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The Multi-Antibiotic Resistance Profile and Phylogenetic Analysis of Bacteria Using 16S rRNA Gene Sequencing and Genotyping Technology

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Abstract

Background: The global surveillance and profiling of the multi-antibiotic-resistant strains of bacteria have been reported by the World Health Organization in 2024 among the top priority threats facing global public health. **Aim:** To determine the multi-antibiotic resistance profile and phylogenetic analysis of bacteria using 16S rRNA gene sequencing and genotyping technology. **Methods:** The bacterial strains were used from the stocks of the routine culturing in the general microbiology laboratory in the Department of Applied Biology, University of Sharjah; these are: *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Streptococcus pyogenes.* The 16S rRNA gene sequences and the phylogenetic genotyping diagnosis were determined followed by determination of the multi-resistance profile of the tested strains using 2 levels of sensitivity testing protocols; diffusion well and VITEK-2 automated system. Three multi-resistance control strains were used for quality assurance. **Results:** The phylogenetic diagnosis was determined using the international sequences library available in the public domain of the NCBI Gene/Bank, USA. Strains were genotyped with 99.9% homology and diagnostic positions on the phylogenetic trees for the strains of *E. coli*, *P. aeruginosa* isolates, *K. pneumoniae*, and *S. pyogenes*, respectively. The multi-antibiotic resistance profiles were as follows: *E. coli* 12.5% (E1), *P. aeruginosa* 37.5% (F1), *P. aeruginosa* 31.25% (H1), *P. aeruginosa* 31.25% (G1), *P. aeruginosa* 37.5% (B2), *K. pneumoniae* 6.25% (A1), *K. pneumoniae* 50% (C1), *K. pneumoniae* 6.25% (D1), *K. pneumoniae* 6.25% (D2), *S. pyogenes* 0% (L1), for the *E. coli*, *P. aeruginosa*, *K. pneumoniae* isolates, *S. pyogenes*, respectively. **Conclusion:** The results show the multi-antibiotic resistance profile of the tested strains and its

phylogenetic genotyping analysis which will improve surveillance of the infectious diseases and molecular diagnosis.

Keywords

Multi-Antibiotic-Resistant Bacteria, Phylogenetic Analysis, 16S rRNA Gene Sequencing

1. Introduction

Currently, there is a worldwide growing interest in the multi-antibiotic resistant bacteria particularly the causative agents of the nosocomial infections for the inpatients as well as for the outpatients [1]-[8]. According to the World Health Organization report [9], antimicrobial resistance (AMR) was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths [10]. A global extended retrospective review has been published on the global burden of bacterial antimicrobial resistance during 1990-2021 as a systematic analysis with forecasts to 2050 [11]. It has been reported that the misuse of antibiotics has led to the emergence of antibiotic resistance in hospitals initially and is now commonly seen in the community due to their misuse of drugs available in the market [12]. A statistical analysis was done on 71 countries and showed an increase of 35% in antibiotic consumption between 2000 and 2010 [13]. Antibiotic resistance development has been seen since their early use, such as the use of penicillin, which spread rapidly, leading to resistance to penicillin [14] [15]. The increase in resistance of these antibiotics led to an increase in morbidity and mortality rate; for example, *Pseudomonas aeruginosa* is solely responsible for approximately 13 - 15% of all hospital-acquired infections [1]. The antibiotic resistance could be either innate or acquired, so either the microorganism itself is resistant to a specific antibiotic or, if it is acquired, its resistance occurred because of multiple events of mutation such as conjugation, transformation, and transduction through horizontal gene transfer [16]-[18]. Multiple clinical strains are causing a worldwide concern because of their resistance to multiple antibiotics, as their resistance can be transmitted via motile genetic elements, strains such as *Acinetobacter, Klebsiella, Pseudomonas aeruginosa, Proteus, Escherichia coli*, and *Enterobacter species* [18].

