

CEFTAZIDIME SENSITIVE BETA HEMOLYTIC *PSEUDOMONAS AERUGINOSA* – AN ESKAPE PATHOGEN

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ABSTRACT

Pseudomonas aeruginosa is the most rebellious hospital bug, causing life threatening diseasesoften challenging to treat. In view of this, a study was undertaken to uncover hemolytic P.aeruginosaand antibiotic susceptibility against selected antibiotics. Air samples from different locations within a hospital were collected by exposure plate technique and surface samples were collected by wiping the sterile moist swabs over the ventilator tube, bed railing, computer mouse/ keyboard, patient table, floor and wall from intensive care unit. Collected samples were subjected to standard isolation, characterization and hemolytic assay procedures. β hemolytic P.aeruginosa was isolated from the collected samples. Antimicrobial susceptibility test was performed according CLSI (2015) against gentamicin (120µg), ampicillin (10µg), amoxicillin (10µg), imipenem(10µg), andceftazidime (30µg). P.aeruginosa was sensitive to all antibiotics tested. The most imperative preventive measure against contagion is to rev- up decontamination protocol and continuous education to generate better understanding about the reservoir of Pseudomonas areuginosa, which is routinely over looked.

Keywords: ESKAPE pathogen, *Pseudomonas aeruginosa*, beta hemolysis, hospital infection, drug resistance.

INTRODUCTION

Antibiotic resistant ESKAPE pathogens are public health threat globally, *P. aeruginosa* is an ESKAPE pathogen known for its ubiquitous nature and antibiotic resistance (Jack et al., 2013).P. aeruginosais termed as nosocomial terrorist causing nosocomial morbidity and mortality(Boucher et al., 2009). It is also called "water bacterium" because it is widely found in and around water sources (Lukeet al., 2016). It is also reported to be found in liquid hand soap contaminated up to 8×10 (5) cfu/g (Blanc et al., 2016). Despite the updated hospital care strategies, hospital infections emerge as the most frequent problem in health care setting world wide especially in developing countries (Simonsenet al., 2004). According to a prevalence study conducted by WHO in 2002, an average of 8.7% hospital acquired infections were reported from four WHO regions namely, Europe, Eastern Mediterranean, East Asia and Western Pacific (Ducel*et* al.. 2002). P.aeruginosa South hassubstitutedStaphylococcus aureus, the common health care setting associated pathogen (Hani et al., 2009). P.aeruginosais known to cause wide spectrum of clinical conditionsviz., ventilator associated pneumonia, urinary tract infection, bacteremia, post operativewound infection and is associated with high mortality rates regardless of appropriate antimicrobial therapy(Rossoliniet al., 2005, Desiree et al., 2010). Several reports claim life threatening diseases caused by *P.aeruginosa in*immunocompromised patients (Driscoll et al., 2007,2010, Gayneset al., 2005, Emoriet al., 1991). Antibiotics used to treat infections caused by *P. aeruginosa* are based on the type and severity of the clinical condition and zonal resistance patterns (Giamarellouet al., 2001). P. aeruginosa was reportedto demonstrate resistance to multiple antibiotics(Nikaido 2003, Farida et al., 2010,). The swift emergence of antibiotic resistant P. aeruginosailluminatesit as one of the most stern health care associated pathogen(Nwamkwoet al., 2010).

MATERIALS AND METHODS

A total of 96 samples were collected from air,ventilator tube, bed railing, computer mouse/keyboard, patient table, floor and wall of intensive care unit from one of the leading hospital in Kuala Lumpur. Air samples were collected by open plate technique while surface samples were collected by wiping the sterile moist swabs over the surface of selected areas(Dharan*et al.*, 2002Javed*et al.*,2008). The collected samples were transported to Research laboratory for isolation and characterization. The isolates were biochemically characterized and identified in accordance with standard procedures (Palleroni, 2015, Haynes, 1951). Hemolytic assay was conducted by inoculating pure culture of *P. aeruginosa*

on blood agar medium and incubated at 37^{0} C overnight to observe the hemolysis post incubation(Pyzh*et al.*, 2011).Suspension of standard reference strain (*P aeruginosa* ATCC 27853) was used in this study.

Out of 76 *P.aeruginosa* isolates, 40 isolates were subjected to antibiotic susceptibility test according to CLSI (2015). Commercially manufactured antibiotic discs were obtained from Oxoid pharmaceutical, Malaysia. Antibiotic discs used in this study were of gentamicin (120 μ g), ampicillin (10 μ g), amoxicillin (10 μ g), imipenem (10 μ g), and ceftazidime (30 μ g) with varied potency respectively. Post incubation, the diameter of inhibition zone was measured and compared with standard antibiotic susceptibility table (CLSI, 2015). Resistance and sensitivity was categorized against the antibiotics tested.

