



PREVALENCE OF NOSOCOMIAL INFECTIONS CAUSED BY *PSEUDOMONAS AERGINOSA* IN ASSIUT UNIVERSITY HOSPITAL

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Pseudomonas (Ps.) aeruginosa is one of the most common pathogens causing nosocomial infections. This pathogen causes several infections including urinary tract infection (UTI), wound infection, pneumonia, bacteremia,...etc. Immunocompromised patients and patients in intensive care unit are at high risk of acquisition of infection, in addition multidrug resistant *Ps. aeruginosa* isolates had been characterized.

This study was planned to determine the frequency of *Ps. aeruginosa* in nosocomially infected patients in Assiut university hospital and to type isolated strains.

In this study, 677 samples collected from 366 nosocomially infected patients admitted to different hospital wards at Assiut University Hospitals, including chest, trauma, neurology, internal medicine, post operative and pediatric ICUs, trauma and hematology units. Identification of bacterial strains was done by cultural and biochemical tests. Biotyping analysis for isolated strains was done using API 20NE.

In our study, a total of 30 (8.2%) *Ps. aeruginosa* strains were isolated. Four API codes profile for *Ps. aeruginosa* isolates were identified, the isolate with API code profile 1154575 was more frequent distributed in Assiut University Hospital.

INTRODUCTION

Ps. aeruginosa is Gram-negative, non-spore forming rods, motile, oxidase positive while indole, vogues proskur and nitrate negative¹. About 10-15% of *Ps. aeruginosa* strains produce pigment only when grown on pigment-enhancing media².

Ps. aeruginosa is an important opportunistic pathogen which plays an important role in hospital intensive care units. The presence of multiple intrinsic and acquired mechanisms of resistance to a wide variety of antibiotics in *Pseudomonas aeruginosa* allows spread of pathogen and makes pathogen control is difficult^{3&4}.

It has been reported that *Ps. aeruginosa* is the second most common cause of nosocomial pneumonia, health care-associated pneumonia, and ventilator-associated pneumonia. *Ps.*

aeruginosa was accountable for 30% of pneumonias, 19% of urinary tract infections, and 10% of bloodstream infections⁴⁻⁶.

A study conducted by Hassan *et al.*⁷ at Assiut University Hospitals, reported that *Ps. aeruginosa* accounts for 17.73% of isolated uropathogens. In another study at Cairo University Hospitals, Wassef *et al.*⁸ reported that the highest isolation of *Ps. aeruginosa* were from lower respiratory tract infections (44.2%), followed by surgical site infections (SSIs), burns & skin infections (37.5%) and urinary tract infections (23.2%).

There are several typing systems for isolated *Ps. aeruginosa* strains; biotyping⁹, antibiogram¹⁰, pyocin typing¹¹, serotyping¹², phage typing¹³ and molecular typing¹⁴.

MATERIAL AND METHODS

Study population

This study was conducted on 366 nosocomially infected patients admitted to different hospital wards at Assiut University Hospitals, including chest, trauma, neurology, internal medicine, post-operative and pediatric ICUs, trauma and hematology units during a period of 12 months from May 2014 to May 2015. Six hundred seventy seven specimens were collected according to the site of infection: endotracheal tubes (n= 218), sputum samples (n= 171), blood (n= 167), urine samples (n= 78), wound swabs (n= 23), and throat swabs (n= 20). There was more than one sample collected from one patient.

Bacteriological examination

All samples were inoculated on blood agar, MacConkey's agar, Mannitol salt agar and Cetrinide agar. Suspected colonies were sub-cultured on Pseudomonas agar (for pyocyanin).

Identification and confirmation of isolates was done by Gram stain, colony morphology, oxidase test, Triple Sugar Iron test (TSI), Simmon's Citrate, Christensen's urea, catalase test and ability to grow at 42°C.

Biotyping of isolated *Ps. aeruginosa* strains

Typing of *Ps. aeruginosa* strains (n= 30) was done using API 20NE kit (BioMerieux, Marcy L Etoile; France).

