Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Sanger sequencing reveals *Pseudomonas aeruginosa* as the most common cross-contamination of gastrointestinal endoscopy

Zainab H Majeed[1]*, Ahmed E Dhamad[2]

[1,2]Department of Biology, College of Science, Wasit University, IRAQ

*Corresponding Author: Zainab Hameed Majeed.

DOI: https://doi.org/10.31185/wjps.608
Received 21 November 2024; Accepted 24 December 2024; Available online 30 March 2025

ABSTRACT: *Pseudomonas aeruginosa* (*P. aeruginosa)* is a common cause of nosocomial infections. It has become a significant pathogen over the last two decades, accounting for 10% to 20% of infections in many hospitals. It demonstrates an inherent resistance to numerous antibiotics. This study aims to utilize the advanced molecular techniques to identify Pseudomonas spp. that causes cross-contamination of stomach biopsies specimens taken by gastrointestinal endoscopy and the resistance it shows to some commonly used antibiotics. A total of sixty-one biopsy tissue samples were taken from patients suffering from gastrointestinal disorder at Al-Karama and Al-Zahraa Teaching Hospitals. Bacterial species were identified by traditional techniques (Gram stain and biochemical tests) and molecular techniques (16S rRNA PCR-based techniques and Sanger sequencing) methods. On average 54.1% showed bacterial growth, 56.0% of them was identified as *Pseudomonas spp.* (*P. aeruginosa* and *P. nitroreducens*). In this study, *P. aeruginosa* was identified as the most prevalent contaminant associated with gastrointestinal endoscopies. It is known to cause opportunistic and severe co-infections in patients. As far as we are aware, this is the first report of its kind identified and studied *P. nitroreducens* as a contaminant in Iraqi patients. Antibiotic susceptibility test was done and isolates showed various resistance and sensitivity patterns (high antibiotic resistance to Azithromycin, Ceftriaxone, Erythromycin and Trimethoprim, and low antibiotic resistance to Amikacin, Gentamicin, Levofloxacin and Piperacillin-Tazobactam). This study highlights attention to the critical issue of contamination in gastrointestinal endoscopes due to inadequate sterilization. Molecular methods, especially Sanger sequencing proved more accurate detection than the traditional methods. To the best of our knowledge, this study reveals new bacterial species in in Iraqi hospitals. Additionally, the isolated bacterial species demonstrated various patterns of resistance and sensitivity based on antibiotic susceptibility testing.

Keywords: *Pseudomonas aeruginosa*, *Pseudomonas nitroreducens*, Gastrointestinal endoscopy contamination, Sanger sequencing, Antibiotic resistance.

1. INTRODUCTION

The *Pseudomonas* genus includes over 140 species, most of them are saprophytic, with over 25 species associated with humans, and the majority of Pseudomonas responsible for human diseases being associated with opportunistic infections [1]. *Pseudomonas* species are Gram-negative, catalase, oxidase and urease producing bacteria, non-glucose fermenter aerobic rods ranging from 0.5 - 0.8 µm in width and 1.5 - 3.0 µm in length. They move using a single polar flagellum [2]. *P. aeruginosa* is rarely present as a component of the typical microbial flora found in healthy individuals [3]. *P. aeruginosa* a nosocomial pathogen, has garnered the most attention due to its frequent role in human

*Corresponding author: zainab.hameed@uowasit.edu.iq

diseases. It is a widespread, an independent bacterium commonly found in diverse moist environments. Although it rarely causes illness in healthy individuals, it poses a significant risk to hospitalized patients, especially those with serious underlying conditions like cancer or burns [4]. It causes the most severe infections such as malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia [5]. The elevated mortality rate linked to *P. aeruginosa* infections is primarily due to compromised immune systems, its antibiotic resistance, and the production of toxic extracellular enzymes and substances [4,6]. *P. nitroreducens*, is a soil bacterium initially discovered in oil brine in Japan, and it can be found in river sediments [7].

P. nitroreducens is well-known for its ability to synthesize polyhydroxybutyrate homopolymers (polyesters) from medium-chain fatty acids, a trait utilized in the industrial production of polyesters [8]. *P. aeruginosa* contaminates medical devices by biofilm formation, which has served as a model organism for the biofilm research [9]. The concept of bacterial biofilms was first proposed in 1936. Bacteria easily attach to the moist surfaces, subsequently forming structured cell colonies embedded in a self-produced matrix, primarily made up of polysaccharides, which aid in attachment to both the surface and one another [10,11]. In clinical settings, various environments promote the ideal conditions for bacterial biofilm formation, including contact lenses, central venous catheters, urinary catheters, and others [12]. Recent studies have also confirmed the existence of biofilm on the surfaces of gastrointestinal endoscope channels [13]. Biofilms act as a reservoir for pathogenic bacteria, which can detach, return to their planktonic state, and infect patients. They also release endotoxins that can enter the bloodstream through damaged mucosal surfaces, potentially leading to systemic complications [14].

