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Molecular Detection Using A Particular Primer For The Isolation Of *Pseudomonas Aeruginosa* From A Persistent Bacterial Co-Infection In A COVID-19 Patient.

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Abstract
Bacterial co-infections increase the severity of respiratory viral infections and are frequent causes of mortality in COVID-19 infected subjects. We aimed to investigate the antimicrobial resistance patterns and molecular typing of Pseudomonas aeruginosa isolates among Coronavirus disease-19 patients. According to some, one of the most nosocomial bacteria is *Pseudomonas Aeruginosal*. Between October 2021 and June 2022, 100 sputum samples were collected for this study from several Wasit hospitals. twenty seven (27%) isolates were identified as *Pseudomonas aeruginosa* by routine biochemical tests, vitek 2 system ; In attempting to the identification of *P. aeruginosa* strains at the DNA level, Polymerase chain reaction (PCR) is used based on specific primer (27F/1492R) for (16S rRNA) gene. The results showed that PCR has found to be rapid and more sensitive and specific in identification of *P. aeruginosa*. During the COVID-19 period, especially at the beginning of the pandemic, an inappropriate use of broad-spectrum antibiotic treatments has been frequently described, mainly due to prolonged hospitalization, and the use of immune-suppressive treatments as steroids. *P. aeruginosa* strains were resistant to imipenem followed by meropenem , ciprofloxacin and levofloxacin .

Keyword : *Pseudomonas aeruginosa*, human, vitek 2 system, antimicrobial .

1. Introduction

A prevalent opportunistic pathogen linked to infections acquired in healthcare settings is the Gram-negative bacteria *Pseudomonas aeruginosa* (Kollef et al ., 2021). And and people with impaired immune systems. Crucially, it is a leading source of morbidity and mortality among hospitalized patients and frequently resistant to antibiotics (Lucena et al ., 2014). Particularly in immunocompromised hosts, *P. aeruginosa* infections can result in serious, life-threatening infections and significantly raise overall healthcare expenses. (Morales, et al ., 2012 ; Kaier et al ., 2019). Antibiotics are usually used to treat P. aeruginosa bacteremia, although in certain

situations, the infection may be hard to cure and may need extensive or protracted treatment. Infection with P. aeruginosa still causes high mortality rates of up to 62% in some patient groups, despite medical advancements and antibiotic therapy (Vidal et al ., 1996). Antibiotics are commonly used to treat P. aeruginosa bacteremia, but in certain situations, the infection may be hard to cure and may need for extensive or protracted treatment. High death rates of up to 62% in some patient groups are still caused by P. aeruginosa infections despite medical advancements and antibiotic therapy (Pfaller, et al ., 1998 , Richards, et al .,1999 , Bodey, et al ., 1985). P. aeruginosa infections linked to healthcare settings are also on the rise. They can manifest as pneumonia, urinary tract infections, surgical site infections, and bacteremia, and they account for around 7% of all nosocomial infections. (Magill, et al ., 2014 , Weiner et al ., 2016). Because P. aeruginosa is an opportunistic infection that prefers immunocompromised patients, this number is even greater in intensive care unit (ICU) settings (Bodey et al ., 1985). Because there aren't many empirical therapy choices in the intensive care unit, P. aeruginosa antibiotic resistance is a major worry. The carbapenem class of medications is frequently used in empirical and conclusive treatment. But these have been rendered useless in the face of Enterobacteriaceae (CRE) and carbapenem-resistant Acinetobacter baumannii (CRA), which have been more common in particular areas during COVID-19 outbreaks. (Gottesman, et al .,2021). This may be partially caused by the overstretched laboratory and human resources for the diagnosis, treatment, and care of COVID-19, which decreases the ability to screen for multidrug-resistant organisms (MDROs). It may also lead to lapses in conventional infection prevention and control procedures, making it impossible to isolate or cohort all patients who test positive for MDROs (Monnet & Harbarth. 2020). Antibiotic stewardship is crucial for both prescribing and de-escalating antibiotics in order to prevent the emergence of antibiotic resistance, which may be caused by these causes (Campion & Scully 2018). in addition to the monitoring activities necessary to guide suggestions. The prevalence of various nosocomial infections in healthcare settings were believed to be positively impacted by better hand cleanliness and increased infection prevention and control methods during the Coronavirus Disease 2019 (COVID-19) pandemic (Zhu et al ., 2022;Sturm et al .,2022) However, this conclusion was not always reached. With conflicting findings from research, it is still unknown if the incidence of P. aeruginosa bacteremia rose, fell, or stayed constant during the pandemic (Amarsy, et al ., 2022; Hirabayashi et al ., 2021; İpek et al ., 2022). Additionally, it is still unclear whether the rise in antibiotic prescriptions during the pandemic (Winders, et al ., 2021). Aided in the development of resistant strains, particularly since antibiotic exposure is a major risk factor for resistance and research indicates that resistant P. aeruginosa strains might appear as soon as eight days after starting meropenem. (Yusufetal ., 2017).

