

Sensitivity pattern of pseudomonas aeruginosa isolates to quinolones

Ronaq Zaman, Muhammad Inam, Bashair Ahmad

Kabir Medical College, Gandhara university, Peshawar; Department of Orthopedic And Trauma Surgery, Post Graduate Medical Institute, Hayatabad Medical Complex, Peshawar and Center of Biotechnology and Microbiology, University of Peshawar, Pakistan

Objective: To determine the sensitivity pattern of *Ps. aeruginosa* against fluoroquinolone antibiotics.

Methodology: This descriptive study was carried out at Microbiology Research Laboratory Center of Biotechnology and Microbiology [COMBAM], University of Peshawar, from September 2011 to April 2012. A total of 296 samples were collected from local hospital and screened for *Ps. aeruginosa*. Samples were collected from different wound infections such as post operative wound, diabetic foot, fire arm injuries, road traffic accidents, acute and chronic osteomyelitis, abscess and bed sores.

Results: Out of 296 samples, *Ps. aeruginosa* was isolated from 100 samples with frequency of 33%. Out of 100 samples, 64 were collected from male patients and 36 from female patients. The patient

age ranged from 11 to 88 years. Infection rate was greater in age range of 31-60 years (63%) followed by age range of 11-30 (28%) and age 60 and above had infection rate of 9%. Out of 100 isolates, 12% showed resistance to all antibiotics. None of the antibiotics showed 100% sensitivity to all the isolates.

Conclusion: This study gives an insight to the current state of sensitivity pattern of *P. aeruginosa* to quinolones antibiotics. There is a need for controlling the emergence of multi drug resistant organisms in the hospitals and community acquired infections. (Rawal Med J 2013;38: 244-248).

Keywords: Sensitivity, resistance, Pseudomonas aeruginosa, quinolones.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic Gram-negative bacterium responsible for a wide range of infections including urinary tract infection (UTI), pneumonia and bacteremia in the immunocompromised host.¹ It is an opportunistic pathogen and represents one of the most resistant nosocomial pathogens because 28 percent of healthy people in hospital environment are carrier for *Ps. aeruginosa*.¹ It contributes a lot to wound related morbidity and mortality especially in hospitalized burnt patients, orthopedic related infection, respiratory tract infections, immunocompromised and catheterized patients. It may be the cause of the chronic pulmonary infection, which is the cause of death in-patients with cystic fibrosis.²

The pathogenicity of *Ps. aeruginosa* depends on its ability to produce different proteases and toxins and on its ability to resist phagocytosis.³ It is a dangerous pathogen because it is resistant to common

antibiotics. They are sensitive to carbenicillin, cephalosporin, colistin, gentamicin, streptomycin, quinolones and polymyxin but cross-resistance between these antibiotics does occur.⁴ It is not a part of the normal flora but it is frequently found in patients with cystic fibrosis, burns and neutropenia.⁵ A postoperative wound infection is a postoperative complication which causes financial burden, embarrassment to surgeon, discomfort to the patient and even death of the patient. In developing countries the increasing incidence of *Ps. aeruginosa* in post operative wound infections is a serious problem due to lack of hygiene and low quality antiseptics.⁶

Quinolone antibiotics are broad spectrum antibiotics first discovered in 1962 and used in 1967 for the treatment of UTI caused by enterococcal bacteria. The first quinolone antibiotic introduced was Nalidixic acid and further research work led to the development of a large and effective family of quinolones; the fluoroquinolones like

ciprofloxacin.⁷ The fluoroquinolones enter the bacterium by passive diffusion through water filled protein channels [porins] in the outer membrane. Once inside the cell they inhibit the replication of bacterial DNA by interfering with the action of DNA gyrase.⁸ They are effective against gram negative organism such as Enterobacteriaceae, Pseudomonas, Haemophilus influenza, Moraxella catarrhalis, Legionellaceae, Chlamydia, Mycoplasma and some Mycobacteria. This study was conducted to determine the sensitivity pattern of P. aeruginosa against fluoroquinolones antibiotics.