RESULTS AND DISCUSSION

Isolation of *P.aeruginosa*

A total of 76 isolates of *P.aeruginosa* were isolated in this study from various areas of the intensive care unit of selected hospital as indicated in Table 1.

Samples	β hemolytic <i>Pseudomonas aeruginosa isolates</i>
Air	28
Ventilator tube	22
Bed Railing	8
Computer Mouse and Keyboard	6
Patient Table	5
Floor	4
Wall	3
Total	76

Table 1: Isolation of β hemolytic *Pseudomonas aeruginosa* from Intensive Care Unit

*P.aeruginosa*showed highest prevalence in the air samples 28 (36.8%) followed by ventilator tubes 22 (28.9%), bed railing 8 (10.5%), Computer mouse and keyboard 6 (7.9%), patient table 5 (6.6%), floor 4 (5.3%) and wall 3 (4%) in accordance with (Felicity *et al.*, 1968, Ana*et al.*,2008). This explains, the P.*aeruginosa* continues to be the hospital bug.Hemolytic assay, revealed beta hemolytic *P.aeruginosa* in line with other report (Piyush*et al.*,2008).

Antibiotic Susceptibility Testing

Table 2, confirms the susceptibility pattern of the confirmed isolates of *P.aeruginosa*. Largely, the isolates were found to be sensitive to all the antibiotics tested.

Sensitive (%)
60.0
42.5
40.0
37.5
32.5

 Table 2: Antibiotic sensitivity of Pseudomonas aeruginosa

Antibiotic susceptibility test revealed sensitivity to all the groups of antibiotics tested *viz.*, gentamycin, ampicillin, amoxicillin, imipenem and ceftazidime. Other reports, demonstratedceftazidime and gentamycin sensitivity Désirée*et al.*, (2010),Siva *et al.*, (2009) in contrast to reports from Malaysia (Pathmanathan*et al.*, 2009). Gentamicin sensitivity wasparallel with the results recorded by Anithaet *al.*, (2016), in contrast to other reports (Herman*et al.*, 1986,Alkalin*et al.*, 1988,Milind D *et al.*, 2014). However, other findings revealed resistance to gentamycin,ampicillin and amoxicillin (Jamshaid*et al.*, 2008).

Ceftazidime sensitivity was in line with other reports(Indu*et al.*,2014, Tarana*et al.*, 2015). The conspicuous observation of this study was ceftazidime sensitive *P.aeruginosa* as opposed to many reports that recorded ceftazidime resistance (Harris *et al.*, 1999,Babay*et al.*, 2007, Tan*et al.*, 2008,Asghar*etal.*, 2009, Bukharie*et al.*, 2010,Al-Agamy*et al.*,2011, Jiancheng*et al.*, 2013, Mohan*et al.*, 2013,Milind*et al.*, 2014,Mubashir*et al.*, 2016,Zakieh*et al.*, 2016).In addition, several studies have reported cephalosporin resistant*P.aeruginosa*(Anitha*et al.*, 2016,Akhabue*et al.*, 2011,Omar *et al.*, 2016).

Amoxillin sensitive *P.aeruginosa*were recorded in this study while the other reports revealed resistance (Anitha*et al.*, 2016).Imipenem sensitivity was similar with other reports (Dinic*et al.*, 2008, Juhi*et al.*, 2009,Siva *et al.*, 2009, Garba*et al.*, 2012, Ravichandra*et al.*, 2012, Randa*et al.*, 2016, Anitha*et al.*, 2016,Premanadham*et al.*, 2016, Sonal*et al.*, 2016).

The variation of sensitivity and resistance is multifactorial, the reports of antibiotic overuse and misuse is ruinous globally, this is in agreement with the reports by (Ozumba, 2003).

CONCLUSION

The credible approach to reduce morbidity and retain hospitals as life saving resources is by combined coordinated effort of hospital personnel and patient. It is important to urge the patient to adhere to the antibiotic strategies which should be reinforced effort by surveillance champions cyclically. In addition, the maintenance of patient number and

clinician ratio may prove to be significant effort in infection control management to address the escalating scare of ESKAPE pathogens.