Although the prevalence of Gram-negative infections in COVID-19 patients was evaluated by many investigations, little information is currently available regarding Pseudomonas aeruginosa-caused infections in hospitalized COVID-19 patients. The purpose of this study is to look into the molecular type and patterns of antibiotic resistance of Pseudomonas aeruginosa isolates from patients with coronavirus disease-19.

2. MATERIALS AND METHODS

2.1. Collection of samples

Between October 2021 and June 2022, one hundred sputum samples were taken from patients with respiratory tract infections who were treated at Wasit Hospitals Province.

2.2. Identification of P. aeruginosa phenotype

MacConkey agar and blood agar were used to cultivate all of the obtained swabs, which were then incubated aerobically for 24 hours at 37°C. Bacterial characterisation was established based on biochemical, microscopy, and culture testing (Saleh et al., 2014). in addition to verifying using a Vittek 2 system identification kit.

2.3 .DNA extraction

Following the manufacturer's instructions, the extraction kit was used to extract and purify the

bacterial genomic DNA from *P. aeruginosa*.

2.4. Primers

Two PCR primers were used to amplify 16S rRNA of P. aeruginosa supplied by Alpha DNA/Canada. To obtained DNA fragment was 1500 bp (10). 27- Forward primer: 5-AGA GTT TGA TCC TGG TCA GAA CGC T-3 1492- Reverse primer: 5-TAC GGC TAC CTT GTT ACG ACT TCA CCC C-3

2.5.PCR master mix

The PCR mixture was made in PCR tubes that had the kit with the PCR components. The remaining components were added to the reaction mixture in accordance with the company's instructions, which are displayed in Table 1.

Table (1): PCR master mix components

Master mix components	Volume1 Sample (µl)
Master Mix	10 µl
Forward primer	1 µl
Reverse primer	10 µM
sterile deionized water	6 µl
DNA	2 µl
Total volume	20

Following the completion of the PCR mixture preparation, the tubes were sealed and gently swirled for five seconds using a vortex rotary mixer. To carry out the PCR thermocycler conditions, the tubes were moved to a PCR thermocycler.

2.6.Programs of PCR Thermocycler

PCR was performed using a PCR thermocycler. The device was programmed as in the following table (2).

Table (2): PCR Thermocycler program steps.

Steps	Temperature C	M : S	Cycle
Initial Denaturation	94	04 : 00	1
Denaturation	94	00 : 45	30
Annealing	*P. aeruginosa* gene 58	01 : 00	
Extension	72	01 : 30	

Final extension	72	10 : 00	1

One and a half percent agarose gel electrophoresis was used to detect the presence of amplification.

2.7.Antibiotic Test (Qualitative Disk Method)

Four antibiotic disks (imipenem , meropenem , ciprofloxacin and levofloxacin) were used to detect the sensitivity of isolates of Pseudomonas aeruginosa according to method described earlier (Bauer. 1966).