Sanger sequencing is a DNA sequencing technique that relies on chain termination during the DNA elongation, utilizing polymerase enzymes and specialized nucleotides [15]. It has long been the benchmark for accurately determining nucleic acid sequences, whether natural or synthetic. In laboratory medicine, sequence analysis is crucial for identifying emerging pathogens, discovering new genotypes of known pathogens, and monitoring significant evolutionary changes in pathogen genomes. It is also indispensable for confirming unusual laboratory findings, such as the detection of a pathogen in a new species or location. In the Sanger sequencing technique, amplified DNA or complementary DNA (cDNA) attaches to an oligonucleotide primer. DNA polymerase extends the strand by incorporating a mixture of four deoxynucleotide triphosphates (dNTPs: dGTP, dATP, dCTP, dTTP) along with chain-terminating dideoxynucleotide triphosphates (ddNTPs: ddGTP ddATP, ddCTP, ddTTP) [16], (figure 1).

This study investigates the contamination rates in gastrointestinal endoscopes at Al-Karama and Al-Zahraa teaching hospitals and explores how this increases the risk of bacterial infections. *P. aeruginosa* is the most common contaminant found in the endoscopy device compared to other growing species. To the best of our knowledge, this study represents the first documented instance of *P. nitroreducens* cultured from human stomach biopsies, as a rare type of bacteria. Furthermore, antibiotic susceptibility testing demonstrated that the isolated bacterial species exhibited different patterns of resistance and sensitivity to the most common therapeutic antibiotics.

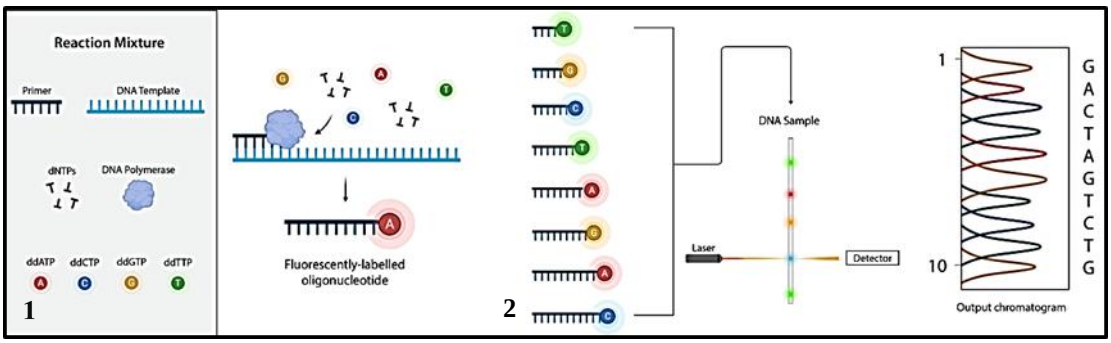

FIGURE 1: A detailed illustration showcasing the Sanger sequencing process conducted through capillary electrophoresis. (1) Chain-termination PCR using fluorescent ddntps, (2) Size separation and sequence analysis using capillary gel electrophoresis and fluorescence detection, adapted from BioRender [17].

2. MATERIALS AND METHODS

2.1 Samples collection

Samples were obtained from patients from both males and females at Al-Zahra and Al-Karama Teaching Hospitals in Wasit Governorate, Iraq. This study was conducted between October 2023 and March 2024. A total of 61 gastric

biopsy samples were collected from the gastric antrum or corpus of patients with gastrointestinal disorders who were suspected of having *Helicobacter pylori* infection. The biopsy samples were placed in tubes containing 2 mL of sterilized brain heart infusion broth (BHI) as a transport medium and were delivered to the laboratory within 2 hours, using a cold box to maintain proper conditions for culturing.

2.2 Bacterial culture and preservation

Biopsy tissues were homogenized and cultured on standard Columbia agar. The agar plates were incubated at 37°C, both in ambient air and in a carbon dioxide-humidified environment. Colonies that grew were stored for short-term and long-term use by transferring the bacteria to Columbia agar slant tubes and 10% glycerol/BHI tubes, respectively. The slant tubes were kept at 2-4°C, while the glycerol/BHI tubes were stored at -80°C.

2.3 Bacterial identification

A. Traditional identification

To identify the bacteria, Gram staining and biochemical tests were carried out. The catalase test involved adding a drop of hydrogen peroxide (H_2O_2) onto a glass slide, then mixing a small part of the bacterial colony with the drop using a disposable loop. The formation of bubbles within 30 seconds indicates a positive result [18]. The oxidase test involved applying a drop of oxidase enzyme reagent to a small amount of bacterial colony on filter paper. A color change to purple within 10 seconds indicates a positive result [19]. For the urease test, several colonies were cultured on urea agar base and were incubated at 37°C for 24 hours. A color change to red or pink signifies a positive result. Positive and negative controls were included to validate the tests [20].