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REFERENCES

- 1. Akhabue, E., Synnestvedt, M., Weiner. M.G, Bilker, W.B. and Lautenbach, E. (2011). Cefepimeresistant *Pseudomonas aeruginosa*. Emerg Infect Dis., Vol 17, 1037-1043.
- Al-Agamy, M.H., Shibl, A.M., Samar, A., Zaki, S.A. andTawfik, A.F. (2011). Antimicrobial resistance pattern and prevalence of metallo-lactamases in *Pseudomonas aeruginosa* from Saudi Arabia. African J Micro Res., Vol 5, 5528-5533.
- 3. Alkalin, H.E., Torun, M. and Alacam, R. (1988). Aminoglycoside Resistance Patterns in Turkey. Scandinavian Journal of Infectious Diseases, Vol20, 199-203.
- Ana, C., Renato, P.M., Ariel, E.S., Everlon, C.R., José, M.M. and Fernando, A.Á. (2008). Isolation of *Pseudomonasaeruginosa*Strains from Dental Office Environments and Units in Barretos, State of Sao Paolo, Brazil and Analysis of Their Susceptibility to Antimicrobial Drugs. Brazilian Journal of Microbiology, Vol 39, 579-584.
- 5. Anitha, M., Moniha, DM., Mohammed, S.A., Pratikshia, K. and Swathy, S.R. (2016). The Frequency of *Pseudomonas aeruginosa*Clinical isolates in a Tertiary Care Hospital. Int. J. Pure App. Biosci., Vol 4(3), 154-159.
- Asghar, A.H and Faidah,H.S. (2009). Frequency and antimicrobial susceptibility of gram negative bacteria isolated from 2 hospitals in Makkah, Saudi Arabia. Saudi Med., Vol 30, 1017-23, 781-786.
- 7. Babay, H.A. (2007). Antimicrobial resistance among clinical isolates of *P.aeruginosa* from patients in a teaching hospital, Riyadh, Saudi Arabia 2001 2005. Japanese Journal Infect Dis., Vol 60 (2-3),123-125.
- Blanc, D.S., Gomes, M.B., Abdelbary, M., Prod'hom, G., Greub, G., Wasserfallen, J.B., Genoud, P., Zanetti, G. and Senn, L. (2016). Hand soap contamination by *Pseudomonas aeruginosa* in tertiary care hospital: no evidence of impact on patients. J. Hosp Infect., Vol, 93 (1), 63-7.
- Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., Scheld, M., Spellberg, B. and Bartlett, J. (2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis., Vol 1, 48(1),1–12.
- 10. Bukharie, H.A. andMowafi, H.A. (2010). Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* and antibiotic use in King Fahd Hospital of the University in Khobar, Saudi Arabia. Sci J King Faisal Univ., Vol11,185-192.
- 11. Clinical and Laboratory Standards Institute (2015): Performance standards for antimicrobial susceptibility testing. Twenty-fifth informational supplement, CLSI

document M100-S25. PA, USA: Wayne.

- Désirée, C., Simone, C., Ottavio, Z., Giulio, Z., Rosaria, M., Susanna, L., Monica, C., Stef ano, F.G., Milano, M., Barbara, C., Maria, L., Chiara, B., Maurizio, A. and Elio, C. (2010). Multidrug Resistant *Pseudomonas aeruginosa* Infection in Children Undergoing Chemotherapy and Hematopoietic Stem Cell Transplantation. Haematologica, Vol 95, 1612-1615.
- 13. Dharan, S. and Pittet, D. (2002). Environmental control in operating theatres. Hosp. Infect., Vol 51 (2), 79-84.
- Dinic, M., Antic, S., Kocic, B., Djordjevic, D. S., Bogdanovic, M. and Jovanovic, T. (2008). Resistance Pattern of Inpatient and Outpatient Isolates of *Pseudomonas aeruginosa* in the City of Nis, 2004 to 2006. ACTA FAC MED NAISS, Vol 25 (4), 205-210.
- 15. Driscoll, J.A., Brody, S.L. andKollef, M.H. (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections," *Drugs*, vol. 67, no. 3, 351–368,67(3) 351-68.
- 16. Ducel, G., Fabry, J. and Nicolle, L. (2002). Prevention of Hospital acquired Infections, A Practical Guide. World Health Organization, Vol2, 4-5.
- 17. Emori, TG., Culver, D.H. and Horan, T.C. (1991). National nosocomial infections surveillance system (NNIS): Description of surveillance methods. American Journal of InfectionControl, Vol 19, no. 1, 19–35, 1991.
- 18. Farida, A. and Asif, M. (2010). Susceptibility Pattern of *Pseudomonas aeruginosa* against various antibiotics. Africal Journal of Microbiology Research, Vol 4 (10), 1005-1012.
- 19. Felicity, P. and Lowbury, E.J.L. (1968). Survival of Wound Pathogens Under Different Environmental Conditions. J. Hyg., Camb., Vol 66, 393-406.
- 20. Garba, I.Y.H., Lusa, E., Bawa, M.B., Tijjani, M.S., Aliyu, U.U., Zango, M.I.O and Raji. (2012). Antibiotics Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Wounds in Patients Attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Nigerian Journal of Basic and Applied Science, Vol 20(1), 32-34.
- 21. Gaynes, R. and Edwards, J.R. (2005). National Nosocomial Infections Surveillance System, "Overview of nosocomial infections caused by gram-negative bacilli," *Clinical Infectious Diseases*, Vol. 41, no. 6, 848–854.
- 22. Giamarellou, H. and Antoniadou, A. (2001). Antipseudomonal antibiotics. Med Clin North Am., Vol 85 (1), 19-42.
- 23. Hani, A.M. and Adnan, S.J. (2009). Incident of *Pseudomonas aeruginosa* in post operative wound infection. American Journal of Infectious Diseases, Vol 5(1), 1-6.
- Harris, A., Torres, V.C., Venkataraman, L., DeGirolami, P., Samore, M. and Carmeli, Y. (1999). Epidemiology and clinical outcomes of patients with multi resistant *Pseudomonas* aeruginosa. Clin Infect Dis., Vol 28, 1128-33.
- 25. Haynes, W.C (1951). *Pseudomonas aeruginosa* its characterization and identification. J.Gen microbial., Vol 5 (5 suppl), 939-950.
- 26. Herman. W., Van Landuyt, H.W., Boelaert, J., Glibert, B., Gordts, B. and Verbruggen, A.M. (1986). Surveillance of Aminoglycoside Resistance. European Data. *The American Journal of Medicine*, Vol80, 76-81.
- 27. Indu, B., Balvinder, S.A., Dimple, K. andNeetushree.(2014). Incidence of multidrug resistant *Pseudomonas aeruginosa* isolated from burn patients and environment of teaching

