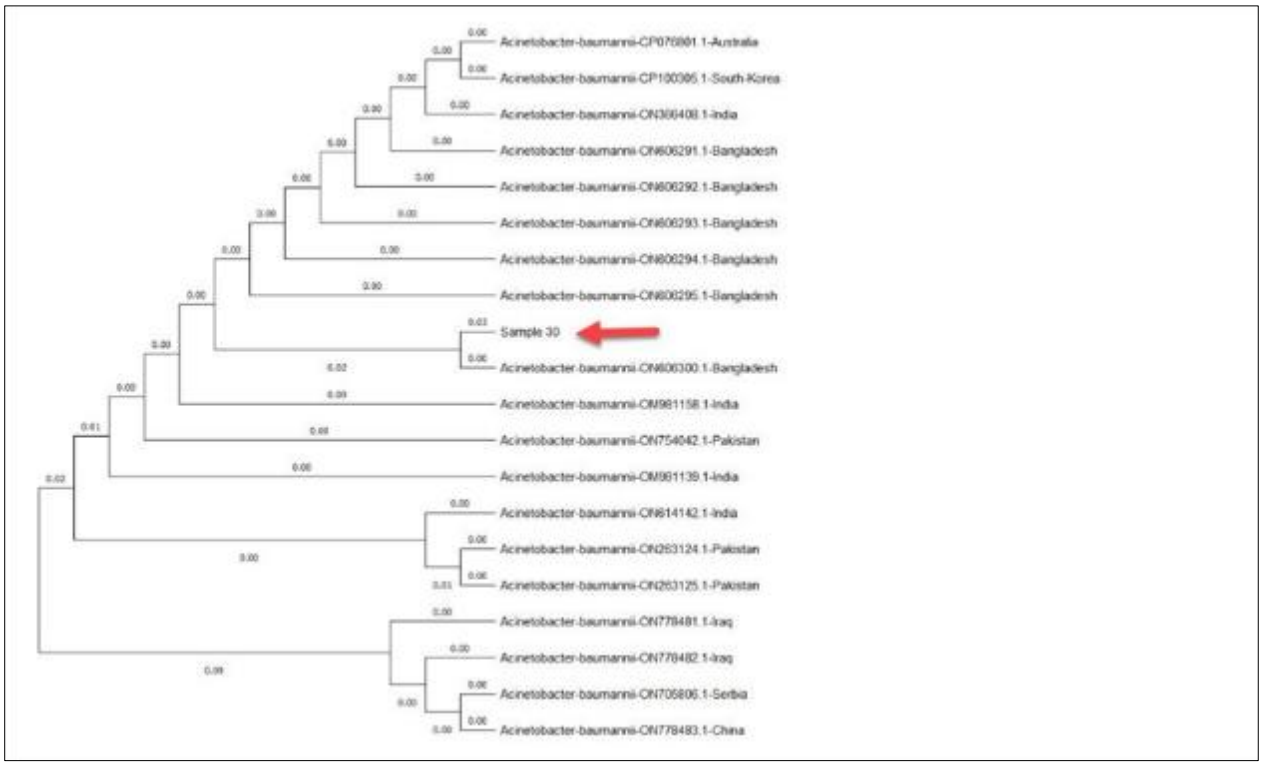



**Figure 5** The comprehensive phylogenetic tree of the *16S rRNA* sequences from NCBI database and sequences from the tested sample

## 5. Conclusion

According to the findings of current study in ICU, The highes frequency of specimens collection from intensive care unit patients were isolated from pus and was Proteus mirabilis. Genotypically highly specific, sensitive and reliable *16S rRNA*-based PCR assay for the identification of *Proteus mirabilis*, *Acinetobacter baumannii*, *Burkholderia cepacia* , *Serratia marcescens* , and *klebsiella oxytoca* and differentiate them from other closely related species.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The authors declare no conflict of interest

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

## References

[1] Salonia, J.S. and Sotelo, A. (2021) Infections Acquired in the Intensive Care Unit. Mount Sinai Expert Guides: Critical Care, Wiley Online Library, , pp., 414–428.

[2] Amaan, A., Dey, S.K. and Zahan, K. (2022) Improvement of Hand Hygiene Practices among the Healthcare Workers in a Neonatal Intensive Care Unit. Canadian Journal of Infectious Diseases and Medical Microbiology, Hindawi, 2022.

[3] Yao, N., Yang, X.-F., Zhu, B., Liao, C.-Y., et al. (2022) Bacterial Colonization on Healthcare Workers' Mobile Phones and Hands in Municipal Hospitals of Chongqing, China: Cross-Contamination and Associated Factors. Journal of epidemiology and global health, Springer, , pp., 1–10.

[4] Morris, S. and Cerceo, E. (2020) Trends, Epidemiology, and Management of Multi-Drug Resistant Gram-Negative Bacterial Infections in the Hospitalized Setting. Antibiotics, Multidisciplinary Digital Publishing Institute, 9(4), p., 196.