B. Molecular identification

- **Extraction of DNA and its integrity checking**

DNA was extracted using specialized kits from Scientific Research Company. Following the provided protocol, bacterial isolates were first cultured on Columbia base agar. Then, 1 ml of BHI broth was added to sterile tubes, and cells were harvested by centrifugation. Lysis and binding were carried out using the appropriate buffers, and the lysate was passed through a spin-DNA column with subsequent washing and drying steps. The purified DNA was eluted in 50 µl of preheated elution buffer and stored at -20ºC. The amount and integrity of the extracted DNA were evaluated using a Quantus™ Fluorometer (Promega, USA). The samples were prepared in 1X TE buffer and loaded into the fluorometer. The manufacturer's instructions were followed to evaluate the DNA integrity.

Polymerase Chain Reaction (PCR)

The PCR technique was utilized for molecular detection based on the 16S rRNA gene. The primer pairs used in this study were as follows: 22F: GCTAAGAGATCAGCCTATGTCC and 22R: [TGGCAATCAGCGTCAGGTAATG. The 25 µl PCR reaction mixture contained 12.5 µl of 2x PCR Master Mix containing Taq polymerase (Promega company, M7822), 1 µl of extracted DNA, 1 µl of each primer, and 9.5 µl of DNase-free water. The PCR thermal cycling settings were as follows: DNA was initially denatured for three minutes at 94°C, this was followed by 40 cycles, including denaturation at 94°C for 30 seconds, primer annealing at 58°C for 30 seconds, extension at 72°C for one minute, and a final extension at 72°C for five minutes. The PCR product was then analyzed by a 0.5% agarose gel containing ethidium bromide was used, along with the GeneRuler 100 bp DNA ladder, for staining and assessing the quantity and quality of the PCR results [21].

Sanger sequencing

DNA sequencing-based bacterial identification offers greater objectivity and precision compared to traditional methods, particularly in classifying rare microorganisms that may emerge as pathogens in immunocompromised individuals [22]. Two primer pairs (758F, 907R, 27F, and 1492R) used in this study targeted the 16S rRNA gene (Table 1). The technique was carried out at Macrogen Inc. (Korea) following their standard protocol.

Table 1. Primer pairs information.

Primers name	Primer sequence (5'-3')	Reference
758F	GGA TTA GAT ACC CTG GTA	Macrogen
907R	CCG TCA ATT CMT TTR AGT TT	
27F	AGA GTT TGA TCM TGG CTC AG	
1492R	TAC GGY TAC CTT GTT ACG ACT T	

2.4 Antibiotic Susceptibility Test

Antimicrobial susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method with eight antibiotics: Gentamicin, Amikacin, Ceftriaxone, Levofloxacin, Azithromycin, Erythromycin, Piperacillin-Tazobactam, and Trimethoprim (Table 2). These antibiotics represent six distinct classes: aminoglycosides, cephalosporin, fluoroquinolones, macrolides, penicillin-β-lactamase inhibitors, and sulfonamides, frequently utilized antibiotics following the guidelines set by the Clinical Laboratory Standards Institute (CLSI) in 2020 [23]. In brief, freshly cultured bacterial colonies on nutrient agar were used to perform the antibiotic susceptibility test. The isolates were suspended in sterile normal saline and standardized to 0.5% McFarland, equivalent to approximately 1.5×10^8 CFU/ml. A volume of 0.2 ml of culture suspension was uniformly applied to a sterile Mueller-Hinton (MH) agar plate (Liofilchem, Italy). Sterile antibiotic discs (Liofilchem, Italy) were placed on the agar using sterile forceps under aseptic conditions, followed by incubation at 37°C for 24 hours. After incubation, the inhibition zones were measured in millimeters (mm) [24].

Table 2: Details of antibiotics, including their classification, abbreviations, and disc concentrations.

Antibiotic Class	Antibiotic Name	Abbreviations	Disc Concentration (µg)
Aminoglycoside	Gentamicin	CN	10
	Amikacin	AK	30
Cephalosporin	Ceftriaxone	CRO	30
Fluoroquinolones	Levofloxacin	LEV	5
Macrolide	Azithromycin	AZM	15
	Erythromycin	E	15
Penicillin-βlactamase	Piperacillin-Tazobactam	TZP	110
Sulfonamides	Trimethoprim	TM	5

3. RESULTS AND DISSCUSIONS

3.1 Isolation and molecular diagnosis

The study involved 61 patients presenting with diagnosed. gastrointestinal disorders, including abdominal pain, diarrhea, vomiting, anorexia, and stomach ulcers. Of these, the majority (58.7%) were females. The collected samples were carefully transported under sterile conditions and immediately cultured on Columbia base agar. Bacterial growth was successfully confirmed in 54.1% of Stomach tissue samples. Despite prolonged preservation, the bacteria retained their viability for growth for both middle and longtime storages. These samples underwent both traditional and molecular identification methods. Gram staining revealed that all isolates were gram-negative and rod-shaped (Figure 2). Additionally, the isolates tested positive for catalase, oxidase and urease activity [25]. The integrity of the extracted DNA, measured using Quantus™ fluorometers, showed an average concentration of 15 ng/µL across all samples. Bacterial species identification was conducted based on the results of PCR and Sanger sequencing. The purified DNA was sent to Macrogen Company, primer pairs (758F, 907R, 27F, and 1492R) were utilized for the analysis. 92.8% were identified as *Pseudomonas aeruginosa* and 7.2% as *Pseudomonas nitroreducens*. The Sanger sequencing results of 16s rRNA gene were compared with worldwide bacteria database from NCBI to draw species trees (Figure 3).