institution. J. Clin. Diagn. Res., Vol8(5), DC26 - DC29.

- 28. Jack, N., Sean, P. G. and Bredan, F. G. (2013). Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther., Vol 11(13), 297-308.
- 29. Jamshaid, A.K., Zafar, I., Saeed, U.R., Kalsoom, F. and Abbas, K. (2008). Prevalence and Resistance Pattern of *Pseudomonas aeruginosa* Against Various Antibiotics. Pakistan Journal of Pharmaceutical Science, Vol 21(3),311-315.
- 30. Javed, I., Hafeez, R., Zubair, M., Anwar, M.S, Tayyib, M. and Husnain, S. (2008). Microbiological surveillance of operation theatres and ICUs of a tertiary care hospital. Lahore. Biomedica., Vol 24, 99-102.
- 31. Jiancheng, Xu, Xiumei, D., Hui, Wu. and Qi, Zhou.(2013). Surveillance and Correlation of Antimicrobial Usage and Resistance of *Pseudomonas aeruginosa*: A Hospital Population-Based Study. PLOS ONE Vol 8. Issue 11. e78604.
- 32. Juhi, T., Bibhabati, M., Archana, T., Poonam, L. and Vinita, D. (2009). *Pseudomonas aeruginosa*meningitis in post neurosurgical patients. Neurology Asia, Vol14(2), 95 – 100.
- 33. Luke, S.P. Moore., Joel Cunnigham. And Hugo, Donaldson. (2016). A clinical approach to managing *Pseudomonas aeruginosa* infections. British Journal of Hospital medicine, Vol 77(4) 50 – 54.
- 34. MilindDavane., NamdevSuryawanshi., Asha Pichare. andBasavrajNagoba. (2014). *Pseudomonas aeruginosa*from hospital environment. Journal of Microbiology and Infectious Diseases, Vol 4 (1), 42-43.
- 35. Mohan, B.S., Lava, R., Prashanth, H.V., Nambiar, V., Basavaraj, M., Nayak, Venkatesh R. and Mahesh, B. (2013). Prevalence and antibiotic sensitivity pattern of *Pseudomonas aeruginosa*: an emerging nosocomial pathogen. Int J Biol Med Res., Vol4, 2729-2731.
- 36. Mubashir, A.K. and Aftab, F. (2016). Antimicrobial resistance patterns of Pseudomonas aeruginosa in tertiary care hospitals of Makkah and Jeddah. Ann Saudi Med., 36 (1), 23-28.
- 37. Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. Microbiol. Mol. Biol. Rev.,67(4), 593–656.
- 38. Nwankwo, E.O.K. and Shuaibu, S.A. (2010). Antibiotic Susceptibility Pattern of Clinical Isolates of *Pseudomonas aeruginosa*in a Tertiary Health Institution in Kano, Nigeria. Journal of Medicine and Biomedical Sciences, Vol 17, 37-40.
- 39. Omar Bashir Ahmed. (2016). Incidence and antibiotic susceptibility pattern of *Pseudomonas aeruginosa*isolated from inpatients in two tertiary hospitals. ClinMicrobiol., Vol 5, 2.
- 40. Ozumba, U.C. (2003). Antibiotic Sensitivity of Isolates of *Pseudomonas aeruginosa* in Enugu, Nigeria. African Journal of Clinical & Experimental Microbiology, Vol 4(1), 48-51.
- 41. Palleroni, N.J. (2015). Pseudomonas. Bergy's manual of systematics of Archea and Bacteria.
- 42. Pathmanathan, S.G., Samat, N.A. and Mohammed, R. (2009). Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian hospital. Malaysian J Med Sci., Vol 16, 28-33.

