DOI: https://doi.org/10.53350/pjmhs22166587

ORIGINAL ARTICLE

Molecular Detection of Pseudomonas Aeruginosa Isolated from Different Clinical Cases and Test Antibiotics Sensitivity on the Bacterial Growth

DUAA ADILABDULHADY[1], HADAF MAHDI KADHIM[2]
[1-2]Department of Biology, College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.
Corresponding author: Duaa Adilabdulhady, Email: sci.bio.mas.20.27@qu.edu.iq

ABSTRACT

150 samples were collected from different clinical causes of hospitals in Al-Diwaniyah city. All samples were cultured on blood agar and Maconkey agar medium and incubated for 24 hat 37°C. The results showed 112 (74.6%) bacterial growth. Initially, identification of the bacterial isolates were carried out viabiochemical tests, 25 isolates Pseudomonasaeruginosa were detected. Identity was confirmed by polymerase chain reaction (PCR) targeting the areE genewith size 498pb. This bacterium was isolated from burn infection 30% and wound infection 20%, while it was isolated from Respiratorytract 16.6%.

The antibiotics susceptibilitytest for 16 bacterial isolates have been tested. It has been found that the bacterial isolates were resistance 100% for Ampicillin, Amoxicillin, Piperacillin, Cefotaxime and Cefepim and 18.75% forMeropenem. While they were sensitive 100% for Amikacin.

Keywords: antibiotics, areE gene, Pseudomonas aeruginosa, PCR.

INTRODUCTION

Pseudomonasaeruginosais Gram negative, motile, straight or curved rods, non spore forming(Ryan and Ray, 2004). This bacterium grows in a thermal range ranges from 37°C- 42°C and its growth at up42°C distinguishes them from the rest of the types of bacteria (Mietzneretal., 2016).P.aeruginosa is positive for catalase and oxidase, it is similar toEnterobacteriace, but differed as obligately aerobes as possible to obtain energy from carbohydrates with oxidation but it isnonfermenters (Levinson, 2016)**.**This bacterium produces two types of useful pigments in clinical and laboratory diagnosis, pyocyanin and Pyoverdin dye (Blue green, an important means of diagnosis of these bacteria, which is the only type that produces pyocyanin(Levinson, 2014).

This organism is opportunistic pathogen, particularly in hospitals; in patients with malignant and hematologic disease; nosocomial infections from lumbar punctures and catheterizations; in patients given prolonged treatment with immunosuppressive agents, radiation and antibiotics; as well as it may contaminate surgical wound, burns, abscesses. It also cause ear infection, cystic fibrosis and lungs of patients treated with antibiotics (Madigan et al., 2003).P.aeruginosais naturally resistance to many of widely used antibiotics

Antibiotics are divided into bactericidaand Bacteriostatic. the bactericida has the ability to kill bacteria and prevent their growth again such as penicillins, cephalosporins andaminoglycosides group, while Bacteriostatic is stopping bacterial grouth such astetracyclines and chloramphenicol(Laurence et al., 1997). This study aimed to detect P.aeruginosathat isolated from different cases in Diwaniyahcity by biochemical tests and PCR as well as test antibiotics sensitivity on the bacterial growth

MATERIALS AND METHODS

Sample collection: 150 samples were collected from some clinical resources in hospitals of Al-Diwaniyah city from Sep. 2021 to Jan. 2022.

Isolation and identification of bacterial isolates: All samples were cultured on blood agar and Maconkey agar, then incubated for 24 hrsat 37°C. All bacterial isolates were streaked on the medium for pure cultures, stained slide smears from these cultures were examined with objective lens zoom power X100 of microscope for Gram stain. Further characters that used for identification of the isolates including the following biochemical tests (Indol production test, Vogesproskauer and methyl red test, Oxidase,Citrate utilization, Motiliy, Urease, Catalase and Triple sugar iron test (TSI)).

Bacterial genomic DNA extraction: Genomic DNA has been extracted from bacterial isolates via (Genomic DNA extraction kit) depended on the protocol supplemented by the manufacturing American company (Geneaid).

Detection of aroE gene by polymerase chain reaction (PCR): All isolates ofP. aeruginosa were tested by specific PCR for aroE gene to confirm identity as P. aeruginosa. Specific primers aroE (F: 5'- ATGTCACCGTGCCGTTCAAG -3') and (R: 5'- TGAAGGCAGTCGGTTCCTTG -3') to target aroE gene (Curran et al., 2004) were used to achieve a specific PCR for identification P. aeruginosa isolates. The PCR reaction mixture was described as follow: 12.5 µl of master mix,1.5 µlof forward primer, 1.5 µlof revers primer,3 µl of DNA template, 6.5 µlof PCR water. The mixture was prepared in PCR tubes then transferred to the thermocycler, first cycle was at 96°C for 5 mints after 30 cycle at 96°C for 30 seconds then at 55°C for one minut. and then at 70°C for 2 mints. . The PCR product was electrophoresed on agarose gel which was already prepared at 1.5% (w/v) concentration, DNA molecular size standard (DNA Ladder 100pb) was included on the gel. The stained bands via ethidium bromide were visualized by exposure to Ultraviolet.