Bacterial identification had evolved from the common workflow that is used in hospitals, as it includes culture and biochemical testing and analysis based on the phenotypes and metabolic features of the bacteria. However, not all pathogenic strains can be cultured successfully, as this method is time-consuming and does not lead to accurate diagnosis [19]. In contrast, the use of the 16S rRNA gene sequencing for accurate bacterial identification and thus profiling of the multi-antibiotic resistant has become inevitable for accurate diagnosis of both culturable and the nonculturable bacteria. The 16S rRNA of about 1500 base pairs long con-

sists of conserved nucleotide sequences interspersed with 9 variable regions that are genus- or species-specific and differ in their hypervariable region, making it an ideal tool for phylogenetic diagnosis [20]-[23]. These advances in technologies make it possible to get the full discriminatory potential of 16S rRNA, improving the taxonomic resolution of bacterial communities at species and strain level. Additionally, the extensive amount of information available on databases on its properties makes this marker a convenient target for broad-range molecular analysis. Therefore, the aim of this study was to determine the multi-antibiotic profile and phylogenetic analysis of the multi-antibiotic resistant bacteria using 16S rRNA gene sequencing and genotyping technology.

2. Methods

2.1. Bacterial Strains

Bacterial strains were isolated from environmental samples collected from within the campus and kept as stocks for routine culturing in the general microbiology laboratory in the Department of Applied Biology, University of Sharjah; these are: *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Streptococcus pyogenes.* For long-term storage, the strains were preserved in 50% glycerol stocks at −80˚C and for experimentation, strains were grown overnight on nutrient agar at 37˚C (Nutrient agar (Himedia Laboratories, Maharashtra, India, Cat. No. M001).

2.2. Determination of the Multi-Resistance Profiling

Both *antibiotic* activity and the multi-resistance profile were conducted for the tested strains using the inhibition zone method and the VITEK-2 system and the automated VITEK® SOLUTIONS—Complete automated ID/AST platform (https://www.biomerieux-diagnostics.com). The multi-resistance of the tested strains was determined manually by dividing the number of the resisted antibiotics on the total number of the tested ones; as this is according to the international standards as stipulated in the Clinical and Laboratory Standards Institute (CLSI) CLSI M100-Ed35, 2025 [24]. Four multi-resistance control strains were used for quality control assurance; these were *Escherichia coli, ATCC®* 25922, *Klebsiella pneumoniae, ATCC®* 13883, and *Streptococcus faecalis, ATCC®* 33186, and were subjected to the same diagnosis protocol as with the tested strains.

The bacterial strains were grown overnight on nutrient agar plates at 37˚C. No less than four colonies were collected by using a sterile loop and resuspended in a 2 mL saline solution and then vortexed to obtain a homogenous mixture. The suspension was adjusted to the 0.5 McFarland standard (~1.5 × 108 CFU/ml) and tested for antibacterial activity using well diffusion.

Antibiotic sensitivity tests were performed using the Kirby-Bauer disk diffusion assay [25]. The bacterial mixtures were seeded on Mueller-Hinton agar plates by using a sterile cotton swab. Antibiotic discs were then placed on the agar plates using sterile tweezers. The plates were incubated overnight at 37˚C. After 24

hours, the inhibition zones were measured using a ruler, and the data were interpreted as sensitive, intermediate, and resistant according to the Clinical and Laboratory Standard Institute and compared also with the manufacturer reference sheet. Additionally, VITEK antibiotic susceptibility testing analysis was conducted by growing the bacterial strains overnight on nutrient agar at 37˚C. Then, using a sterile loop, four to five colonies were collected and resuspended in a 2 mL saline solution. The density of the colonies was checked using a Densi CHEK Plus device; the reading should range from 0.58 to 0.62. The mixture was put on the cassette, then VITEK® 2 Antibiotic Susceptibility Testing Gram-Negative (AST-GN70) cards and VITEK® 2 Antibiotic Susceptibility Testing Gram-Positive (AST-GP75) cards were placed accordingly in the VITEK® 2 Compact diagnostic system (bioMérieux https://www.biomerieux.com/).