METHODOLOGY

This descriptive study was carried out at Microbiology Research Laboratory Center of Biotechnology and Microbiology [COMBAM], University of Peshawar, from September 2011 to April 2012. A total of 296 samples were collected from Department of Orthopedic and Spine Surgery, Hayatabad Medical Complex, Peshawar and screened for P. aeruginosa. Samples were collected from post operative wound, diabetic foot, fire arm injuries, road traffic accidents, acute and chronic osteomyelitis, abscess and bed sores. The samples were collected by sterile swab sticks from different wounds.

Mac Conkey agar was used for identification of *Ps. aeruginosa*. For identification of isolates gram staining was done. Nutrient broth and Muller Hinton media was used to refresh cultures and to determine sensitivity pattern by disc diffusion and minimum inhibitory concentration (MIC) methods. Antibiotic discs were used for disc diffusion method. The list of antibiotic disc is given in Table 1. For MIC, ciprofloxacin, levofloxacin and ofloxacin were used in concentrations 0.5- 64mg/L.

Table 1. List of Antibiotic Discs.

	Antimicrobial Agent	Potency(mcg)
1	Nalidixic Acid	5
2	Ciprofloxacin	30
3	Levofloxacin	5
4	Ofloxacin	5
5	Moxifloxacin	5
6	Norfloxacin	10
7	Enoxacin	10

MIC of antibiotics was determined by an agar dilution technique. Data were recorded with the help of a Proforma and was analyzed using SPSS version 10.

RESULTS

Out of 296 samples, *Ps. aeruginosa* was isolated from 100 samples with frequency of 33%. Out of 100 samples, 64 were collected from male patients and 36 from female patients. Male-female ratio was 2:1 (Table 2)

Table 2. Frequency of gender of patients.

Gender	Number	Percent
Female	36	36.0
Male	64	64.0
Total	100	100.0

The patient age ranged from 11 to 88 years. Infection rate was greater in age range of 31-60 years (63%) followed by age range of 11-30 (28%) and age range of 60 and above had infection rate of 9%. The infection rate was greater in hospitalized patient (79%) than the outdoor patients (21%). The ratio of indoor patient to outdoor patient is 4:1. The frequency rate of different types of wounds is given in Table 3.

Table 3. Frequency of different types of wounds.

Nature of wound	Number	Percent
Abscess	1	1.0
Acute osteomyelitis	6	6.0
Bomb blast injury	8	8.0
Bed sores	2	2.0
Chronic osteomyelitis	8	8.0
Crush injury	1	1.0
Diabetic foot	19	19.0
Fire arm injury	8	8.0
Post operative wound	30	30.0
Road traffic accident	16	16.0
Septic arthritis	1	1.0
Total	100	100.0

Out of 100 isolates, 12% showed resistance to all antibiotics. None of the antibiotics showed 100% sensitivity to all the isolates. There was very high resistance to nalidixic acid. Ninety-two percent of isolates showed resistance, 4% showed intermediate resistance while 4% were sensitive (Figures 1 & 2).

Fig 1. Percent sensitivity of different antibiotics (disc diffusion method)