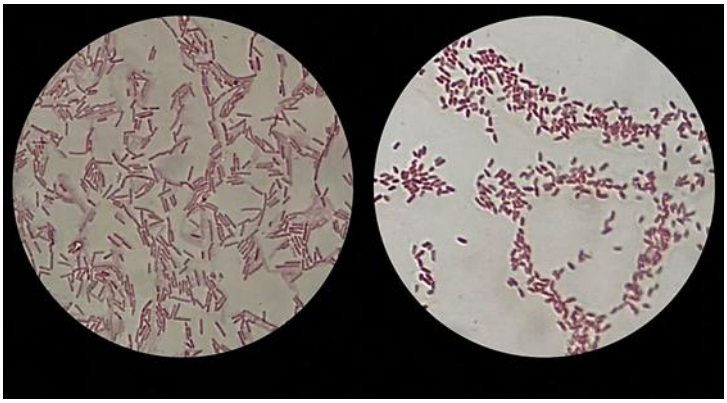

FIGURE 2: Gram staining results of, (a) *P. aeruginosa* and (b) *P. nitroreducens.*

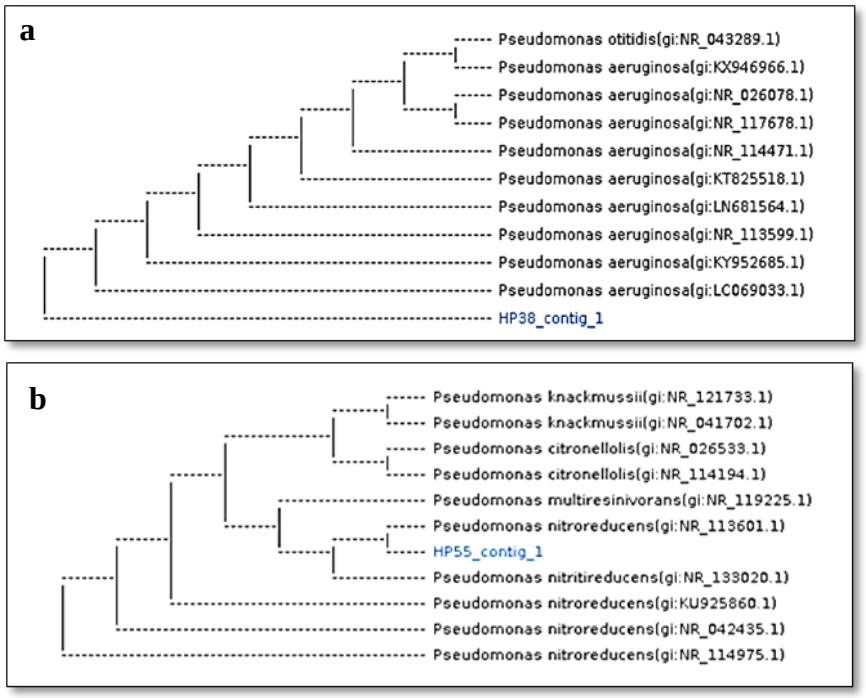

FIGURE 3: Phylogenetic trees. These trees illustrate the sanger sequence results for isolated and identified bacteria (a) *P. aeruginosa* and (b) *P. nitroreducens.*

Pseudomonas spp. are widespread microorganisms capable of affecting individuals with compromised immune systems and are the common cause of hospital-acquired infections [26]. *Pseudomonas sp.* exhibits exceptional metabolic versatility and adaptability, enabling them to colonize a wide range of ecological environments, including water, soil, and animals. It is also notable for their inherent resistance to various antimicrobial agents [27]. *P. aeruginosa* and *P. nitroreducens* cause many opportunistic and nosocomial infections such as, endocarditis, meningitis, pneumonia, and septicemia. They cause severe infection like malignant external otitis and endophthalmitis as well [28]. Also, Previous studies have demonstrated that the urease activity of bacteria that colonize in gastric mucosa, (such as, *Pseudomonas spp.*), affect the diagnosis of *Helicobacter pylori* and give a false positive result in urea breath urease [29], This may cause a serious medical and treatment problem. Based on statistical analysis (Figure 4), of endoscopy-related contamination in AL-Karama Teaching Hospital and AL-Zahraa Teaching Hospital, both hospitals have high contamination rates due to improper sterilization protocols and improper cleaning practices during endoscopy procedures. While AL-Zahraa hospital still faces significant issues, its contamination rate is lower compared to AL-Karama hospital. This slight difference may be attributed to more consistent, albeit still insufficient, sterilization efforts or marginal improvement in staff compliance with hygiene procedures.