2.3. Determination of the Minimum Inhibition Concentration (MIC) and Biochemical Characteristics Using the VITEK-2 System

The Minimum Inhibition Concentration (MIC) and biochemical characteristics were conducted using the VITEK® SOLUTIONS—Complete automated ID/AST platform (https://www.biomerieux-diagnostics.com). Bacterial strains were grown overnight on nutrient agar at 37˚C by using a sterile loop; four to five colonies were collected and resuspended in 2 mL saline solution. Density of the colonies was checked using a DensiCHEK plus device; the reading should range from 0.58 to 0.62. The mixture was put on the cassette, and VITEK 2 Identification Card Gram-Negative (ID GN) cards and VITEK 2 Identification Card Gram-Positive (ID GP) cards were placed accordingly. Samples were placed in the VITEK 2 Compact device.

2.4. Genomic DNA Extraction and 16S rRNA Gene Amplification

Genomic DNA was extracted from 1 ml of pure bacterial culture using the Isolate II Genomic DNA kit (Bioline, BIO-52066) according to the manufacturer's protocol. The purity and concentration of extracted genomic DNA were assessed using the NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific) (Table 1). To amplify the 16S rRNA gene, the MicroSEQ Full Gene 16S rDNA PCR Kit (Thermo Fisher Scientific, 4349155) was used. In brief, 60 ng of genomic DNA was used in three separate PCR reactions, thereby amplifying three overlapping fragments to ensure that a high-quality and full-length gene sequence was obtained. PCR was carried out according to the following conditions: 95˚C for 10 minutes; 30 cycles of 95˚C for 30 seconds, 60˚C for 30 seconds, and 72˚C for 45 seconds, followed by a final extension at 72˚C for 10 minutes. Generated amplicons were then verified by visualizing them on a 2% agarose gel. Moreover, 5 µL of each PCR product was treated with ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, 78200.200.UL) according to the supplied protocol and used for sequencing. For contamination checks during DNA extraction, PCR, or sequencing, negative and positive control as provided by the manfcturer within the kit were run in parallel with samples.

Table 1. The genomic DNA concentration and the A260/A280 purity ratio of all tested strains using the NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific) are given in the following Table.

	Bacterial strain	Genomic DNA concentration (ng/uL)	A260/A280 purity ratio
1	*Kelbsiella Pneumonia* (A1)	43	1.87
2	*Pseudomonas aeruginosa* (B2)	23	2.00
3	*Kelbsiella Pneumonia* (C1)	28	1.95
4	*Kelbsiella Pneumonia* (D1)	38.5	1.84
5	*Kelbsiella Pneumonia* (D2)	31.8	1.99
6	*Escherichia coli* (E1)	50	2.00
7	*Pseudomonas aeruginosa* (F1)	25.6	1.82
8	*Pseudomonas aeruginosa* (G1)	52	1.81
9	*Pseudomonas aeruginosa* (H1)	60	1.99
10	*Streptococcus pyogenes* (L1)	15	1.89

2.5. 16S rRNA Gene Sequencing

Sequencing reactions were conducted using the MicroSEQ Full Gene 16S rDNA Sequencing Kit (Thermo Fisher Scientific, 4347484) which includes the primers in its master mix solution according to the manufacturer's protocol. In short, each of the three purified PCR products was sequenced using both their respective forward and reverse primers, resulting in six reactions. Sequencing reactions were carried out using the following conditions: 25 cycles of 96˚C for 10 seconds, 50˚C for 5 seconds, and 60˚C for 4 minutes. Sequencing reactions were then purified using the ethanol/EDTA/sodium acetate precipitation method. Afterward, capillary sequencing was carried out by the Genetic Analyzer 3500 (Applied Biosystems, Thermo Fisher Scientific, USA). Obtained forward and reverse sequences were then analyzed using the Sequencing Analysis Software 6 (Applied Biosystems, Thermo Fisher Scientific, USA) and assembled into one contiguous sequence using the CAP3 software (http://doua.prabi.fr/software/cap3). Finally, the assembled sequence was aligned to published sequences using Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Pairwise Sequence Alignment

The full gene sequence of the bacterial strain extracted was aligned automatically using the BLAST tool against the gene libraries available for bacterial species in the NCBI (https://www.ncbi.nlm.nih.gov/), Sanger Institute (http://www.sanger.ac.uk), DDBJ (http://www.ddbj.nig.ac.jp), and EMBL-EBI GeneBank (http://www.ebi.ac.uk) databases.