RESULTS AND DISCUSSION

Results

Ps. aeruginosa was identified as Gram-negative, non-sporing rods, oxidase +ve, reduce nitrate to nitrite, not ferment sugars and citrate positive, urease -ve, catalase +ve and can grow at 42°C.

Ps. aeruginosa grew on blood agar as mucoid colonies, grew on MacConkey's agar as non-lactose fermenting colonies (NLF), grew on Cetrinide agar and grew on Pseudomonas agar (for pyocyanin) showing greenish blue colonies as shown in figures 1a & 1b.

In this study, 162/366 (44.26%) females and 204/366 (55.74%) males were included. The age of patients ranged from two months to 82 years. One hundred sixty patients aged above 40 years (43.7%), 120 patients aged 18-

40 years (32.8%) and 86 patients were children (<18 years) (23.5%).



Fig. 1a: Colonies of *Ps. aeruginosa* on Cetrinide agar.

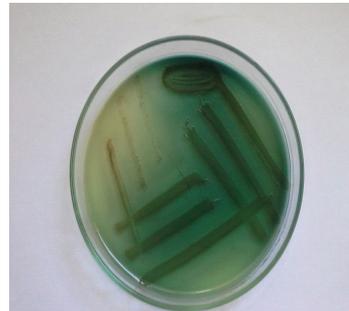


Fig. 1b: Greenish blue colonies of *Ps. aeruginosa* on Pseudomonas agar (for pyocyanin)

One thousand and one hundred forty eight pathogens (1148) were isolated. Of these, 515 (44.86%) were Enterobacteriaceae, 317 (27.6%) were *Staphylococci* spp., 120 (10.45%) were *Candida* spp., 30 (2.63%) were *Ps. aeruginosa*, and 166 (14.46%) were other non-Enterobacteriaceae which include *Acinetobacter* spp., *Stenotrophomonas* spp., and other *Pseudomonas* spp. (Fig. 2).

Ps. aeruginosa represented 2.63% (30/1148) from total isolates, 4.43% (30/677) from total number of samples, and 8.2% (30/366) from number of nosocomially infected patients. The frequency of *Ps. aeruginosa* from different samples is summarized in table 1.

Ps. aeruginosa isolates were mostly isolated from neurology ICU by 33.33% (10/30) followed by chest ICU representing 26.67% (8/30), trauma ICU and internal medicine ICU by 10% (3/30) each, hematology unit and pediatric ICU by 6.68% (2/30) each, and then post-operative ICU and trauma unit by 3.33% (1/30) (Fig. 3).

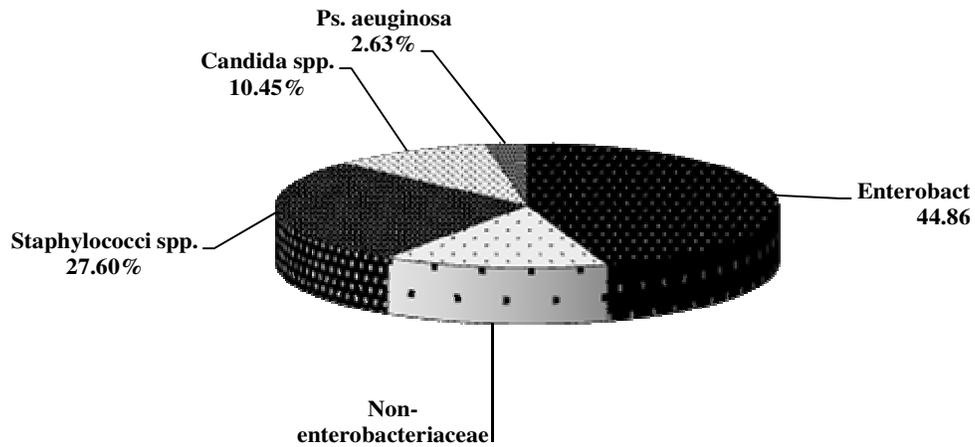


Fig. 2: Types of pathogens identified out of total 1148 isolates from nosocomially infected patients.

Table 1: Frequency of *Ps. aeruginosa* in 677 samples from nosocomially infected patients.