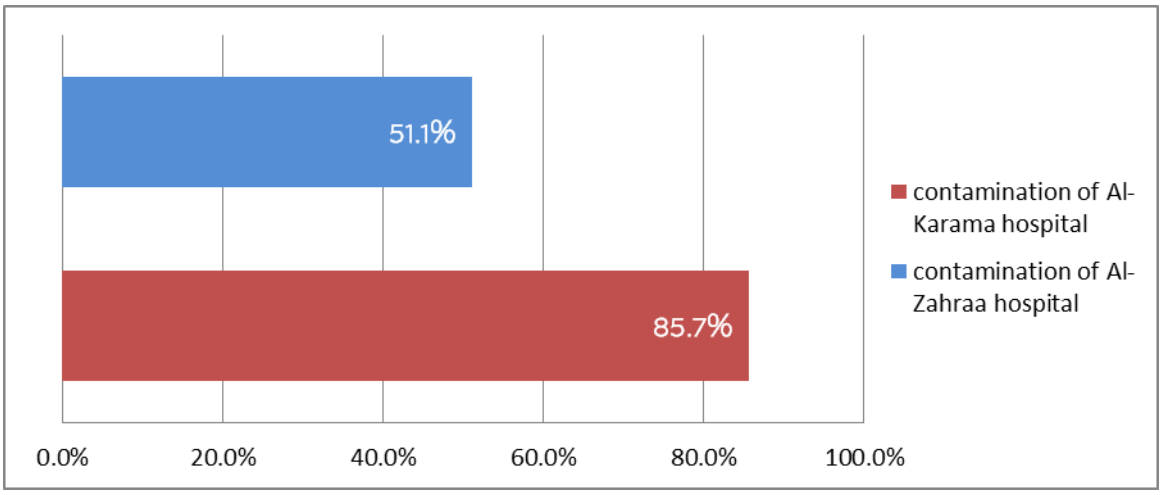

FIGURE 4: Shows the ratio of contamination to the number of samples taken in Al-Karama and Al-Zahraa teaching hospital.

3.2 Antibiotic susceptibility test

The bacterial isolates exhibited varying levels of susceptibility and resistance to the antibiotics tested.(Table 4), (figure 5).

Bacterial species	Antibiotics															
	Azithromycin		Amikacin		Ceftriaxone		Erythromycin		Gentamicin		Levofloxacin		Piperacillin-Tazobactam		Trimethoprim	
	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)
P. aeruginosa	0	91.6	100	0	8.3	91.6	0	100	83.3	8.3	100	0	100	0	0	100
P. nitroreducens	0	100	100	0	0	100	0	100	100	0	100	0	100	0	0	100

NOTE: 8.3% of *P.aeruginosa* had an intermediate response to Azithromycin and Gentamycin.

According to the results which were illustrated in (table 4), *P. aeruginosa* isolates showed 100% sensitivity to Amikacin, Levofloxacin and Piperacillin-Tazobactam, and 83% to Gentamicin. *P. nitroreducens* was 100% sensitive to Amikacin, Levofloxacin and Piperacillin-Tazobactam, but it differed from *P. aeruginosa* in its 100% sensitivity to Gentamicin and Trimethoprim. Amikacin mechanism of action attaches to the 30S subunit of the bacterial ribosome. disrupting the reading of the genetic code and inhibiting protein synthesis. This interference leads to premature termination of protein production and the incorporation of incorrect amino acids [30]. Levofloxacin disrupts essential bacterial processes, including DNA replication, transcription, repair, and recombination, by inhibiting type II topoisomerases. It serves as an effective therapeutic option for treating severe Gram-negative hospital acquired infections [31]. Piperacillin, a β-lactam antibiotic, combined with Tazobactam, a β-lactamase inhibitor, functions as a non-reversible inhibitor of bacterial β-lactamases. This combination is highly effective in treating intra-abdominal infections, infections of the skin and soft tissues, lower respiratory tract infections, and complicated urinary tract infections. offering broad-spectrum coverage against resistant pathogens [32]. Gentamicin crosses the gram-negative membrane through an oxygen-dependent active transport mechanism [33]. The results of current study are in line with the recent research by Farhan, *et al*. (2021), Anderson, *et al*. (2008), Daley, *et al* (1996) and Charles *et al.* (1971) [34,35,36,37]. Nageeb, *et al.* (2022), Lepe, *et al.* (2022), Gin, *et al.* (2007), and Ahmed, *et al.* (1989), on the other hand, reported the high-level resistance to these antibiotics [38,39,40,41].

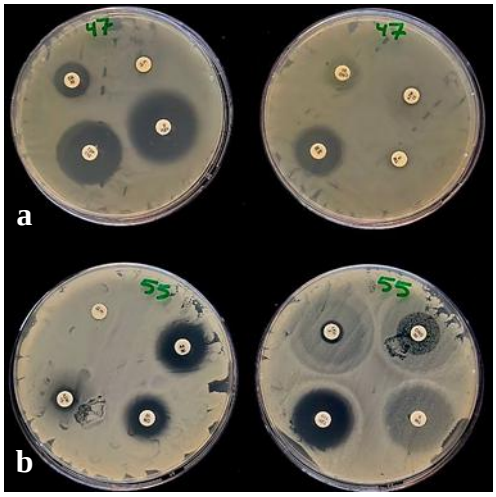

FIGURE 5: Sensitivity of (a) *P. aeruginosa* and (b) *P. nitroreducens* to Amikacin, Gentamycin, Levofloxacin and Piperacillin-Tazobactam.