3.RESULTS AND DISCUSSION

3.1. Sampling

Sputum samples from one hundred patients at Wasit City hospitals were gathered. as shown in table (3). Twenty-seven (27%) of the local isolates were described based on microscopic and cultural characteristics. As shown in table (4) and figure (1), genus and species were identified through biochemical testing and confirmation using the vitek 2 system identification kit.

Table (3): Types of sample, number and Percentage of *Pseudomonas aeruginosa* isolated from Sputum samples of human .

Sample	Type	The number	Percentage
Clinical	Sputum	27	27 %

Table (4): VITEK ®2 technique, used for identification of *P. aeruginosa*

Test	Human
APPA	-
ADO	-
PyrA	-
IARL	-
Dcel	-
BGAL	-
H2S	-
BNAG	-
AGLTp	-
dGLU	+
GGT	+
OFF	-
BGLU	-
dMAL	-
dMAN	+

dMNE	+
BXYL	-
BAlap	+
ProA	+
LIP	+
PLE	-
TyrA	+
URE	-
dSOR	-
SAC	-
dTAG	-
dTRE	-
CIT	+
MNT	+
5KG	-
ILATK	+
AGLU	-
SUCT	+
NAGA	-
AGAL	-
PHOS	-
GIy A	-
ODC	-
LDC	-
IHISa	-
CMT	+
BGUR	-
O129R	-
GGAA	-
IML Ta	-
ELLM	-
ILATa	-

Selected Organism	99% Probability	Pseudomonas aeruginosa	
	Bionumber: 0003453103500240	Confidence:	Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																		
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Figure (1) : VITEK ®2 technique, used for identification of Pseudomonas aeruginosa

Suarez-Cuartin and other, (Suarez-Cuartin, et al ., 2018 ;Ali & Assafi . 2024 ;Rahman . 2006) agrees with our result of burn infections as indicated only 28.1% of the total, also Dos et al., (Dos ., 2023 ; Kolmos et al ., 1993). Established that burn represents 25-29%. This may be due to the high distribution of this organism around the hospital environment hence exposure risk to the burn wounds (Hancock . 1998). The main objective of the collection of samples was to isolate the bacteria *P.aeruginosa* and isolated the depending on the phenotypic characteristics of the developing colonies as the appearance of the center of Agar color pale color of the inability to ferment the sugar lactose located in the center of the plant and has a smell similar to the smell of grape fermented while the colonies appeared dark color and most surrounded by A clear halo on the center of the blood agars, indicating its ability to decompose blood The results of the biochemical tests showed positive results for the oxidase test. All the isolates were characterized by their inability to produce hydrogen sulfide gas and they were not fermented for sucrose and lactose and agrees with Mangalagiri and other (Mangalagiri, et al ., 2023 ; Su, et al ., 2018; Arai et al .,1970; Bongiovanni & Barda . 2023).

3.2. Antimicrobial susceptibility

The *P.aeruginosa* antimicrobial susceptibility test isolates shows that resistant to 100 % (imipenem , meropenem , ciprofloxacin and levofloxacin). The prevalence of MDR was 100% . agree with Lu He .,et al 2021 (Li et al ., 2022; Li et al 2022 ; de et al ., 2023; Heet al ., 2021; Onguru et al ., 2008; Aloush et al ., 2006). and in 2023 found Pseudomonas aeruginosa resistant to imipenem , meropenem , ciprofloxacin (de et al ., 2023) and other find meropenem-resistant *Pseudomonas Aeruginosa* (Gobezie, et al ., 2024) . Rehman and other isolated Pseudomonas aeruginosa and found resistant to ciprofloxacin (Rehmanet al ., 2019).