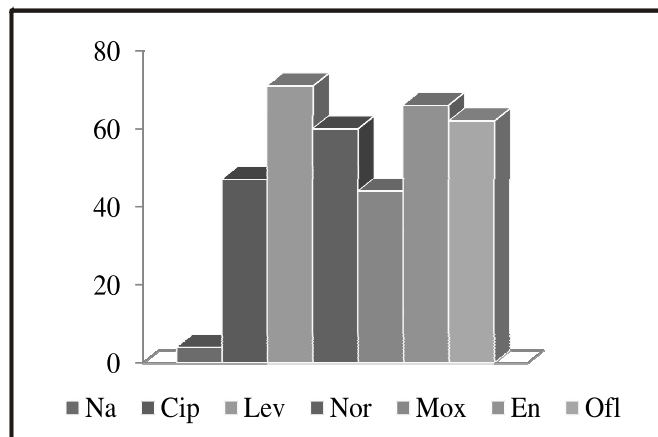
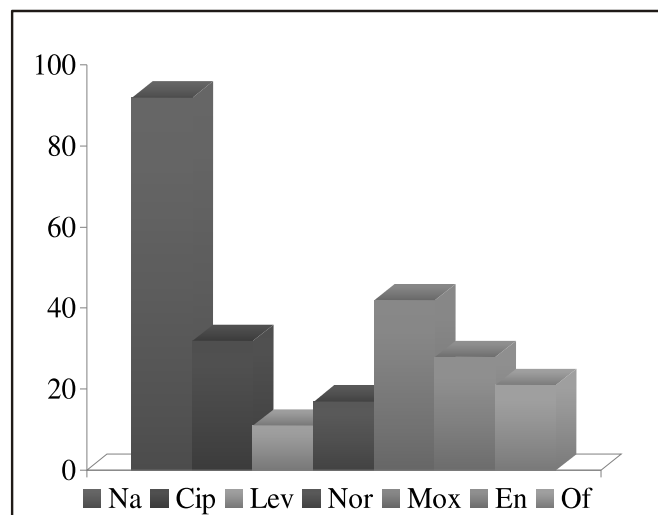


Fig 2. Percent Resistance of different antibiotics (disc diffusion method).



The study showed that 32% isolates were resistant to ciprofloxacin, 21% were intermediate resistant while 47% isolates were sensitive (Figures 1&2). By MIC method 43% were sensitive. Intermediate resistant were 18% and resistant were 39% (Figures 3 & 4).

Fig 3. Percent Sensitivity by MIC.

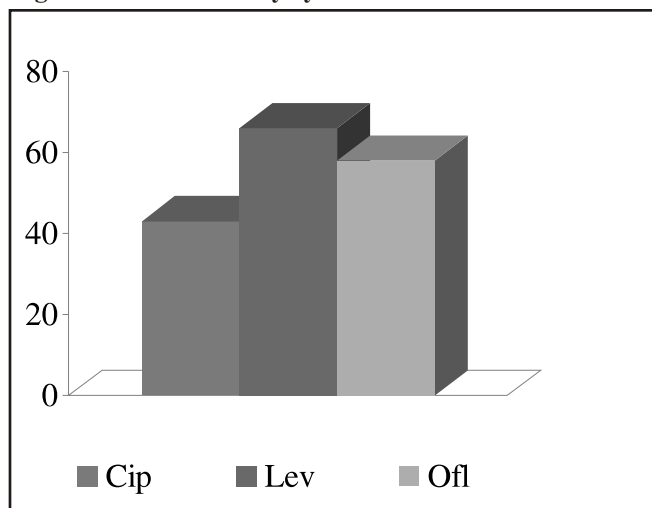
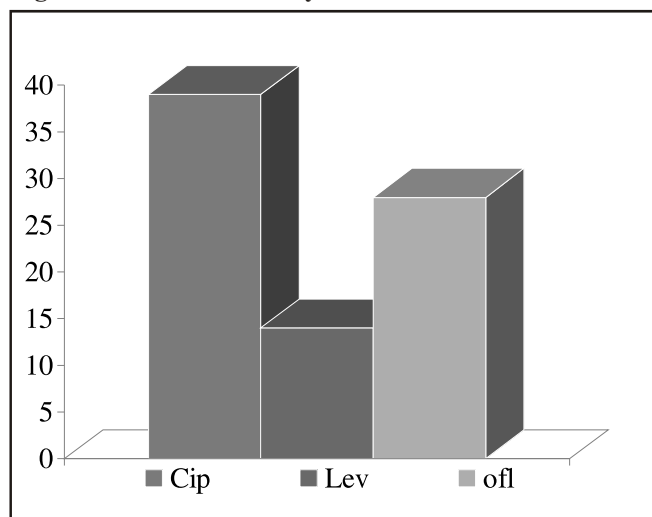


Fig 4. Percent Resistance by MIC.



