JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. **7**(Spl. Edn.), p. 465-469

Pseudomonas aeruginosa: Need for Optimization of Antibiotic Use

Sadia Zafar[1]*, Syed Baqir Shyum Naqvi[2], Faaiza Qazi[1], Nusrat Bano[3]and Rabia Sheikh[4]

[1]Faculty of Pharmacy, Jinnah University for Women, Karachi, P.O.Box 74600, Pakistan.
[2]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Pakistan.
[3]Faculty of Pharmacy, ZiauddinCollege of Pharmacy, ZiauddinUniversity, Karachi - 75600 Pakistan.
[4]Nigehban Compounding Pharmacy, Karachi, Pakistan.

(Received: 25 August 2013; accepted: 24 October 2013)

Pseudomonas aeruginosa **is an opportunistic pathogen, accounted for causing large number of serious infections. Due to emergence of multidrug resistant species the treatment of pseudomonal infections has become complicated. The cross-sectional observational study was performed from March 2012 to July 2012. 100 clinical isolates of *Pseudomonas aeruginosa* were obtained from in-patient and out-patient's samples collected from various sites by clinical laboratory of reputed tertiary care hospital of Karachi. The Kirby Bauer Disc Diffusion Method was used to determine the antibiotic susceptibility. The highest percentage of Organism was isolated from Urine sample (23%). Prevalence of pseudomonal infection gender-wise showed Female (58%) and Male (47%) were found to be infected. The age group of 71-100 yrs. in Male and 51-70 yrs. in Female were found to be more prone to pseudomonal infections. *Pseudomonas aeruginosa* sp. showed highest resistance against Colistin (42%) followed by Sulzone (39%), while it exhibited intermediate resistance to Aztreonem (32%). Tazo-pipercillin and Amikacin conferred sensitivity of 83%, 74% respectively. The sp. isolated from urine sample was highly sensitive to Amikacin (73.9%); More than 85% of sp. isolated from Tracheal aspirate was sensitive to Colistin and Sulzone. Imipenem and Tazo-pip, both were highly effective against more than 80% of sp. isolated from Pus specimen.**

Key words: *Pseudomonas aeruginosa*, Prevalence, Resistance.

Multiple mechanisms of antibiotic resistance in *Pseudomonas aeruginosa* was perceived as the worst nightmare since the beginning of this decade[1]. The pathogen is noted for its intrinsic capability of antimicrobial resistance as it attains genes encoding resistant determinants[2].Penicillins and cephalosporins have long lost efficacy against the pathogen as it produces extended spectrum beta lactamases (ESBL)[3]. *Pseudomonas aeruginosa* is considered to be an opportunistic gram-negative rod. It is accounted for causing large number of serious infections[4]. Nowadays severity of infections has been increased and treatment of these infections has become complicated due to emerging resistance among these clinical isolates especially the multidrug resistant species has worsen the situation[5]. The physicians today are left with fewer choices to effectively treat pseudomonal infections and they are opting to select the regimen of combination therapy because this microorganism has shown resistance to antibiotics by different mechanisms[4]. This study is based on the data acquired from the local population of Karachi, Pakistan and is designed to report the pattern of resistance of the pathogen against array of antibiotics meanwhile the susceptibility with few antimicrobials is also taken into account.

* To whom all correspondence should be addressed.
E-mail: sadiazafarnew@yahoo.com

MATERIALS AND METHODS

Disc Diffusion Method was used to evaluate the sensitivity of *Pseudomonas aeruginosa* to different antibiotics according to CLSI standards [6].

No. of isolates: 100 clinical specimens were obtained from various sites of infections of inpatient and outpatient.

Period of sample collection: isolates of *Pseudomonas aeruginosa* were collected from period March,2012-July2012from a clinical laboratory of reputed tertiary care hospitalof Karachi.

Identification of Species

The isolates were identified by routine standard procedures.

Antimicrobial agents

Standard (Oxoid) discs of Amikacin 30µg, Aztreonam 30µg, Ceftazidime 30µg, Gentamycin 10 µg, Imipenem 10 µg, Tazo-pip 10/100 µg,Colistin10 µg, sulzone 100 µg.

Culture Media

Mueller Hinton Agar was used, the preparation and storage of media were done according to Manufacturer's instructions (Oxoid, U.K).

Growth of culture

Mueller Hinton Broth (Oxoid, U.K) were used for growth of inoculum at 37^0C for 2-6 hrs.

McFarland Standard

The broth culture is incubated until the turbidity of the 0.5 McFarlandstandards was achieved.

Inoculation of Mueller Hinton Agar Plates

Sterile cotton swabs were used to inoculate the plates with culture of *Pseudomonas aeruginosa* by dipping it in inoculums suspension and after removing excess fluid it was then streaked evenly over the surface of agar medium.