- 43. Piyush, BH., Naresh K., Tasleem, A., Mandal, T.K., Akhilesh, V.K., Sharma, G.L. and Rajesh, D. (2008).*In Vitro* Anti-Bacterial Activity of a Novel Isoquinoline Derivative and its Post Antibacterial Effect on *Pseudomonas aeruginosa*. African Journal of Microbiology Research, Vol 2, 126-130.
- 44. Premanadham, N., Srinivasulu, R. P., Jithendra, K. B, Siva, P. R. and Vasundhara, P. (2016). Antibiotic Susceptible Pattern of *Pseudomonas aeruginosa* Isolated from Clinical Specimens in a tertiary care centre Hospital Narayana Medical College and Hospital Nellore, AP, India. Int. J. Curr. Microbiol. App. Sci., Vol 5(10), 324-329.
- 45. Pyzh, A.E. and Nikandrov, V.N. (2011). Contribution of blue green pigments to hemolytic activity of *Pseudomonas aeruginosa* cultural fluid. Zh.MikrobiolEpidemiolImmunobiol., Vol (1), 19-25.
- 46. Randa Ahmed Gasimelseed Mohammed and AbdelbagiElnagi Mohammed (2016). *In vitro* sensitivity of *Pseudomonas aeruginosa* to piperacillin, Azlocillin, Imipenem and Meropenem. American Journal of Research Communication, Vol 4(3), 107 -117.
- 47. Ravichandra, P. H., Rashmi, B., Neena, K., Suresh, S., Anitha, M.R. and Vijayanath, V. (2012). Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* strains isolated from clinical sources. Journal of Pharmaceutical and Biomedical Sciences Vol4,(05).
- 48. Rossolini, G.M. and Mantengoli, E. (2005). Treatment and control of severe infectionscaused by multiresistant*Pseudomonas aeruginosa*. Clinical Microbiology and Infection, Vol 11(4), 17-32.
- 49. Simonsen, G.S., Tapsall, J.W., Allegranzi, B., Talbot, E.A. andLazzari, S. (2004). The antimicrobial resistance containment and surveillance approach a public health tool. Bull World Health Organization, (12),82, 928–34.
- 50. Siva, G.P., Azura, S. and Ramelah, M. (2009). Antimicrobial Susceptibility of Clinical Isolates of *Pseudomonas aeruginosa* from Malaysian Hospital. Malaysian Journal of Medical Sciences, Vol 16(2), 27-32.
- 51. Sonal, L., Anita, Himani, P., Krunal, S and Lakhani, S.J. (2016). Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* at the tertiary care center, Dhiraj Hospital, Piparia, Gujurat. International Archives of Integrated Medicine, Vol 3(5), 133-137.
- 52. Tan, T.Y.,Hsu, L.Y.,Koh, T.H, SY Ng, L., Tee, N.W. and Krishnan, P. (2008). Antibiotic Resistance in Gram-negative bacilli: A Singapore perspective. Ann Acad Med Singapore, Vol 37, 819-25.
- 53. Tarana, S., Mohammed, R., Vichal, R. and Yogesh, C. (2015). A Comparative Study of Antibiogram of *Pseudomonas aeruginosa*in Hospital and Community Acquired Infections. *Int.J. Curr.Microbiol.App.Sci.*, Special Issue-1, 286-291.
- 54. The Burden of Health Care-Associated Infection Worldwide (2010). A Summary. World Health Organization.
- 55. Zakieh, R., Mahshid, M. and Alireza, R. (2016) Investigation of *Pseudomonas aeruginosa*Resistance Pattern against Antibiotics in Clinical Samples from Iranian Educational Hospital. Advances in Microbiology, Vol6, 190-194.