DISCUSSION

The discovery of antibiotics in 1940s is considered as one of the greatest achievements in the clinical medicine. As a result, the mortality rate decreased due to bacterial infections. Unfortunately, as more antibiotic has appeared the microorganisms have developed resistance to them.² The frequency of *Ps. aeruginosa* in wound infections was 33% in our study. Ranjan et al reported 29.6% prevalence which is in agreement with current study.⁹ This frequency is also in agreement with the work done by Dhan Raj¹⁰ (27.5%) as well as by Revathy et al¹¹ (36%). Male-female ratio was 2:1 in the current study. Hena

et al¹² showed the male:female ratio of 2:1 which is the same as in current study. Ratio of 1:1.16 of female to male was found in the study conducted by Ullah et al.¹³ Higher rate of infection in males as shown in current study is because of more exposure of males to the outside environment in our country as compared to females.

Ogiwara et al as well reported a high resistance (over 90%) of the organisms to nalidixic acid.¹⁴ Anthony et al reported 100% resistance.¹⁵ Both of these studies are comparable to the current study. The higher rate of resistance of nalidixic acid is because of low potency against *Ps. aeruginosa*. In a study from Tunis by Bousseimi et al, 53.4% of isolates were resistant to ciprofloxacin which is higher than the current study.¹⁶ Meenakumari et al reported 33% of resistance which is in strong agreement with the present study.¹⁷ Norfloxacin sensitivity of 87.5% was reported by Abraham et al which is higher than the current study.¹⁸ Iffat et al reported resistance of 20% to moxifloxacin which is not in accordance with the current study.¹⁹ Ahmed et al reported a resistance of 37% to enoxacin which is comparable to the present study.²⁰ The study conducted by Bouza et al in Spain on ofloxacin showed 30% resistance which is in accordance with the current study.²¹ A higher sensitivity (80%) was reported by Fedeyi et al.²²

Physicians consider antibiotics as ordinary drugs and prescribe them frequently. The programs launched for the purpose of reduce consumption of antimicrobials have effects for transient period because of lack of repeated and sustained incentives. A program launched in 2000 in France had a very good effect as it caused 23% decrease in antibiotic consumption during time period of five years.²³ In Belgium, a campaign launched by Belgian Antibiotic Policy Coordination Committee (BAPCOC) resulted in a 36% decrease in antibiotic prescription in the community between 1999 and 2007 and decreased antibiotic resistance in *Streptococcus Pneumoniae* and *S. Pyogenes*.⁸

CONCLUSION

By MIC method, levofloxacin showed 66% sensitivity, ofloxacin 58% and ciprofloxacin 43%. Twelve percent of isolates showed resistance to all

the antibiotics. The bacterial isolates did not show 100% sensitivity to any one of antibiotics. We suggest that clinicians should choose the antibiotic to which micro-organism is sensitive.

Author Contributions:

Conception and design: Ronaq Zaman
Collection and assembly of data: Ronaq Zaman
Analysis and interpretation of the data: Muhammad Inam
Drafting of the article: Muhammad Inam
Critical revision of the article for important intellectual content: Bashir Ahmad
Statistical expertise: Muhammad Inam
Final approval and guarantor of the article: Ronaq Zaman
Corresponding author email: dr_mohammadinam@yahoo.co.uk
Conflict of Interest: None declared
Rec. Date: Mar 12, 2013 Accept Date: May 05, 2013