[5] Dahwash, S.M., Raheema, R.H., Albahadili, M.A. and Maslat, A.H. (2021) Distribution of Phylogenetics and Virulence Genes of Uropathogenic Escherichia Coli among Urinary Tract Infection in Pregnant Women. Biochem. Cell. Arch, 21(1), pp., 449–456.

[6] ALjeelizy, Z.A.H., Raheema, R.H., Abood, S.F. and khalaf, A.A. (2022) Phylogenetic Tree Analysis Based on the 16S Sequence Alignment for Proteus Mirabilis Isolated from UTI Patients in Wasit Province, Iraq. World Journal of Biology Pharmacy and Health Sciences, 12(1), pp., 125–132. https://doi.org/10.30574/wjbphs.2022.12.1.0154

[7] Abdul-hussein, Z.K., Raheema, R.H. and Inssaf, A.I. (2018) Molecular Diagnosis of Diarrheagenic E. Coli Infections among the Pediatric Patients in Wasit Province, Iraq. Journal of Pure and Applied Microbiology, Oriental Scientific Publishing Company, 12(4), pp., 2229–2241.

[8] Hashim, H.O., Mohammed, M.K., Mousa, M.J., Abdulameer, H.H., et al. (2020) Infection with Different Strains of SARS-CoV-2 in Patients with COVID-19. Archives of Biological Sciences, 72(4), pp., 575–585.

[9] Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. (2000) A Greedy Algorithm for Aligning DNA Sequences. Journal of Computational biology, Mary Ann Liebert, Inc., 7(1–2), pp., 203–214.

[10] Sievers, F. and Higgins, D.G. (2014) Clustal Omega, Accurate Alignment of Very Large Numbers of Sequences. Multiple Sequence Alignment Methods, Springer, 105–116, pp., 105–116.

[11] SAS Institute. (2004) SAS/STAT 9.1 User's Guide. Volume 1-7. SAS Institute.

[12] Abdurehman, D.A. and Abdurahman, M.K. (2022) Isolation, Assessments of Risk Factors, and Antimicrobial Susceptibility Test of Klebsiella from Gut of Bee in and around Haramaya University Bee Farm, East Hararghe, Oromia Regional State, Ethiopia. Veterinary Medicine International, Hindawi, 2022.

[13] Lin, M.-F. (2014) Antimicrobial Resistance in Acinetobacter Baumannii : From Bench to Bedside. World Journal of Clinical Cases, 2(12), p., 787.

[14] Jassim, A.T., Raheema, R.H. and Melek, H.K. (2022) Characterization of Multidrug Resistant Bacteria Isolated from Patients with Diabetic Foot Ulcers in Wasit Province. Biochem. Cell. Arch, 22(1), pp., 000–000. https://connectjournals.com/03896.2022.22.000

[15] Raheema, R.H. and Mahood, S.A. karium. (2016) The Effect of Partial Purified Plantaricin against Urinary Tract Infection (UTI) Induced by Escherichia Coli and Proteus Mirabilis in Experimental Rat Model.. European Journal of Pharmaceutical and Medical Research, 3(8), pp., 623–630. https://www.researchgate.net/publication/324416223

[16] Rebecca, L.J., Susithra, G., Sharmila, S. and Das, M.P. (2013) Isolation and Screening of Chitinase Producing Serratia Marcescens from Soil. Journal of chemical and pharmaceutical research, Journal of Chemical and Pharmaceutical Research, 5(2), pp., 192–195.

[17] Siddiqui, T., Sahu, C., Patel, S.S. and Ghoshal, U. (2022) Clinical and Microbiological Profile of Patients with Bloodstream Infections Caused by Burkholderia Cepacia Complex. Journal of Laboratory Physicians, Thieme Medical and Scientific Publishers Pvt. Ltd.

[18] Raheema, R.H. (2016) Experimental Study for the Effect of Trigonellafoenum-Graecum (Fenugreek) Seeds Extract on Some Biochemical and Histo Pathological Study in Induced Diarrhea in Mice by Klebsiella Pneumoniae . IOSR Journal of Pharmacy, 6(2), pp., 4–13.

[19] Al-Saeedi, R.H.A. and Raheema, R.H. (2019) Molecular Diagnosis of Some Virulence Genes in Pseudomonas Aeruginosa Clinical Isolates in Wasit Province. Indian Journal of Public Health, 10(04).

[20] Raheema, R.H. (2016) Effect of Pomegranate Peel Extract on Some Biochemical and Histopathological Parameters in Experimental Induced Mice with Staphylococcus Aureus . Journal of Animal Health and Production, 4(2), pp., 42–49.

[21] Raheema, R.H. and Abed, K.A. (2019) Molecular Identification of Virulence Genes of Staphylococcus Aureus Isolated from Different Clinical Isolates. Indian Journal of Public Health Research & Development, 10(2).

[22] Raheema, R.H., Melek, H.K. and Atheer, T.J. (2021) Characterization and Molecular Study to Detect Multidrug Resistance Bacteria Isolated from Patients with Diabetic Foot Ulcers in Wasit Province. International Journal of Science and Research Archive, 3(2), pp., 201–208.