Antibiotic susceptibility: The sensitivity of antibiotics was performed by Disc diffusion method onagar of Muller-Hinton according to Bauer and Kirby (1966). The inoculum was prepared by adding a single colony of bacterial isolates to brain heart infusion broth (5ml)then incubated for 24 hrsat 37°C. A sterile swab was used to streak the inoculum on a Muller Hinton agar plate. The each disc of antibiotic was placed on the medium surface and incubated for 18-24 hrsat 37°C. A ruler was used to measure the antibiotic inhibition zones and then compared with standard criteria in the clinical laboratory standard institute (CLSI).

RESULTS AND DISCUSSION

150 samples were obtained from some clinical cases of hospitals in Al-Diwaniyah city. The samples were cultured onMaconkey agar and blood agar then incubated for 24 hat 37°C. The results showed 112 (74.6%) bacterial growth. Initially, identification of the isolates were carried out via biochemical tests, 25 isolates Pseudomonasaeruginosa were detected as positive for oxidase, catalase and citrate, while negative for indol, methyl red and vogesproskauer. Identity was confirmed by PCR targeting the areE gene with size 498pb as shown in Figure 1.

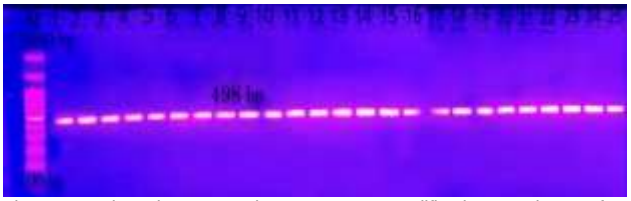

Figure 1: The chromosomal areE gene amplification products of P. aeruginosa, electrophoresis was carried out at voltage 90 on 1.5% agarose gel for one hour, the M column M represents DNA Ladder.

This bacterium was isolated from burn infection 30% , wound infection 20%, ear and nose 22.2% and 19% respectively, while it was isolated from Respiratorytract 16.6% as reported in Table 1. The results of current study were supported with another study reported byManhar(2011).

Table 1: Numbers and percentages of P. aeruginosa isolated from different clinical cases

Clinical source	Number of bacterial isolates	Number of P. aeruginosa	Percentage (%)
Wounds	25	5	20
Burns	30	9	30
Ear	18	4	22.2
Nose	21	4	19
Respiratory tract	18	3	16.6
Total	112	25	22.3

The antibiotics susceptibility for 16 isolates of P. aeruginosa have been tested. The percentages of antibiotic resistance were shown in Figure 2. It has been found that the bacterial isolates were resistance 100% for Ampicillin, Amoxicillin, Piperacillin. This result is supported by other studies, Al-Ghanimi (2015), AL-Salihi (2014) and Al-Sudani (2014).Suggesting that P. aeruginosaisolated in this study could not effected by these antibiotics because of the antibiotics were randomly used as a treatment for the infection with P. aeruginosa.

This bacterium was resisting 75% for Amoxicillin-Clavuulante, this result is in agreement with Fadhel (2013) who demonstrated that P. aeruginosa has 71.5% resistance to Amoxicillin-Clavuulante. However, this organism was 100% resistance to Cefotaxime and Cefepimwhich are classified from Cephalosporin the fourth generation. This results were supported with Al-Bayati et al., (2021), Al-Ghanimi (2015) and Jabbar et al., (2017).

Furthermore,isolates ofP. aeruginosa was resistance62.5% to Levofloxacin, this result was close to Al-Naimi (2015) who reported that this bacterium was resistance 75% to this antibiotic. Moreover, the isolates were resistance 18.75% to Meropenem antibiotic which belongs to carbapenam group, this result was closed to Mahdi (2020). Interestingly, all P. aeruginosa isolates were sensitive 100% for Amikacin, this was close to Sulaiman and Abdulhasan (2020). This result lead to suggest that the best antibiotic for P. aeruginosa that isolated in this study is Amikacin.

In conclusion, PCR targeting the areE gene was successfully used to detect all P. aeruginosa isolates with size 498pb. This bacterium causes different infections including wounds, burns, ear, nose and Respiratory tract. Interstingly, the optimal antibiotic for P. aeruginosa that isolated in this study is Amikacin.