3.3. Molecular Identification of P. aeruginosa

Ten strains identified as P. aeruginosa after amplification of 16S rRNA genes using PCR technique. Molecular identification was done on human samples. When using 27- Forward primer and 1492-Reverse primer that detected Pseudomonas Aeruginosa , a band of approximately 1500 bp (Base pairs) band was observed on the agarose gel for isolates, (Figure : 2) that confirmed these isolates belonged to Pseudomonas Aeruginosa . This result is agreement with which mentioned by shuqian and other (Hu et al .,2022 ; Hussein et al .,2022 ; Altaai et al., 2014). In 2023 isolated from Contaminated Soil With Oil Residues and Identification Pseudomonas aeruginosa by 16s rRNA gene (Hussein et al ., 2023).

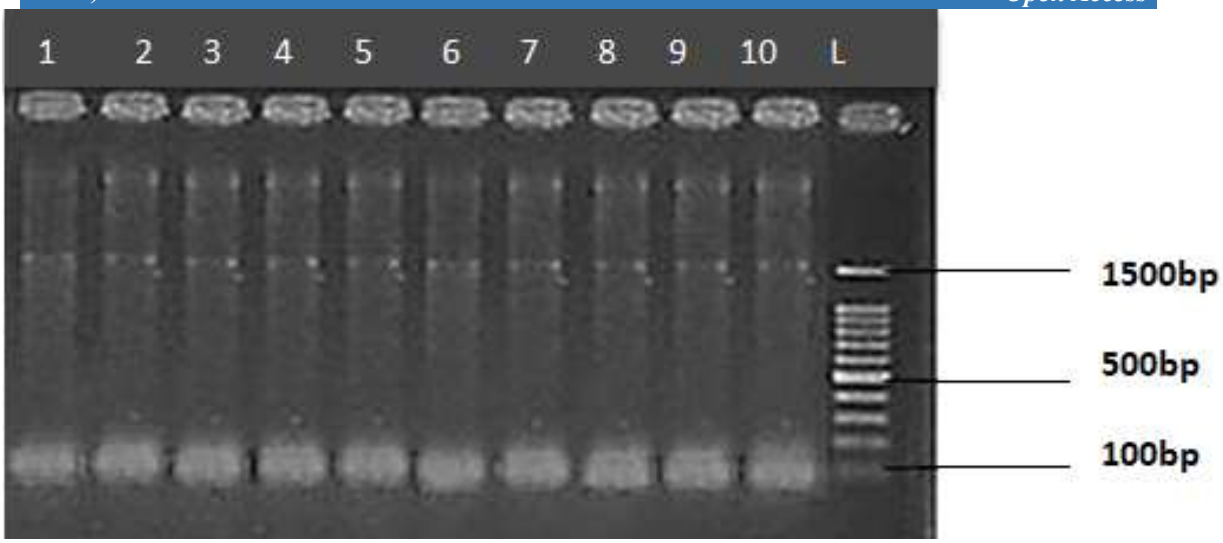

Figure (2): Amplification of Pseudomonas species primers for Pseudomonas Aeruginosa of human isolate was fractionated on 1.5 % agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Bind 1 resemble 1500 bp .

References

1 Kollef, M. H., Torres, A., Shorr, A. F., Martin-Loeches, I., & Micek, S. T. (2021). Nosocomial infection. Critical care medicine, 49(2), 169-187

2 Lucena, A., Dalla Costa, L. M., Nogueira, K. S., Matos, A. P., Gales, A. C., Paganini, M. C., ... & Raboni, S. M. (2014). Nosocomial infections with metallo-beta-lactamase-producing Pseudomonas aeruginosa: molecular epidemiology, risk factors, clinical features and outcomes. *Journal of Hospital Infection*, *87*(4), 234-240.

3 [Morales, E., Cots, F., Sala, M., Comas, M., Belvis, F., Riu, M., ... & Castells, X. (2012). Hospital costs of nosocomial multi-drug resistant Pseudomonas aeruginosa acquisition. BMC health services research, 12, 1-8.

4 Kaier, K., Heister, T., Götting, T., Wolkewitz, M., & Mutters, N. T. (2019). Measuring the in-hospital costs of Pseudomonas aeruginosa pneumonia: methodology and results from a German teaching hospital. BMC Infectious Diseases, 19, 1-8.