No. Of <i>Ps. aeruginosa</i> isolates	Samples
15	Endotracheal aspirates (n= 218)
8	Sputum samples (n= 171)
1	Blood culture (n= 167)
3	Wound swabs (n= 23)
2	Urine samples (n= 78)
1	Throat swabs (n= 20)
Total (n= 30)	Total = (677)

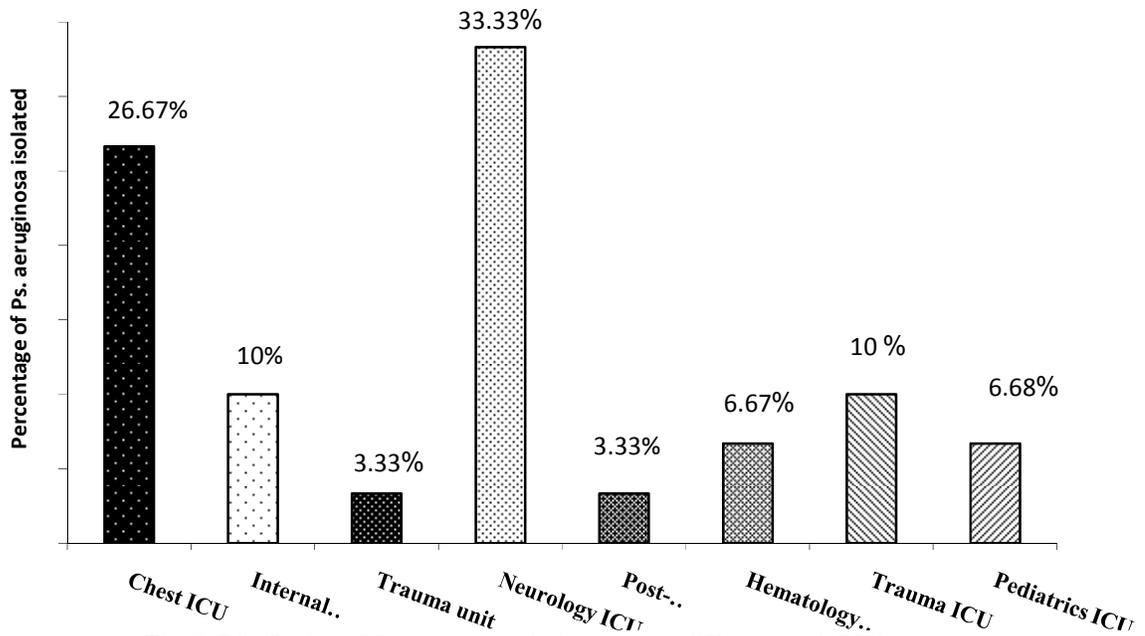


Fig. 3: Distribution of *Ps. aeruginosa* isolates among different wards/ICUs.

Results of API 20NE

The API 20NE Index system was performed to identify 30 isolated strains of *Ps. aeruginosa*.

The API 20 NE Index system identified 30 isolates of *Ps. aeruginosa* with four different

analytic profile index numbers (nine strains with 1154575 code, eight strains with 0354575 code, eight strains with 0154575 code, and five strains with 0144575 code) as shown in table 2 and figure 4.

Table 2: Biotyping of *Ps. aeruginosa* isolates with API 20 NE:

Infection Site	No. of isolates	API code profile
Chest ICU (n= 8)	4	1154575
	2	0354575
	2	0154575
Trauma ICU (n= 3)	2	0144575
	1	1154575
Neurology ICU (n= 10)	4	0354575
	3	1154575
	3	0154575
Trauma unit (n= 1)	1	1154575
Pediatrics ICU (n= 2)	2	0154575
Internal medicine ICU (n= 3)	3	0144575
Postoperative ICU (n= 1)	1	0154575
Hematology unit (n= 2)	2	0354575