2.7. Multiple Sequence Alignment

The phylogenetic analysis was constructed using the Neighbor-Joining tree of the

isolated strain using BLAST and CLUSTAL W (1.83) available in the following international GeneBanks: National Center for Biotechnology Information (NCBI), International Nucleotide Sequence Databases (INSD) (DDBJ GenBank), and EMBL-EBI Bank (European Bioinformatics Institute and the European Molecular Biology Laboratory). The closely related homologous strains were identified, retrieved, and compared to the sequence of the strain extracted using CLUSTAL W (version 3.2) available on the Biology StudyBench (https://workbench.sdsc.edu/).

2.8. Statistical Criteria for Species Identification

Genotyping identification of species through sequence similarity was determined based on the international diagnostic criteria as set by the international Gene Banks (International Nucleotide Sequence Databases (INSD) (DDBJ GenBank), EMBL-EBI Bank (European Bioinformatics Institute and the European Molecular Biology Laboratory), and the National Center for Biotechnology Information (NCBI), where if the difference between the query and the compared strain is 1 - 1.5% (14 - 22 bp), 1.5 - 5.0% (23 - 72 bp), and 5.0 - 7.0% (72 - 98 bp), then the query strain should be given to the same species, genus, or a different genus, respectively.

3. Results

3.1. Biochemical Characteristics Diagnosis of the Tested Strains

The biochemical characteristics diagnosis of the tested strains were tested against 64 biochemical reactions as included in the VITEK-2 system (data not shown). Several quality control strains were used for assurance and were subjected to all diagnosis protocols as with the tested strains, including *Escherichia coli, ATCC®* 25922, *Klebsiella pneumonia, ATCC®* 13883, and *Streptococcus faecalis, ATCC®* 33186.

3.2. Multi-Resistance Profiling

The multi-resistance profile of strains revealed a high prevalence level of resistance as follows: *E. coli* 12.5% (E1), *P. aeruginosa* 37.5% (F1), *P. aeruginosa* 31.25% (H1), *P. aeruginosa* 31.25% (G1), *P. aeruginosa* 37.5% (B2), *K. pneumoniae* 6.25% (A1), *K. pneumoniae* 50% (C1), *K. pneumoniae* 6.25% (D1), *K. pneumoniae* 6.25% (D2), *S. pyogenes* 0% (L1), for the isolates *E. coli* isolate, *P. aeruginosa* isolates, *K. pneumoniae* isolates, *S. pyogenes* isolate, respectively (Table 2 and Table 3). The most resisted antibiotics by the strains were ampicillin (Amp) 10 mcg, ciprofloxacin (CIP) 40 mcg, nitrofurantoin (NIT) 100 mcg, and co-trimoxazole (sulfa/trimethoprim) (COT) for the strains *K. pneumoniae*; *Pseudomonas aeruginosa* and 12.5% for *E. coli* respectively. For quality assurance, the ATCC strains, including *Escherichia coli, ATCC®* 25922, *Klebsiella pneumoniae, ATCC®* 13883, and *Streptococcus faecalis, ATCC®* 33186, were used as controls as subjected to the diagnosis protocol as with the tested isolates. The inhibition zones varied from 0 mm for the multi-resistance strains for as low as 10 mcg of ampicillin (Amp) to 40 mm for the sensitive strains as high as 40 mcg ciprofloxacin (Table 2 and Table 3).