5 Vidal, F., Mensa, J., Almela, M., Martínez, J. A., Marco, F., Casals, C., ... & de Anta, M. T. J. (1996). Epidemiology and outcome of Pseudomonas aeruginosa bacteremia, with special emphasis on the influence of antibiotic treatment: analysis of 189 episodes. *Archives of internal medicine*, *156*(18), 2121-2126.

6 Pfaller, M. A., Jones, R. N., Doern, G. V., Kugler, K., & Group, T. S. P. (1998). Bacterial pathogens isolated from patients with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). Antimicrobial agents and chemotherapy, 42(7), 1762-1770.

7 Richards, M. J., Edwards, J. R., Culver, D. H., & Gaynes, R. P. (1999). Nosocomial infections in medical intensive care units in the United States. *Critical care medicine*, *27*(5), 887-892.

8 Bodey, G. P., Jadeja, L., & Elting, L. (1985). Pseudomonas bacteremia: retrospective analysis of 410 episodes. *Archives of internal medicine*, *145*(9), 1621-1629

9 Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., ... & Fridkin, S. K. (2014). Multistate point-prevalence survey of health care–associated infections. *New England Journal of Medicine*, *370*(13), 1198-1208.

10 Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J., ... & Sievert, D. M. (2016). Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *infection control & hospital epidemiology*, *37*(11), 1288-1301.

11 Bodey, G. P., Jadeja, L., & Elting, L. (1985). Pseudomonas bacteremia: retrospective analysis of 410 episodes. Archives of internal medicine, 145(9), 1621-1629.
12 Gottesman, T., Fedorowsky, R., Yerushalmi, R., Lellouche, J., & Nutman, A. (2021). An outbreak of carbapenem-resistant Acinetobacter baumannii in a COVID-19 dedicated hospital. *Infection prevention in practice*, *3*(1), 100113
13 Monnet, D. L., & Harbarth, S. (2020). Will coronavirus disease (COVID-19) have an impact on antimicrobial resistance?. *Eurosurveillance*, *25*(45), 2001886.
14 Campion, M., & Scully, G. (2018). Antibiotic use in the intensive care unit: optimization and de-escalation. *Journal of intensive care medicine*, *33*(12), 647-655.
15 Zhu, N. J., Rawson, T. M., Mookerjee, S., Price, J. R., Davies, F., Otter, J., ... & Holmes, A. (2022). Changing patterns of bloodstream infections in the community and acute care across 2 coronavirus disease 2019 epidemic waves: a retrospective analysis using data linkage. Clinical Infectious Diseases, 75(1), e1082-e1091
16 Sturm, L. K., Saake, K., Roberts, P. B., Masoudi, F. A., & Fakih, M. G. (2022). Impact of COVID-19 pandemic on hospital onset bloodstream infections (HOBSI) at a large health system. *American journal of infection control*, *50*(3), 245-249.