(A)Code 1154575



(B) Code 0154575

Fig. 4: Biotyping of *Ps. aeruginosa* isolate with API 20 NE

Discussion

In our study, *Ps. aeruginosa* strains represented 4.43% from total number of samples in Assiut university hospital. Our results are comparable to previous results reported by Morrison and Wenzel¹⁵, (8.5%) and Nadeem *et al.*¹⁶, (10.1%). However, higher *Ps. aeruginosa* infection rate were reported by others; Mansour *et al.*¹⁷, reported the rate of *Ps. aeruginosa* isolation from patient samples in Egypt and Saudi Arabia, was 32.8% and 30.0% respectively, also Wassef *et al.*⁸, reported that the percentage of *Ps. aeruginosa* isolates in clinical samples was 20.7% and Gad *et al.*¹⁸ detected 18.2% of *Ps. aeruginosa* in different clinical samples.

In the current study, *Ps. aeruginosa* isolates represented 8.2% (30/366) from number of nosocomially infected patients. Our results are similar to some extent to previous results reported by Abbas *et al.*¹⁹ (12.5%). However, higher *Ps. aeruginosa* percentage (18.8%) were reported by Mahmoud *et al.*²⁰.

In the present study, *Ps. aeruginosa* was detected in 13.04% (3/23) of infected wounds. Similar findings (11-12%) were reported by other groups Gad *et al.*¹⁸; Cayci *et al.*²¹. While, higher rates were reported by Mahmoud *et al.*²⁰ who found *Ps. aeruginosa* in 5% (3/54) of wound swab samples collected from El-Minia University Hospital, Egypt and Jamasbi and Proudfoot¹² who detected *Ps. aeruginosa* in 32.9% (55/167) of wound exudate samples were obtained from a Northwest Ohio hospital, USA.

In this work, *Ps. aeruginosa* isolates were recovered from 5.9% (23/389) of respiratory tract infection cases. These results agreed to some extent with Gad *et al.*¹⁸, who detected *Ps. aeruginosa* in 6% of samples collected from patients suffering from respiratory tract infections in El-Minia University Hospital, Egypt. However, these results were lower than the findings by Mahmoud *et al.*²⁰ who detected *Ps. aeruginosa* in 14.8% (8/54) of sputum samples collected from patients suffering from respiratory tract infections over a 3-year study period in Menofia University Hospitals, Egypt and also Fatima *et al.*²² detected *Ps. aeruginosa* in 24% of sputum samples of lower respiratory tract infection patients admitted to different hospitals of Karachi, Pakistan over a 14 months period from January 2010 to March 2011.

In this study, *Ps. aeruginosa* were recovered from 2.6% (2/78) of urinary tract infection cases. These findings were less than the results published by Hassan *et al.*⁷, who detected *Ps. aeruginosa* in 8.6% (50/581) in samples collected from urinary tract infections patients at Urology Department in Assiut University Hospital, Egypt.

In the current study, *Ps. aeruginosa* was detected in 0.6% (1/167) of blood cultures (1/167). Our results were lower than those reported by Cayci *et al.*²¹, who detected *Ps. aeruginosa* in 3.3% of samples.

The difference in prevalence rate in each unit and/or sample in our results than other previously reported results may be attributed to difference in patient population, underlying diseases, environments, study periods, and the number of investigated specimens.

Identification and biotyping of *Ps. aeruginosa* was done by API 20NE. Four API codes profile for *Ps. aeruginosa* isolates were identified 1154575, 0154575, 0354575 and 0144575. *Ps. aeruginosa* with API code profile 1154575 was most frequent isolated, followed by strains with API code 0154575 and 0354575. While isolates characterized by API code 0144575 was less frequent distributed.

Conclusion

Ps. aeruginosa is a common cause of nosocomial infection in Assiut university hospitals. *Ps. aeruginosa* with API code profile 1154575 was mostly identified.

REFERENCES

- 1- J. W. Govan, "*Pseudomonas*", In: "Mackie & McCartney Practical Medical Microbiology", J. G. Collee, J. P. Duguid, A. G. Fraser, and B. P. Marmion (Eds.), 13th Ed., Edinburgh: Churchill Livingstone, Vol. 2, 1989, pp. 491-502.
- 2- J. W. Govan, "*Pseudomonas* and Non-Fermenters", In: "Medical Microbiology: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control", D. Greenwood, R. Slack, J. Peutherer, & M. Barer (Eds.), 7th Ed., Churchill Livingstone-ELSEVIER, 2007, pp. 293-299.
- 3- D. Landman, J. M. Quale, D. Mayorga, A. Adedeji, K. Vangala, J. Ravishankar, C.