Table 2. The multi-antibiotic-resistance profile of bacteria which were determined as: Sensitive (S), Intermediate (I), Resistance (R) and Not available (−). The Resistance Criteria were determined according to the Clinical and Laboratory Standard Institute (CLSI), and also according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

No.	Antibiotic Name	Antibiotic concentration (mcg)	MR Strain-E1 *Escherichia coli* strain	Interpretative Criteria	MR Strain-L1 *Streptococcus pyrogenes* strain	Interpretative Criteria	MR Strain-C1 *Klebsiella pneumonia* strain	Interpretative Criteria	MR Strain-A1 *Klebsiella pneumonia* strain	Interpretative Criteria	MR Strain-D1 *Klebsiella pneumonia* strain	Interpretative Criteria	MR Strain-D2 *Klebsiella pneumonia* strain	Interpretative Criteria
1	Ampicillin (AMP)	10 mcg	0	**R**	40	S	0	**R**	8	**R**	11	**R**	10	**R**
2	Ampicillin (AMP)	2 mcg	0	**R**	40	S	0	**R**	10	-	10	-	10	-
3	Amoxyclav (Amoxycillin/ Clavulanic acid) (AMC)	30 mcg	20	S	30	S	15	I	20	S	20	S	26	S
4	Amikacin (AK)	10 mcg	19	-	22	-	20	#	21	-	22	-	19	-
5	Amikacin (AK)	30 mcg	20	S	25	-	21	S	21	S	20	S	24	S
6	Aztreonam (AT)	30 mcg	30	S	24	-	15	**R**	30	S	35	S	31	S
7	Ciprofloxacin (CIP)	5 mcg	35	S	30	-	19	**R**	30	S	32	S	29	S
8	Ciprofloxacin (CIP)	30 mcg	36	-	37	-	25	#	34	-	30	-	30	-
9	Cefuroxime (CXM)	30 mcg	26	S	40	-	0	**R**	24	S	26	S	26	S
10	Ceftriaxone (CTR)	30 mcg	34	S	43	S	10	**R**	32	S	25	S	27	S
11	Cefepime (CPM)	30 mcg	33	S	45	S	16	**R**	30	S	28	S	25	S
12	Nitrofurantoin (NIT)	100 mcg	20	*S	24	*S	15	*S	17	*S	20	*S	20	*S
13	Gentamicin (HLG)	120 mcg	26	-	30	-	16	-	25	-	25	-	21	-
14	Meropenem (MRP)	10 mcg	35	S	20	S	32	S	30	S	30	S	30	S
15	Co-Trimoxazole (Sulpha/ Trimethoprim) (COT)	25mcg	27	S	32	S	0	**R**	30	S	30	S	29	S
16	Piperacillin/ Tazobactam (PIT)	100/ 10 mcg	30	S	45	-	26	S	26	S	26	S	25	S
	Frequency %	-	12.5%	-	0%	-	50%	-	6.25%	-	6.25%	-	6.25%	-

Table 3. The multi-antibiotic-resistance profile of *Pseudomonas aeruginosa* bacteria which were determined as: Sensitive (S), Intermediate (I), Resistance (R) and Not available (−). The Resistance Criteria were determined according to the Clinical and Laboratory Standard Institute (CLSI), and also according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

No.	Antibiotic Name	Antibiotic concentration (mcg)	MR Strain-F1 *Pseudomonas aeruginosa strain*	Interpretative Criteria	MR Strain-H1 *Pseudomonas aeruginosa strain*	Interpretative Criteria	MR Strain-B2 *Pseudomonas aeruginosa strain*	Interpretative Criteria	MR Strain-G1 *Pseudomonas aeruginosa strain*	Interpretative Criteria
1	Ampicillin (AMP)	10 mcg	0	R	0	R	0	R	0	R
2	Ampicillin (AMP)	2 mcg	0	R	0	R	0	R	0	R
3	Amoxyclav (Amoxycillin/Clavulanic acid) (AMC)	30 mcg	0	R	0	R	0	R	0	R
4	Amikacin (AK)	10 mcg	25	-	25	-	24	-	25	-
5	Amikacin (AK)	30 mcg	26	S	26	S	25	S	20	S
6	Aztreonam (AT)	30 mcg	26	S	22	S	23	S	26	S
7	Ciprofloxacin(CIP)	5 mcg	35	S	35	S	40	S	40	S
8	Ciprofloxacin (CIP)	30 mcg	40	-	35	-	35	I	30	-
9	Cefuroxime (CXM)	30 mcg	0	R	0	R	0	R	0	R
10	Ceftriaxone (CTR)	30 mcg	19	I	20	S	18	-	20	S
11	Cefepime (CPM)	30 mcg	27	S	26	S	27	S	30	S
12	Nitrofurantoin (NIT)	100 mcg	0	R	0	R	0	R	0	R
13	Gentamicin (HLG)	120 mcg	30	-	29	-	27	-	24	-
14	Meropenem (MRP)	10 mcg	37	S	30	S	20	S	36	S
15	Co-Trimoxazole (Sulpha/Trimethoprim) (COT)	25 mcg	0	R	10	-	0	R	15	-
16	Piperacillin/Tazobactam (PIT)	100/10 mcg	30	S	27	S	28	S	32	S
	Frequency %	-	37.5%	-	31.25%	-	37.5%	-	31.25%	-