P. aeruginosa and *P. nitroreducens* showed high resistant rates to Azithromycin, Ceftriaxone, Erythromycin and Trimethoprim. The mechanisms of these antibiotics include: suppression of bacterial protein synthesis, reduction of proinflammatory cytokine production, prevention of neutrophil infiltration, and alteration of macrophage polarization by Azithromycin,[42]. However; Ceftriaxone inhibits the bacterial cell wall synthesis by disturbing the mucopeptide synthesis [43]. They specifically target the 23S rRNA in the bacterial 50S ribosomal subunit. Erythromycin prevents the synthesis of proteins [44], and just the same, Trimethoprim acts by Inhibiting the transformation of dihydrofolate into tetrahydrofolate**,** the biologically active form of the folic acid, in susceptible organisms [45]. *Pseudomonads* employs a variety of innate and acquired resistance mechanisms, including antibiotic inactivation, alteration of drug targets, reduceducing the membrane permeability, activating efflux pump systems, biofilm formation, and quorum-sensing pathways. These strategies work in tandem to significantly decrease its susceptibility to antibiotics, making it highly resistant to treatment [46]. Antivirulence therapies focus on neutralizing these factors to reduce bacterial harm while allowing the immune system to eliminate the infection. Unlike traditional antibiotics, this approach exerts less selective pressure, thereby reducing the likelihood of drug resistance development [47]. Biofilm formation enables pathogenic bacteria to survive under adverse conditions such as temperature changes, limited nutrients,exposure to antibiotics, and enhance their persistence on both living and non-living surfaces [48]. Biofilm formation is a defining characteristic of *P. aeruginosa*, with its complex and organized biofilms frequently observed in patients suffering from chronic infections [49]. It may be the primary reason for the widespread of *P. aeruginosa* as contaminants in medical devices, especially gastrointestinal endoscopes, as inefficient sterilization and not drying them well during storage, providing a suitable environment for biofilm formation.

4. CONCLUSIONS

This study highlights the critical problem of contamination in gastrointestinal endoscopy linked to *P. aeruginosa*, emphasizing the role of insufficient sterilization protocols in contributing to hospital-acquired infections. The results show that *P. aeruginosa* is the most common contaminant, presenting a significant threat to patient safety due to its strong resistance to various antibiotics. Additionally, the study identifies *P. nitroreducens* as a rare but noteworthy contaminant, detected using advanced molecular techniques like Sanger sequencing, underscoring the value of modern tools for precise bacterial identification. The antibiotic resistance patterns identified in these bacterial isolates highlights the urgent need for stronger infection control measures, prudent antibiotic usage, and the exploration of alternative treatment options. Additionally, the ability of these bacteria to form biofilms further complicates their removal, as it enables them to withstand unfavorable conditions and resist standard sterilization methods. To address these challenges, future efforts should focus on enhancing sterilization protocols, especially for medical equipment like endoscopes, to reduce contamination rates. Research into new therapies, particularly those targeting bacterial biofilms and resistance mechanisms, is essential to prevent the spread of these infections. This study provides valuable insights into the risks associated with current practices and the urgent need for innovative solutions in healthcare setting.

REFERENCES

[1] Iglewski BH. Pseudomonas. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 27. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8326/.

[2] Yang L, Jelsbak L, Marvig RL, Damkiær S, Workman CT, Rau MH, et al. Evolutionary dynamics of bacteria in a human host environment. Proc Natl Acad Sci U S A. 2011; 108:7481–6. doi: 10.1073/pnas.1018249108.

[3] Lusis, P. I., & Soltys, M. A. (1971). Pseudomonas aeruginosa.

[4] Fazeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. J Res Med Sci. 2012 Apr;17(4):332-7. PMID: 23267393; PMCID: PMC3526125.

[5] Bodey GP, Bolivar R, Fainstein V, Jadeja L. Infections caused by Pseudomonas aeruginosa. Rev Infect Dis. 1983 Mar-Apr;5(2):279-313. doi: 10.1093/clinids/5.2.279. PMID: 6405475.

[6] Harold C. Neu, The role of Pseudomonas aeruginosa in infections, Journal of Antimicrobial Chemotherapy, Volume 11, Issue suppl_B, 1983, Pages 1–13, https://doi.org/10.1093/jac/11.suppl_B.1.

[7] Itoh N, Akazawa N, Yamaguchi M, Murakami H, Ohkusu K. Cholangitis with bacteremia due to Pseudomonas nitroreducens in a patient with pancreatic neuroendocrine tumors: a case report. BMC Infect Dis. 2024 Feb 9;24(1):180. doi: 10.1186/s12879-024-09092-8. PMID: 38336644; PMCID: PMC10858522.

[8] Yao J, Zhang G, Wu Q, Chen GQ, Zhang R. Production of polyhydroxyalkanoates by Pseudomonas nitroreducens. Antonie Van Leeuwenhoek. 1999; 75:345–9. doi: 10.1023/A:1002082303615.