- Flores and S. Brooks, "Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: The preantibiotic era has returned", *Archives of Internal Medicine*, 162, 1515-1520 (2002).
- 4- P. D. Lister, D. J. Wolter and N. D. Hanson, "Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms", *Clinical Microbiology Reviews*, 22, 582-610 (2009).
 - 5- M. H. Kollef, A. Shorr, Y. P. Tabak, V. Gupta, L. Z. Liu and R. Johannes, "Epidemiology and outcomes of health-care-associated pneumonia: Results from a large US database of culture-positive pneumonia", *CHEST Journal*, 128, 3854-3862 (2005).
 - 6- R. A. Weinstein, R. Gaynes and J. R. Edwards, "Overview of nosocomial infections caused by gram-negative bacilli", *Clinical Infectious Diseases*, 41, 848-854 (2005).
 - 7- I. A. E. S Hassan, S. S. Seif El-din, A. M. Nafei and M. A. Abd El-Hafiz, "Comparative studies of different efflux pump inhibitors effect on *pseudomonas aeruginosa* resistant to fluoroquinolone", *New Egyptian Journal of Microbiology*, 32 (2012).
 - 8- M. Wassef, H. El Mahallawy, M. M. Zafer, G. Doaa and R. Abdel hamid, "Lab based surveillance of multidrug resistant *pseudomonas aeruginosa* in Cairo University Hospitals, Egypt", *Journal of Microbiology & Experimentation*, 2, 5 (2015).
 - 9- A. Freitas and A. L. Barth, "Typing of *pseudomonas aeruginosa* from hospitalized patients: A comparison of susceptibility and biochemical profiles with genotype", *Brazilian Journal of Medical and Biological Research*, 37, 77-82 (2004).
 - 10- Clinical and Laboratory Standards Institute (CLSI), "Performance Standards for Antimicrobial Susceptibility Testing, Informational Supplement M100-S18", 18th Ed., CLSI, Wayne, PA, (2008).
 - 11- J. Fyfe, G. Harris and J. Govan, "Revised pyocin typing method for *Pseudomonas aeruginosa*", *Journal of Clinical Microbiology*, 20, 47-50 (1984).
 - 12- R. J. Jamasbi and E. M. Proudfoot, "Phenotypic and genotypic characteristics of clinical isolates of *pseudomonas aeruginosa*: Rate of occurrence and distribution of different serotypes, antimicrobial susceptibility profiles, and molecular typing", *Lab Medicine*, 39, 155-161 (2008).
 - 13- B. Ojeniyi, C. Wolz, G. Döring, J. Lam, V. Rosdahl and N. Hoiby, "Typing of polyagglutinable *Pseudomonas aeruginosa* isolates from cystic fibrosis patients", *APMIS*, 98, 423-431 (1990).
 - 14- T. J. Kidd, K. Grimwood, K. A. Ramsay, P. B. Rainey and S. C. Bell, "Comparison of three molecular techniques for typing *Pseudomonas aeruginosa* isolates in sputum samples from patients with cystic fibrosis", *Journal of Clinical Microbiology*, 49, 263-268 (2011).
 - 15- A. J. Morrison and R. P. Wenzel, "Epidemiology of infections due to *Pseudomonas aeruginosa*", *Review of Infectious Diseases*, 6, S627-S642 (1994).
 - 16- S. Nadeem, S. Qasmi, F. Afaque, M. Saleem and S. Hakim, "Comparison of the in vitro susceptibility of Clinical isolates of *Pseudomonas aeruginosa* in a local hospital setting in Karachi, Pakistan", *British Journal of Medical Practitioners*, 2, 35-39 (2009).
 - 17- S. Mansour, O. Eldaly, A. F. Jiman, M. Mohamed and E. Ibrahim, "Epidemiological Characterization of *P. aeruginosa* Isolates of Intensive Care Units in Egypt and Saudi Arabia", (2013).
 - 18- G. F. Gad, R. A. El-Domany, S. Zaki and H. M. Ashour, "Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: Prevalence, antibiogram and resistance mechanisms", *Journal of Antimicrobial Chemotherapy*, 60, 1010-1017 (2007).
 - 19- S. H. Abbas, M. Naeem, M. Adil, S. M. Naz, A. Khan and M. U. Khan, "Sensitivity patterns of *Pseudomonas aeruginosa* isolates obtained from clinical specimens in Peshawar", *Journal of Ayub*