3.3. Minimum Inhibition Concentration (MIC)

The Minimum Inhibition Concentration (MIC) was determined for the tested strains against 17 commercial antibiotics available in the market. The MIC ranged between 0.25 - 64, 0.25 - 128, and 0.25 - 32 for the strains *K. pneumoniae, P. aeruginosa,* and *E. coli,* respectively.

3.4. Phylogenetic Diagnosis Using 16S rRNA Sequences

The phylogenetic trees of the sequences were constructed for the top 5 nearest homologous strains, and the resulting phylogenetic diagnosis of each strain with a homology of 99.9% is shown for the nearest top 5 strains (**Figures 1-3**). The tested strains were fully diagnosed down to the species level as shown in the phylogenetic trees based on the sequences homology percentage of 99.9% or above for *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. pyogenes*, respectively.

4. Discussion

The frequency of the multi-resistance profile reached 50% for the strains *K. pneumoniae* and 37.5% and 31.25% for *Pseudomonas aeruginos*, whilst it was 12.5%

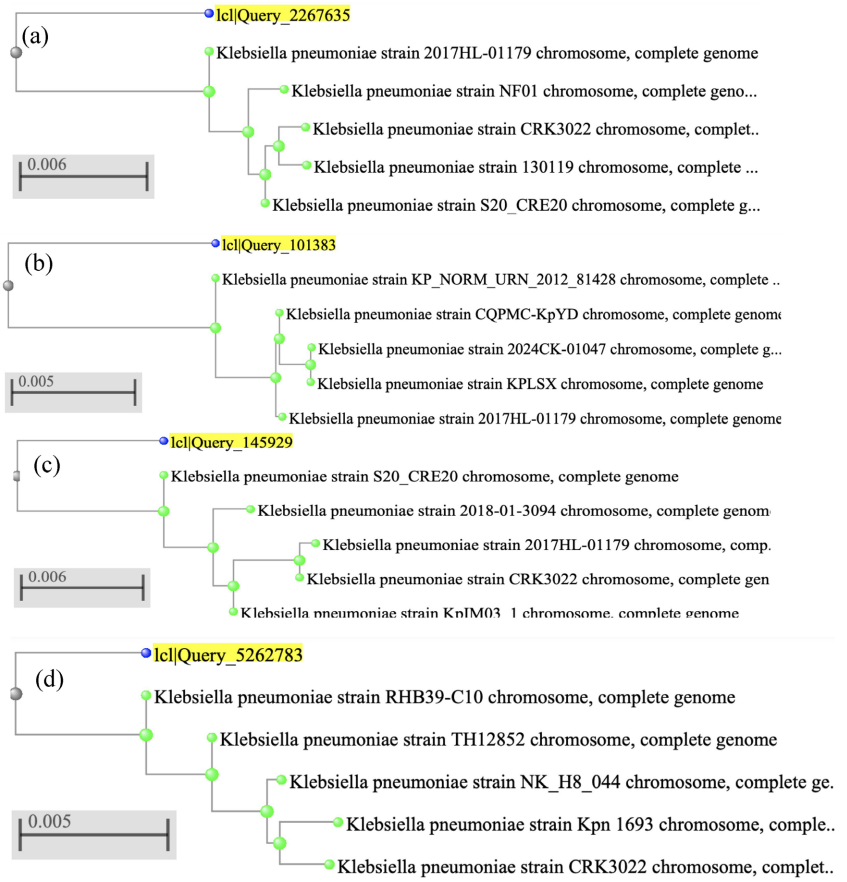

Figure 1. Neighbor-joining phylogenetic tree of the *Kelbsiella Pneumonia* strains with the top-5 homologous strains using the sequence diagnosis of the 16S rRNA gene, (a) *Kelbsiella Pneumonia* strain (A1), (b) *Kelbsiella Pneumonia* strain (D1), (c) *Kelbsiella Pneumonia* strain (D2), and (d) *Kelbsiella Pneumonia* (C1).

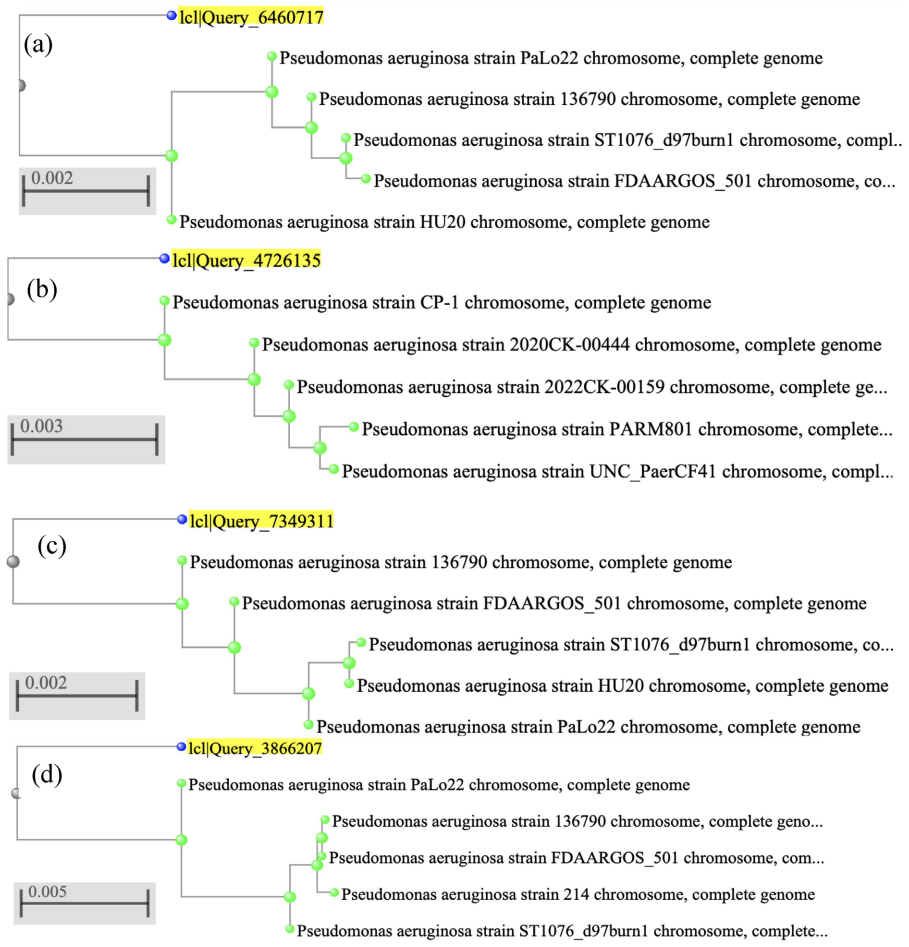

Figure 2. Neighbor-joining phylogenetic tree of the *Pseudomonas aeruginosa* strains causing with the top-5 homologous strains using the sequence diagnosis of the 16S rRNA gene, (a) *Pseudomonas aeruginosa* (F1), (b) *Pseudomonas aeruginosa* (B2) and (c) *Pseudomonas aeruginosa* (H1) and (d) *Pseudomonas aeruginosa* (G1).