Placement of Discs

After drying of inoculum, with the help of sterile forceps the discs of different antibiotics were placed on agar surface.

Interpretation of results

The results were recorded after 24 hrs.ofincubation and were interpreted according to guidelines stated by CLSI.

Reference Strain

To control the Precision and accuracy of the disk diffusion test procedures, quality control strain *Pseudomonas aeruginosa*® 27853 has been used.

RESULTS

In our study, resistant-sensitivity pattern of total 100 clinical isolates of *Pseudomonas aeruginosa* collected from various sites of infectionswas studied using 8 broad spectrum antibiotics.Gender specific distribution of Pseudomonalinfection is shown in Table1 and 2,Theresistance/sensitivity pattern is presented in Table 3.The results were interpreted by the help of CLSI Interpretive standards for *Pseudomonas aeruginosa* as shown in Table 4.The outcomes of the study showed that *Pseudomonas aeruginosa* was highly resistant to Colistin(42%),whereas, Tazo-Pip(83%) and amikacin (74%) were found to

Table 1. Age and gender specific distribution of *Pseudomonas aeruginosa* among patients

Age	Gender	
Year	Male N=47	Female N=53
0-30	13	12
31-50	7	12
51-70	11	17
71-100	16	12

Table 2. Distribution of *Pseudomonas aeruginosa* isolates at various sites of infections

Site of	Male		Female	
Infection	N	%	N	%
Tracheal aspirate	10	21.28	8	15.09
Pus	11	23.40	7	13.21
Urine	9	19.15	14	26.42
Blood	4	8.51	3	5.66
Wound Swab	0	0.00	7	13.21
Bronchial tap	0	0.00	1	1.89
Sputum	6	12.77	6	11.32
BAL	0	0.00	1	1.89
Ear swab	1	2.13	2	3.77
Tissue	2	4.26	0	0.00
Right aural swab	1	0.00	1	1.89
Pericardial fluid	0	0.00	1	1.89
Pleural fluid	1	2.13	0	0.00
Others	2	4.26	2	3.77

Table 3. Resistance-Sensitivity pattern of *Pseudomonas aeruginosa* against different antibiotics

Antibiotics	Resistance %	Sensitive %
Amikacin	26	74
Azteronem	32	68
Ceftazidime	37	63
Gentamicin	37	63
Imipenem	29	71
Tazo-Pip	17	83
Colistin	42	58
Sulzone	39	61

Table 4. Zone diameter interpretive standards for Pseudomonas aeruginosa.CLSI standards table of antibiotics for *Pseudomonas aeruginosa*

Disc Content	Resistance	Intermediate	Sensitive
Amikacin 30µg	≤14	15-16	≥22
Azteronem 30µg	≤15	16-21	≥22
Ceftazidime 30µg	≤14	15-17	≥18
Gentamicin 10µg	≤12	13-14	≥15
Imipenem 10µg	≤13	14-15	≥16
Tazo-Pip 10µg/100µg	≤17		≥18
Colistin 10µg	≤10		≥1
Sulzone 100µg	≤15	16-20	≥21

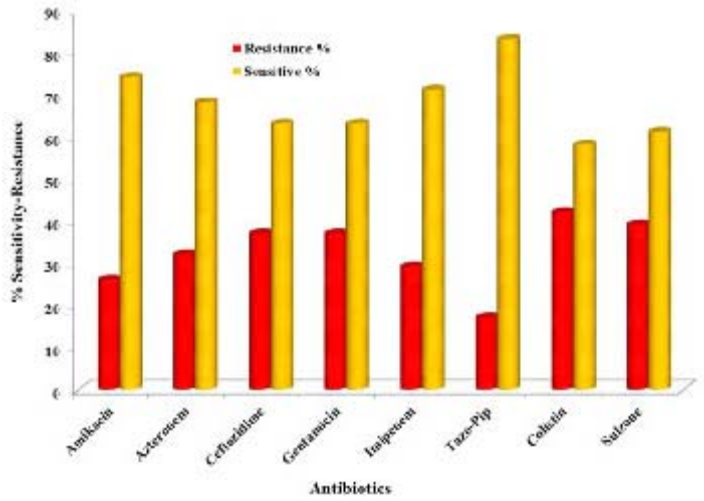

Fig. 1. Sensitivity-Resistance pattern of *Pseudomonas aeruginosa* against broad spectrum antibiotics