[9] Ethan E. Mann, Daniel J. Wozniak, Pseudomonas biofilm matrix composition and niche biology, FEMS Microbiology Reviews, Volume 36, Issue 4, July 2012, Pages 893–916, https://doi.org/10.1111/j.1574-6976.2011.00322.x.

[10] Zobell CE, Anderson DQ. Observations on the multiplication of bacteria in different volumes of stored seawater and the influence of oxygen tension and solid surfaces. Biol Bull Woods Hole. 1936; 71:324–342.

[11] Vickery K, Pajkos A, Cossart Y. Removal of biofilm from endoscopes: evaluation of detergent efficiency. Am J Infect Control. 2004; 32:170–176. doi: 10.1016/j.ajic.2003.10.009.

[12] Donlan RM. Biofilms and device-associated infections. Emerg Infect Dis. 2001;7:277–281. doi: 10.3201/eid0702.010226.

[13] Alvarado CJ, Reichelderfer M. APIC guideline for infection prevention and control in flexible endoscopy. Association for Professionals in Infection Control. Am J Infect Control. 2000;28:138–155.

[14] Bloss R, Kampf G. Test models to determine cleaning efficacy with different types of bioburden and its clinical correlation. J Hosp Infect. 2004;56 Suppl 2: S44–S48. doi: 10.1016/j.jhin.2003.12.029.

[15] Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74(12):5463-5467.

[16] Sanger F, et al. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5463–5467.

[17] AAT Bioquest. (n.d.). Sanger sequencing. Retrieved November 29, 2024, from AAT Bioquest catalog.

[18] Cappuccino, J. G., & Sherman, N. (2014). Microbiology: A Laboratory Manual (10th ed.).

[19] Madigan, M. T., Martinko, J. M., & Bender, K. S. (2014). Brock Biology of Microorganisms (14th ed.). Pearson.

[20] Jorgensen, J. H., & Pfaller, M. A. (2015). Manual of Clinical Microbiology (11th ed.). American Society for Microbiology.

[21] Kaiser, K., & Wang, X. (2020)."Gel Electrophoresis of Nucleic Acids," in Methods in Molecular Biology, Vol. 2061, Springer.

[22] Binnicker, M. J. (2020). "Molecular diagnostic methods for the identification of bacterial pathogens." Clinical Microbiology Reviews, 33(3), e00059-19.

[23] CLSI. Performance standard for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100 Wayne, PA:Clinical and Laboratory Standard Institute; 2020.

[24] Wikipedia contributors. (2024, July 13). Disk diffusion test. In Wikipedia, The Free Encyclopedia. Retrieved13:27,November21,2024,from https://en.wikipedia.org/w/index.php?title=Disk_diffusion_test&oldid=1234356214.

[25] Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A, et al. Genetic features of pseudomonas aeruginosa isolates from Cystic Fibrosis patients compared with those isolates from other origins. J Med Microbiol. 2004;53:73–81. doi: 10.1099/jmm.0.05324-0.

[26] Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A, et al. Genetic features of pseudomonas aeruginosa isolates from Cystic Fibrosis patients compared with those isolates from other origins. J Med Microbiol. 2004;53:73–81. doi: 10.1099/jmm.0.05324-0.

[27] Corona-Nakamura AL, Miranda-Novales MG, Leaños-Miranda B, Portillo-Gómez L, Hernández-Chávez A, Anthor-Rendón J, et al. Epidemiologic Study of Pseudomonas aeruginosa in Critical Patients and Reservoirs. Arch Med Res. 2001;32:238–42. doi: 10.1016/s0188-4409(01)00267-3.

[28] Bodey GP, Bolivar R, Fainstein V, Jadeja L. Infections caused by Pseudomonas aeruginosa. Rev Infect Dis. 1983 Mar-Apr;5(2):279-313. doi: 10.1093/clinids/5.2.279. PMID: 6405475.

[29] T1 - Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection, VL - 57, DO - 10.1099/jmm.0.47768-0 JO - Journal of medical microbiology ER -.

[30] Sizar O, Rahman S, Sundareshan V. Amikacin. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430908/.

[31] Rang HP, Ritter JM, Flower RJ, Henderson G. Rang & Dale's Pharmacology. 9th ed. Elsevier; 2019.

[32] Lau WMercer D, Itani KM, Nicolau DP, Kuti JL, Mansfield D, Dana A.2006.Randomized, Open-Label, Comparative Study of Piperacillin-Tazobactam Administered by Continuous Infusion versus Intermittent Infusion for Treatment of Hospitalized Patients with Complicated Intra-Abdominal Infection. Antimicrob Agents Chemother50:.https://doi.org/10.1128/aac.00329-06.

[33] Chaves BJ, Tadi P. Gentamicin. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2023. PMID: 32491482.

[34] Farhan, S. M., Raafat, M., Abourehab, M. A. S., Abd El-Baky, R. M., Abdalla, S., EL-Gendy, A. O., & Azmy, A. F. (2021). Effect of Imipenem and Amikacin Combination against Multi-Drug Resistant Pseudomonas aeruginosa. Antibiotics, 10(11), 1429. https://doi.org/10.3390/antibiotics10111429.