- Medical College Abbottabad*, 27, 329-332 (2015).
- 20- A. B. Mahmoud, W. A. Zahran, G. R. Hindawi, A. Z. Labib and R. Galal, "Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods", *Journal of Virology & Microbiology*, 2013, 10-13 (2013).
- 21- Y. T. Cayci, A. Coban and M. Gunaydin, "Investigation of plasmid-mediated quinolone resistance in *Pseudomonas aeruginosa* clinical isolates", *Indian Journal of Medical Microbiology*, 32, 285 (2014).
- 22- A. Fatima, S. Nagvi and S. Khalig, "Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infections", *Springerplus*, 1, 70 (2012).



نشرة العلوم الصيدلانية جامعة أسيوط



تحديد وتصنيف السودومونس ايرجينوزا بين مرضى العدوى المكتسبه للمستشفيات في مستشفى جامعة أسيوط

اسماعيل صديق سليمان - احسان عبد الصبور حسن - نها عبد الحليم عفيقي -
شيرين عبد الرحمن - آيات مصطفى كامل

قسم الميكروبيولوجيا الطبية والمناعه ، كليه الطب ، جامعه اسيوط ، اسيوط ، مصر

السودومونس ايرجينوزا هي واحدة من مسببات الأمراض الأكثر شيوعا و التي تسبب عدوى
المستشفيات المكتسبه. هذا الكائن يسبب العديد من الأمراض بما في ذلك التهاب المسالك البولية عدوى
الجرح والالتهاب الرئوي وتجرثم الدم، ... الخ. ومرضى نقص المناعة في وحدة العناية المركزة
معرضون لمخاطر عالية من اكتساب العدوى.

هذه الدراسة هدفت إلى تحديد السودومونس ايرجينوزا في المرضى الذين يعانون عدوى
المستشفيات المكتسبه في مستشفى جامعة أسيوط وتصنيف السلالات المعزولة باستخدام API20NE .
هذه الدراسة شملت ٦٧٧ عينه والتي تم جمعها من ٣٦٦ من المرضى الذين يعانون عدوى
المستشفيات المكتسبه المقبولين في وحدات العناية المركزه و عنابر مستشفيات جامعة أسيوط والتي
تضمنت وحدة العناية المركزة عصبية، وحدة العناية المركزة الطب الباطني، وحدة العناية المركزة
أطفال، وحدة العناية المركزة بعد العمليات، وحدة العناية المركزة الصدر، وحدة أمراض الدم، ثم وحدة
العناية المركزة اصابات ، واخيرا وحدة الاصابات. وقد تم التعرف على السلالات البكتيرية عن طريق
زرعها على مستنبتات بكتريولوجية مختلفة. وقد تم تصنيف السلالات المعزولة باستخدام API 20NE .
في هذه الدراسة تم عزل ٣٠ سلاله من السودومونس ايرجينوزا وتم تصنيف السلالات
المعزولة باستخدام API 20NE وكانت الرموز الأكثر شيوعا لسلالات السودومونس ايرجينوزا المعزولة
من عينات المرضى برمز ١١٥٤٥٧٥.

السودومونس ايرجينوز هي سبب شائع لعدوى المستشفيات المكتسبه في جامعة أسيوط. و كانت
الرموز الأكثر شيوعا لسلالات السودومونس ايرجينوزا المعزولة من عينات المرضى برمز
١١٥٤٥٧٥.