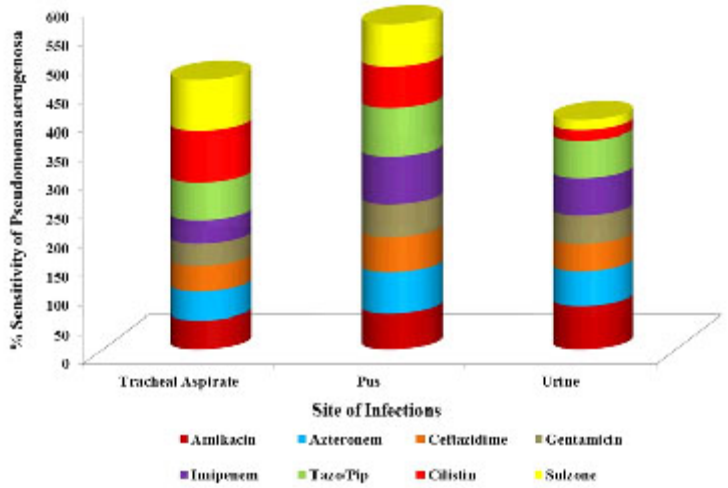

Fig. 2. % Sensitivity of *Pseudomonas aeruginosa* isolated from various sites against different antibiotics.

be most effective against *Pseudomonas aeruginosa* as indicated by the Figure 1. The Present study also revealed that the specie isolated from urine sample was highly sensitive to Amikacin (73.9%); More than 85% of sp. isolated from Tracheal aspirate was sensitive to Colistin and Sulzone which is obvious in Figure 2.

DISCUSSION

The identification and extent of resistance pattern of *Pseudomonas aeruginosa* in Pakistan is necessary. The objective of this study was to observe the trend of sensitivity-resistance pattern in 100 clinical isolates of *Pseudomonas aeruginosa* from the wide range of clinical specimens against 8 broad spectrum antibiotics belonging to different groups. The pathogen predominated in Urine specimen followed by Pus and Tracheal aspirate.Uropathogens exhibit a high degree of antibiotic resistance[7].

The old patients especially females were found to be more susceptible to the pseudomonal infections. Organisms exhibited high resistance against Colistin (42%) which is an alarming situation since Colistin therapy (inhalation and parenteral) is a mainstay and first line agent in treatment of P*seudomonas aeruginosa*[8]. It is considered a superior choice in cystic fibrosis patient with long term *Pseudomonas aeruginosa* lung infection [9]. Increased acquired pathogenic resistance with Colistin reported during present study, renders the ineffective use of this antibiotic as inhalation agent in airway infections.

Low bacterial resistance against Sulzone(Cefapeozone+Sulbactum) (7%) is reported by Nadeem*et al*[10], whereas we report manifold higher resistance againstSulzone treatment up to 39%, which indicates the continual emergence and prevalence of pseudomonas resistance against sulzone.

In present study we have found that pathogen is highly sensitive to Tazo/Pip (83%) and Amikacin(74%). Resistant isolates from urine specimen (Uropathogens) against Colistin and Sulzone were also highly sensitive to Amikacin. Our results were in resemblance to the study of Ogbolu*et al* who reported the susceptibility pattern of *Pseudomonas aeruginosa* to Amikacin (74%) [11].Gonluger*et al* observed that resistance of *Pseudomonas aeruginosa* isolates against common antibiotics have increased in Turkey that is Ceftazidime(50.8%), Gentamicin(57.5%),and Amikacin(25.4%) [12].

Our results were in contradiction to findings of Fadeyi*et al.* who observed increasing resistance of *Pseudomonas aeruginosa* to Ceftazidime (50.7%) and Gentamicin (40.2%)[13]. However, our findings are in confirmation with the results reported in a respective study by Gill that is isolates were more sensitive to Amikacin and Tazo-pip.[14].Our observation also contradicts the results of Ranjbar*etal.* who reported resistance of *Pseudomonas aeruginosa* against Imipenem (97.5%), Amikacin(90%), Gentamycin(67.5%), Ceftazidime (57.5%)[15].

Fatima *etal*. observed high resistance against Pip/Tazo (42%),in his studies Imipenem was found to be most effective (76% Sensitive). However, she reported continuously increasing resistance of Pseudomonas*aeruginosa* against Amikacin [16].Similarly, Jain andKhelyin one of their research conducted over period of 2 yrs. revealed that pip/tazo and Amikacin have been proven to be most active against *Pseudomonas aeruginosa*[17].The findings of Zoghlami*et al* further substantiated our results since they reported resistance of isolates to Ceftazidime(34%), Imipenem (37.1%) and Amikacin(29.6%)[18].

CONCLUSION

The conclusion of our findings is that, Amikacin is the best choice antibiotic for rational and appropriate use against pseudomonal infections. Our results also indicate the importance of adherence to guidelines for appropriate use of antibiotics as laid down by Infectious Disease Control Committee to control the emergence of resistance in strains of *Pseudomonas aeruginosa*, Hence it is recommended that physicians must determine the sensitivity profile prior to prescribing antibiotics to prevent the resistance and treatment failures.