[35] Anderson, V.R., Perry, C.M. Levofloxacin. Drugs 68, 535–565 (2008). https://doi.org/10.2165/00003495-200868040-00011.

[36] D. Daley, L. Mulgrave, R. Munro, S. Neville, H. Smith, W. Dimech, An evaluation of the in vitro activity of piperacillin/tazobactam, Pathology, Volume 28, Issue 2,1996, Pages 167-172, ISSN 0031-3025, https://doi.org/10.1080/00313029600169813.

[37] Charles F. T. Snelling, Allan R. Ronald, Claudette Y. Cates, William C. Forsythe, Resistance of Gram-Negative Bacilli to Gentamicin, The Journal of Infectious Diseases, Volume 124, Issue Supplement_1,December1971,PagesS264S270, https://doi.org/10.1093/infdis/124.Supplement_1.S264.

[38] Nageeb WM, Hetta HF (2022) The predictive potential of different molecular markers linked to amikacin susceptibility phenotypes in Pseudomonas aeruginosa. PLoS ONE 17(4): e0267396. https://doi.org/10.1371/journal.pone.0267396.

[39] J.A. Lepe, L. Martínez-Martínez, Resistance mechanisms in Gram-negative bacteria, Medicina Intensiva (English Edition), Volume 46, Issue 7,2022,Pages 392-402,ISSN 2173-5727, https://doi.org/10.1016/j.medine.2022.05.004.

[40] Gin, A., Dilay, L., Karlowsky, J. A., Walkty, A., Rubinstein, E., & Zhanel, G. G. (2007). Piperacillin–tazobactam: a β-lactam/β-lactamase inhibitor combination. Expert Review of Anti-Infective Therapy, 5(3), 365–383. https://doi.org/10.1586/14787210.5.3.365.

[41] Ahmed Moaz, Kevin Shannon, Ian Phillips, Mechanisms of gentamicin resistance in Gram-negative bacilli in Riyadh, Kingdom of Saudi Arabia, Journal of Antimicrobial Chemotherapy, Volume 24, Issue 5, November 1989, Pages 689–698, https://doi.org/10.1093/jac/24.5.689.

[42] Heidary M, Ebrahimi Samangani A, Kargari A, Kiani Nejad A, Yashmi I, Motahar M, Taki E, Khoshnood S. Mechanism of action, resistance, synergism, and clinical implications of azithromycin. J Clin Lab Anal. 2022 Jun;36(6):e24427. doi: 10.1002/jcla.24427. Epub 2022 Apr 21. PMID: 35447019; PMCID: PMC9169196.

[43] Samantha R. Phillips Pharm.D., BCIDP, ... Sara Groome Pharm.D, in Side Effects of Drugs Annual, 2021

[44] Aniqa Ashraf, Guijian Liu, Balal Yousaf, Muhammad Arif, Rafay Ahmed, Samina Irshad, Ayesha Imtiyaz Cheema, Audil Rashid, Humaira Gulzaman, Recent trends in advanced oxidation process-based degradation of

erythromycin: Pollution status, eco-toxicity and degradation mechanism in aquatic ecosystems, Science of The Total Environment, Volume 772,2021,145389, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2021.145389.

[45] Gleckman R, Blagg N, Joubert DW. Trimethoprim: mechanisms of action, antimicrobial activity, bacterial resistance, pharmacokinetics, adverse reactions, and therapeutic indications. Pharmacotherapy. 1981 Jul-Aug;1(1):14-20. doi: 10.1002/j.1875-9114. 1981.tb03548. x. PMID: 6985448.

[46] Dao, N., Joshi-Datar, A., Lepine, F., Bauerle, E., Olakanmi, O., Beer, K., et al. (2011). Active Starvation Responses Mediate Antibiotic Tolerance in Biofilms and Nutrient-Limited Bacteria. Science 334 (6058), 982–986. doi: 10.1126/science.1211037.

[47] Liao Chongbing, Huang Xin, Wang Qingxia, Yao Dan, Lu Wuyuan, Virulence Factors of Pseudomonas Aeruginosa and Antivirulence Strategies to Combat Its Drug Resistance, JOURNAL=Frontiers in Cellular and Infection Microbiology, VOLUME=12,2022, URL=https://www.frontiersin.org/journals/cellular-and-infection, microbiology/articles/10.3389/fcimb.2022.926758, DOI=10.3389/fcimb.2022.926758.

[48] Rollet, C., Gal, L., Guzzo, J. (2009). Biofilm-Detached Cells, a Transition from a Sessile to a Planktonic Phenotype: A Comparative Study of Adhesion and Physiological Characteristics in Pseudomonas Aeruginosa. FEMS Microbiol. Lett. 290 (2), 135–142. doi: 10.1111/j.1574-6968.2008.01415. x.

[49] Mann, E. E., Wozniak, D. J. (2012). Pseudomonas Biofilm Matrix Composition and Niche Biology. FEMS Microbiol. Rev. 36 (4), 893–916. doi: 10.1111/j.1574-6976.2011.00322. x.

.