17 Amarsy, R., Trystram, D., Cambau, E., Monteil, C., Fournier, S., Oliary, J., ... & Billarant, S. V. (2022). Surging bloodstream infections and antimicrobial resistance during the first wave of COVID–19: a study in a large multihospital institution in the Paris region. *International Journal of Infectious Diseases*, *114*, 90-96.,
18 Hirabayashi, A., Kajihara, T., Yahara, K., Shibayama, K., & Sugai, M. (2021). Impact of the COVID-19 pandemic on the surveillance of antimicrobial resistance. *Journal of Hospital Infection*, *117*, 147-156.,
19 İpek, S., Şahin, A., Gungor, S., Yurttutan, S., Güllü, U. U., Inal, S., & Demiray, Ş. (2022). Nosocomial infections in non-COVID-19 pediatric patients prior to and during the pandemic in a pediatric intensive care unit. *Cureus*, *14*(1).
20 Winders, H. R., Bailey, P., Kohn, J., Faulkner-Fennell, C. M., Utley, S., Lantz, E., ... & Al-Hasan, M. N. (2021). Change in antimicrobial use during COVID-19 pandemic in South Carolina hospitals: a multicenter observational cohort study. *International Journal of Antimicrobial Agents*, *58*(6), 106453
21 Yusuf, E., Van Herendael, B., Verbrugghe, W., Ieven, M., Goovaerts, E., Bergs, K., ... & Goossens, H. (2017). Emergence of antimicrobial resistance to Pseudomonas aeruginosa in the intensive care unit: association with the duration of antibiotic exposure and mode of administration. *Annals of intensive care*, *7*, 1-7.
22 Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disc method. Am J clin pathol, 45, 149-158.
23 Saleh, I. A. A., Saleh, I. A. A., Saleh, N. A. A., & Mb, M. S. (2014). Bacteria and fungi associated with Acute Otitis media. International J. of Curr. t Engin. and Tech, 4(1), 316-318
24 Suarez-Cuartin, G., Giner, J., Merino, J. L., Rodrigo-Troyano, A., Feliu, A., Perea, L., ... & Sibila, O. (2018). Identification of Pseudomonas aeruginosa and airway bacterial colonization by an electronic nose in bronchiectasis. Respiratory Medicine, 136, 111-117.
25 ;Ali, S., & Assafi, M. (2024). Prevalence and antibiogram of Pseudomonas aeruginosa and Staphylococcus aureus clinical isolates from burns and wounds in Duhok City, Iraq. Journal of infection in developing countries, 18(1), 82–92. https://doi.org/10.3855/jidc.18193
26 Rahman, M.F. (2006). Genetic analysis of protease from Pseudomonas aeruginosa isolated from different human infections. M.Sc. thesis in Microbiology. College of science. University of Babylon, Iraq
27 Dos Santos Costa, J., Dos Santos, P. B., de Souza, A. T. H. I., Morgado, T. O., Cândido, S. L., Silva, T. R. D., Nakazato, L., & Dutra, V. (2023). KPC-2-producing Pseudomonas aeruginosa isolated from wild animals in Brazil. *Brazilian journal of microbiology :*