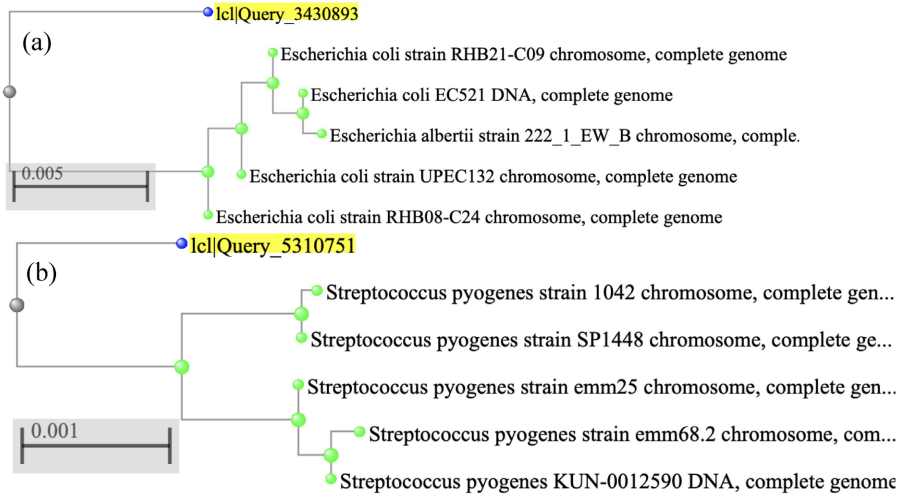

Figure 3. Neighbor-joining phylogenetic tree of the strains causing with the top-5 homologous strains using the sequence diagnosis of the 16S rRNA gene, (a) *Escherichia coli* (E1), and (b) *Streptococcus pyogenes* (L1).

for *E. coli*. Our present results of the multi-antibiotic resistance profile agree with the latest reported data on the global surveillances by the WHO [26]. Furthermore, in comparison with the latest regional and global infections caused by the multi-resistance strains [27] [28]. Our results revealed high similarity among other bacteria strains which have been reported causing the most common infections: ear, nose, respiratory, and urinary tract infections (UTI), respectively.

The phylogenetic analysis of the top-5 species shows conclusively the accurate genotypic diagnosis for all strains with homology of 99.9% and above. This underlined the importance of using phylogenetic genotyping in the diagnosis of such pathogenic strains causing various infections reported worldwide [29]. The multi-resistance profiling order was ampicillin (AMP) 10 mcg, cefuroxime (CXM) 30 mcg, nitrofurantoin (NIT) 100 mcg, and co-trimoxazole (sulfa/trimethoprim) (COT) for the pathogenic strains *K. pneumoniae* and *Pseudomonas aeruginosa*, respectively.

The importance of these results in this study shows conclusively its valuable proven medical implications to enable clinicians to quickly optimize antimicrobial therapy and implement infection control policies, including the right medical decision for the right antibiotic in the right dose at the right time. Furthermore, it improves on the multi-resistance profiling on surveillance programs of the infectious diseases in the UAE and worldwide.

5. Conclusions

Our results show conclusively the accuracy of using 16S rRNA sequencing in bacterial diagnosis and therefore importance of the use of the phylogenetic genotyping in clinical diagnosis of pathogenic bacteria which may cause various infections in the UAE and worldwide, assuring the pathogenic multi-resistance profiling for accurate prescriptions and treatments.

The frequency of the multi-resistance profile in the present study is highly comparable to the multi-resistance control strains that have been reported worldwide such as *K. pneumoniae,* as the most common causative agent for the respiratory infections, *Pseudomonas aeruginosa,* as the most common causative agent for the skin, ear, and urinary tract infections (UTI), while *E. coli* is among the most common causative agents for urinary tract infections (UTI).

Contributions

IM: Conceptualization, design, phylogenetic analysis, writing up the manuscript and intellectual content, project administration and supervision, investigation, and methodology. **NM**: experiments investigation, results presentation. **MM**: Sequencing 16SrRNA gene.

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Conflicts of Interest

The authors declare that they have no conflict of interest as well as no competing interests.

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