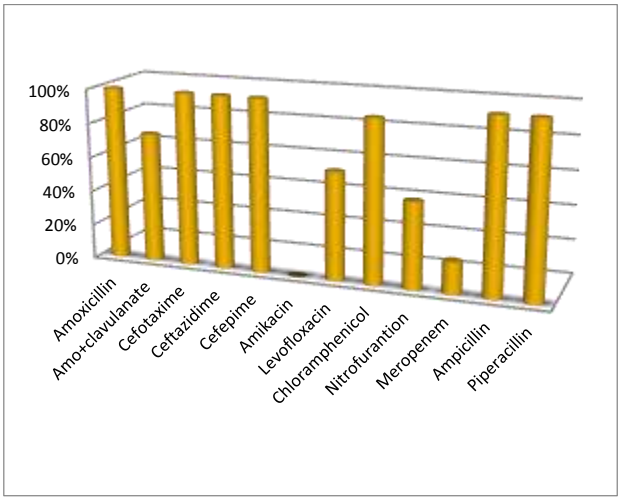

Figure 2: Persentagesresistance ofP.aeruginosafor some of the antibiotics

REFERENCES

1. Al-Ghanimi, HaninZuhair Ali. (2015). Effect of some antibiotics in inhibiting a number of virulence factors of bacteria pseudomonas aeruginosa and Proteus mirabilis isolated from urinary tract infection, master's thesis, College of Science, University of Karbala.
2. Al-Naimi, Ola Abdel-KarimKazim (2015) Study of the effect of probiotics on biofilm formation and protease production of pseudomonas aeruginosa isolated from burns and wounds, Master Thesis, College of Basic Education, Al-Mustansiriya University.
3. AL-Salihi, S. S., Hameed, B. H., &Hameed, B. H. (2014). Antibiosis resistant of Pseudomonas aeruginosa isolated from different clinical specimens. kirkuk university journal for scientific studies, 9(2).
4. Al-Obaidi, NibrasReda Muhammad. (2015). Molecular investigation and gene expression of efflux pumps in multi-antibiotic resistant bacteria Pseudomonas aeruginosa, Master's Thesis, College of Science, Al-Mustansiriya University.
5. Al-Sudani, Ihsan Ali Rahim. (2014). Inhibition of growth and biofilm formation of Pseudomonas aeruginosa in proteins produced from Saccharomyces cerevisiae, Master's thesis, College of Science, Al-Mustansiriya University.
6. Al-Bayati, Qahtan, Adnan Qadir, Muhammad Al-Hassoun. (2021). Detection of virulence factors of Pseudomonas aeruginosa isolated from wound infections and studying the effect of silver nanoparticles on them, Master's thesis, College of Science, Tikrit University.
7. Curran, B., Jonas, D., Grundmann, H., Pitt, T., & Dowson, C. G. (2004). Development of a multilocus sequence typing scheme for the opportunistic pathogen Pseudomonas aeruginosa. Journal of clinical microbiology, 42(12), 5644-5649.
8. Daboor, S. M., Budge, S. M., Ghaly, A. E., Brooks, S. L., & Dave, D. (2010). Extraction and purification of collagenase enzymes: a critical review. Am J BiochemBiotechnol, 6(4), 239-263.
9. Fadhel, R. A. (2013) Inhibition of Biofilm Production of Pseudomonas aeruginosa Isolated from Patients with Diabetic Foot Ulcer Grade II,Thesis, college of science , The university of Mustansiriya.
10. Jabbar, AbeerHamoudi and Al Ramahi, SeifKhoman. (2017). Isolation and identification of Pseudomonas aeruginosa from different clinical samples with sensitivity study to some antibiotics, Al-Qadisiyah Journal of Pure Sciences, Volume 22 Issue. 3.
11. Laurence , D.R .; Bennett, P.N.; and Brown , M.J.(1997) . Clinical Pharmacology . (8th) ed . Churchill Livingstone London.
12. Levinson, W.(2014). Review of medical microbiology and immunology. McGraw-Hill Education.
13. Levinson, W.(2016). Medical Microbiology and Immunology .14th McGraw Hill Education . USA.821.
14. MacFaddin , J.F.(2000) .Biochemical Tests for Identification of Medical Bacteria 3rd ed. Lippincott Williams and Wilkins , USA.
15. Madigan, M.T., J.M. Martinko and J. Parker (2003). Brock Biology of Microorganisms. Pearson Education international, tenth Edition.
16. Mahdi, R. J. (2020) .Detection of some virulence factor of Pseudomonas aeruginosa isolated from Burn' Patients and their surrounding environment and the biological activity of some extracts on it . A thesis ,College of Science, University of Basrah.
17. Manhar, Lama Fouad. (2011). Study of some physiological and genetic aspects of pyocin-producing pseudomonas aeruginosa isolated from clinical cases in Al-Diwaniyah city, Master's thesis, College of Education, University of Al-Qadisiyah.
18. Mietzner , T.A.,Carroll,K.C.,Hobden , J.A.,Miller, S.A., Mitchell, T.G.,… Detrick, B. (2016). Jawetz, Melnick and Adelberg,s Medical Microbiology . McGraw-Hill Education.
19. Rayan, K J and Ray, C.G(2004). Introduction to infectious Diseases : Sherris Medical Microbiology. (4th ed.) McGraw-Hill , New York.
20. Satlin, M. J., Lewis, J. S., Weinstein, M. P., Patel, J., Humphries, R. M., Kahlmeter, G.&Turnidge, J. (2020). Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing position statements on polymyxin B and colistin clinical breakpoints. Clinical Infectious Diseases, 71(9), e523-e529.
21. Sulaiman, S. D., &Abdulhasan, G. A. (2020). Curcumin as Efflux Pump Inhibitor Agent for Enhancement Treatment Against Multidrug Resistant Pseudomonas aeruginosaIsolates. Iraqi Journal of Science, 59-67.