[publication of the Brazilian Society for Microbiology], *54*(4), 3307–3313. https://doi.org/10.1007/s42770-023-01143-7
28 Kolmos, H.J.; Thuesen, B; Nielsen, S.V.; Lohmann, M.; Kristofferesen, K. and Rosdahi, V.T. (1993). Outbreak of infection in burns unit to P. aeruginosa originating from contaminated tubing used for irrigation of patients. J. of Hospitsl Infection. 24:11-21
29 Hancock, R.E. (1998). Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative Gramnegative bacteria. Clinical Infectious Diseases. 27: (Suppl. 1) s93-s99
30 Mangalagiri, S., Sureshkhannan, S., Porteen, K., & Ezhilvelan, S. (2023). Isolation and Molecular Confirmation of Pseudomonas aeruginosa in Chicken meat, Mutton and Pork. ;
31 Su, S. S., Lae, K. Z. W., & Ngwe, H. (2018). Isolation and Identification of Pseudomonas aeruginosa from the Clinical Soil. *University of Yangon Res J*, *8*, 271-275.
32 Arai, T., Otake, M., Enomoto, S., Goto, S., & Kuwahara, S. (1970). Determination of Pseudomonas aeruginosa by biochemical test methods: II. Acylamidase test, a modified biochemical test for the identification of Pseudomonas aeruginosa. *Japanese journal of microbiology*, *14*(4), 279-284.
33 Bongiovanni, M., & Barda, B. (2023). Pseudomonas aeruginosa bloodstream infections in SARS-CoV-2 infected patients: a systematic review. Journal of clinical medicine, 12(6), 2252.)
34 Li, W. R., Zhang, Z. Q., Liao, K., Wang, B. B., Liu, H. Z., Shi, Q. S., ... & Xie, X. B. (2022). Pseudomonas aeruginosa heteroresistance to levofloxacin caused by upregulated expression of essential genes for DNA replication and repair. *Frontiers in Microbiology*, *13*, 1105921.
35 Li, W. R., Zhang, Z. Q., Liao, K., Wang, B. B., Liu, H. Z., Shi, Q. S., ... & Xie, X. B. Pseudomonas aeruginosa Heteroresistance to Levofloxacin Caused by Upregulated Expression of Essential Genes Involved in DNA Replication and Repair.
36 de Sousa, T., Silva, C., Alves, O., Costa, E., Igrejas, G., Poeta, P., & Hébraud, M. (2023). Determination of Antimicrobial Resistance and the Impact of Imipenem + Cilastatin Synergy with Tetracycline in Pseudomonas aeruginosa Isolates from Sepsis. Microorganisms, 11(11), 2687. https://doi.org/10.3390/microorganisms11112687 ,
37 He, L., He, A., & Qian, Y. (2021). Imipenem-resistant Pseudomonas aeruginosa treated with piperacillin/tazobactam in a patient with severe pneumonia: a case report and related literature review. Annals of Palliative Medicine, 10(1), 81017-81817.
38 Onguru, P., Erbay, A., Bodur, H., Baran, G., Akinci, E., Balaban, N., & Cevik, M. A. (2008). Imipenem-resistant Pseudomonas aeruginosa: risk factors for nosocomial infections. Journal of Korean medical science, 23(6), 982–987. https://doi.org/10.3346/jkms.2008.23.6.982
39 Aloush, V., Navon-Venezia, S., Seigman-Igra, Y., Cabili, S., & Carmeli, Y. (2006). Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. *Antimicrobial agents and chemotherapy*, *50*(1), 43–48. https://doi.org/10.1128/AAC.50.1.43-48.2006
40 de Sousa, T., Silva, C., Alves, O., Costa, E., Igrejas, G., Poeta, P., & Hébraud, M. (2023). Determination of Antimicrobial Resistance and the Impact of Imipenem + Cilastatin Synergy with Tetracycline in Pseudomonas aeruginosa Isolates from Sepsis. Microorganisms, 11(11), 2687. https://doi.org/10.3390/microorganisms11112687
41 Gobezie, M. Y., Hassen, M., Tesfaye, N. A., Solomon, T., Demessie, M. B., Kassa, T. D., ... & Belayneh, Y. M. (2024). Prevalence of meropenem-resistant Pseudomonas Aeruginosa in Ethiopia: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control*, *13*(1), 37.
42 Rehman, A., Patrick, W. M., & Lamont, I. L. (2019). Mechanisms of ciprofloxacin resistance in Pseudomonas aeruginosa: new approaches to an old problem. *Journal of medical microbiology*, *68*(1), 1–10. https://doi.org/10.1099/jmm.0.000873

43 Hu, S., Li, X., Yin, X., Li, R., Zhang, R., Zang, J., & Liu, Y. (2022). Species-specific identification of Pseudomonas based on 16S–23S rRNA gene internal transcribed spacer (ITS) and its combined application with next-generation sequencing. BMC microbiology, 22(1), 188.
44 Hussein, A. M., Muhialdin, A. J., Faraj, R. K., Majeed, N. R., & Hamad, W. N. (2022). 16 s rRNA sequencing for Pseudomonas aeruginosa isolated form clinical samples. Materials Today: Proceedings, 65, 2899-2903.
45 Altaai, M. E., Aziz, I. H., & Marhoon, A. A. (2014). Identification Pseudomonas aeruginosa by 16s rRNA gene for Differentiation from Other Pseudomonas Species that isolated from Patients and environment. *Baghdad Science Journal*, *11*(2), 1028-1034.
46 Hussein, Z. T., Mukhlif, S. S., Taha, M., & Ali, S. A. (2023, July). Identification and Characterization of Pseudomonas aeruginosa 16S rRNA Gene Isolated from Contaminated Soil With Oil Residues. In IOP Conference Series: Earth and Environmental Science (Vol. 1215, No. 1, p. 012001). IOP Publishing