REFERENCES

1. Read R.C, Cornaglia G, Kahlmeter G. European Society of Clinical Microbiology and Infectious Diseases Professional Affairs Workshop Group: Professional challenges and opportunities in clinical microbiology and infectious diseases in Europe. *Lancet Infect Dis* 2011;11:408-15.
2. Nwanko E, K Shuaibu SA. Antibiotic susceptibility pattern of clinical isolates of *P. aeruginosa* in a tertiary health institution in Kano Nigeria. *J Med Biomed Sci* 2010;4:2073-8.
3. Khan J, Iqbal Z, Saeed R, Kalsoom F. Prevalence of resistance pattern of *P. aeruginosa* against various antibiotics. *Pak J Pharm* 2002;3:311-5.
4. Meir S, Weber R, Zbinden R, Ruef C, Hasse B. Extended- spectrum beta-lactamases-producing Gram negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. *Infection* 2011;39:333-40.
5. Shriyan S, Sheetal R, Nayak N. Aerobic microorganism in post operative wound infections and their antimicrobial susceptibility pattern. *J Clin Diag Res* 2010;4: 3392-6.
6. Bertrand XM, Thouverez C, Party P, Talon BB. Antibiotic susceptibility and genotype characterization of strains isolated in the intensive care unit. *Clin Micro Infec* 2002;7:706-8.
7. Bradley JS, Jackson MA. The use of systemic and topical Fluoroquinolones. *Paediatrics* 2011;128:1034-45.
8. Huttner B, Goossens H, Verheij T, Harbarth S. Characteristics and outcomes of public campaigns aimed at improving the use of antibiotics in outpatients in high-income countries. *Lancet Infect Dis* 2010;10:17-20.
9. Ranjan KP, Ranjan N, Bansal SK, Arora DR. Prevalence of *Pseudomonas aeruginosa* in Post-

- operative Wound Infection in a Referral Hospital in Haryana. *Indian J Lab Phys* 2010;2:74-7.
10. Nakade Dhanraj BJ. Antibiotic Sensitivity of common Bacterial Pathogens against selected Quinolone. *Biological Sci* 2012;1:77-9.
 11. Revathy G, Puri J, Jain BK. Bacteriology of Burns. *Burns* 1998;24:347-9.
 12. Hena JV, Growth L. Studies on bacterial infections of diabetic foot ulcer. *Afr J Exper microbial* 2010;11:146-9.
 13. Ullah F, Malik SA, Ahmed J. Antimicrobial Susceptibility and ESBL Prevalence in *P. aeruginosa* isolated from burn patients in the North West of Pakistan. *Burns* 2009;35:1020-5.
 14. Ogiwara M, Ishibashi K, Yoshida H, Imafuku Y, Murai M, Igari J, et al. Comparative studies on activities of antimicrobial agents against the causative organisms isolated from patients with urinary tract infections. *Jpn J Antibiot* 1997;52:177-267.
 15. Anthony A, Mvuyo T, Anthony O, Steve J. Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. *Sci Res Essays* 2010; 5:3876-81.
 16. Bousseimi K, Thabet L, Ouesiat S, Jaber OB, Ouchtati A, Cherif S, et al. Epidemiological profile and antibiotic susceptibility of *Pseudomonas aeruginosa* isolates within the hospitalized burned patient. *Critical care* 2005;9:10-11.
 17. Meenakumari S, Verma S, Absar A, Chaudhary A. Antimicrobial Susceptibility Pattern of Clinical Isolates of *Pseudomonas aeruginosa* in an Indian Cardiac Hospital. *Inter J Sci Tech* 2011;3:7117-24.
 18. Abraham Y, Wamisho BL. Microbial susceptibility of bacteria isolated from open fracture wounds. *Can J Mic Res* 2009;3:939-51.
 19. Iffat W, Shoaib MH, Muhammed IN, Rehana TS, Gauhar S. Antimicrobial sensitivity testing of newer quinolones against gram positive and gram negative clinical isolates. *Pak J Pharm* 2010;23:245-9.
 20. Ahmed J, Jan AH, Nawaz G, Khan M. Epidemiology and antibiotic susceptibility of bacterial isolates from Northern Pakistan. *Afr J Micro Res* 2011;5:4949-55.
 21. Bouza E, Gercia-Garrote F, Cercenado E, Marin M, Diaz MS. *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in Spain. *Antimicrob Agents Chemo* 1999;43:981-2.
 22. Fadeyi A, Adigun IA, Rahman GA. Bacteriological Pattern of Wound Swab Isolates in patients with chronic leg ulcer. *Intern J Health Res* 2008;1:183-8.
 23. Sabuncu E, David J, Bemede-Bauduin C, Pépin S, Leroy M, Boëlle PX, et al. Significant reduction of antibiotic use in the community after a nationwide campaign in France, 2002-2007. *PLoS Med* 2009;6:1000008.