REFERENCES

1. Livermore,D. M. “Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare?” *Clin Infect*

Dis, 2002; **34**(5):634-640.

2. Bonomo,R.A.,Szabo,D."Mechanisms of multidrug resistance in Acinetobacter species and *Pseudomonas aeruginosa*,"*Clin Infect Dis*, 2006; **43**(2): S49-S56.
3. Ullah,F.,MalikS. A., Ahmed,J. "Antimicrobial susceptibility and ESBL prevalence in*Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan," *Burns*, 2009; **35**(7):1020-1025.
4. Ryan,K. J. "Pseudomonas and other opportunistic Gram-Negative Bacilli.In: *Sherris Medical Microbiology:An introduction to Infectious diseases*(Ryan,K. J. andRay,C. G., ed.),McGraw Hill, 2004;pp385-90.
5. Mesaros,N.,Nordmann,P.,Plesiat, P., Roussel-Delvaller,M., Van Eldere,J.,Glupczynski, Y.,Van Laethem,Y.,Jacobs,F.,lebecque,P.,Malfroot,A.,TulkensP. M., Van Bambeke, F. "*Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium," *Clin Microbiol Infec*, 2007; **13**(6):560-578.
6. Clinical and Laboratory Standards Institute, "Performance standards for antimicrobial susceptibility testing,"*in proceedings of the 22nd Informational Supplement*,Wayne, Pennsylvania,USA, M100-S22, 2012.
7. Gupta, V.,Yadav A.,Joshi, R. M. "Antibiotic resistance pattern in uropathogens," *Indian J Med Microbiol.*, 2002; **20**(2):96-98.
8. Doring,G.,Conway,S. P., Heijerman,H. G.,Hodson,M. E.,Hoiby,N.,Smyth,A.,Touw, D. J. "Antibiotic therapy against *Pseudomonas aeruginosa*in cystic fibrosis: a European consensus," *EurRespir J.*, 2000;**16**(4): 749-767.
9. Tim,J.,Svend,P. S., Susanne,G.,Carsten,H. ,Niels, H., Christian, K. "Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection," *J Antimicrob Chemoth*,1987; **19**(6): 831-838.
10. Nadeem,S. G.,Qasmi,S. A.,Afaque,F.,Saleem, M. ,Hakim,S. T. "Comparison of the in vitro susceptibility of Clinical isolates of *Pseudomonas aeruginosa* in a local hospital setting in Karachi, Pakistan," *B J M P*, 2009; **2**(4):35-39.
11.. Ogbolu,D. O.,Ogunledun, A.,Adebiyi,O. E.,Dainji, O. A.,Alli,A. O. "Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* to available antipseudomonal drugs in Ibadan, Nigeria," *Afr J Med Med Sci*, 2008; **37**(4): 339-344.
12. Gonluger,U.,Bakici,M. Z.,Ozdemir,L. ,Akkurt,I., Icagasioglu,S.,Gultekin, F." Retrospective analysis of antibiotic susceptibility patterns of respiratory isolates of *Pseudomonas aeruginosa* in a Turkish University Hospital," *Ann Clin Microbiol Antimicrob*, 2003; **2**: 5.
13. Fadeyi,A.,Akanbi,A. A. 2nd,Nwabuisi C., Onile,B. A. "Antibiotic disc sensitivity pattern of *Pseudomonas aeruginosa* isolates obtained from clinical specimen in IIorin, Nigeria," *Afr J Med Med Sci*, 2005; **34**(3):303-306.
14. Gill,M. M.,Usman,J. ,Kaleem,F., Hassan,A., Khalid,A.,Anjum,R., Fahim, Q. "Frequency and antibiogram of multi-drug resistant *Pseudomonas aeruginosa*," *J Coll Physicians Surg Pak*, 2011; **21**(9): 531-534.
15. Ranjbar, R., Owlia, P., Saderi, H., Mansouri, S., JonaidiJafari, N., Izadi, M., FarshadS., Arjom and Zadegan, M. "Characterization of *Pseudomonas aeruginosa* strains isolated from burned patients hospitalized in a major burn center in Tehran," *Acta Medica Iranica* ., 2011; **49**(10):657-659.
16. Fatima,A.,Naqvi,S. B.,Khaliq, S.A. Perveen S., Jabeen, S."Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infections,"*Springer plus*, 2012; **1**(1):70.
17. Jain,S., Khety,Z. "Changing antimicrobial resistance pattern of isolates from an ICU over a 2 year period," *J Assoc Phys India*, 2012;**60**:27-33.
18. Zoghlami,A.,Kanzari, L.,Boukadida,J.,Messadi, A. A.,Ghanem, A. "Epidemiological profile and antibiotic resistance of *Pseudomonas aeruginosa* isolates in burn and traumatology center in Tunisia over a three-year period," *Tunis Med*, 2012; **90**(11):803-806.