

TO STUDY THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM BURN PATIENTS

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Abstract

Burns provide an appropriate site for bacterial proliferation and are more suitable site of infection than surgical wounds. Patients of burn injuries are more susceptible to opportunistic pathogens like *P.aeruginosa*. It has been related with numerous nosocomial and community acquired infections. This capacity of *P. aeruginosa* to produce such a multiple number of infections is due to the virulence factors. The present study was planned to check the extent of prevalence of *P. aeruginosa* in burn patients and to study their antibiotic susceptibility pattern which will later help in efficient drug designing. In the present study, out of 20 samples, 13 samples (65%) showed presence of *P. aeruginosa*. Other isolates obtained were *Proteus sp.* (15%), *Staphylococcus sp.* (15%) and *Klebsiella sp.* (5%). Biochemical characterization led to the final confirmation of the occurrence of *Pseudomonas aeruginosa* in the burn patients. Gram positive cocci were found to be 15% and gram-negative bacilli were found to be 85%. Antibiotic susceptibility pattern for the isolates was observed as Ciprofloxacin (100%) followed by Gentamicin (92%), Ampicillin (46.15%). While the frequency of resistance was highest to Bacitracin (70%) followed by Ampicillin (53.84%), Gentamicin (8%). Zone of inhibition was determined for each antibiotic. Percentage of multi drug resistant isolates was determined which showed that 62% isolates were sensitive to 2 antibiotics used out of 4. While 38% isolates were sensitive to 3 antibiotics out of 4 antibiotics used. These isolates are mainly resistant to two antibacterial named Ampicillin and Bacitracin.

Introduction

Burn injury is one of the significant problems threatening public health in both developed and developing nations (Gupta *et al.*, 1993). Individuals affected during burns require long periods of hospitalization and acquire physical as well as psychosocial disabilities. Various sources of bacterimia include extent of injury, depth of burns and various other microbial factors. The various micro-organisms responsible for bacterimia include *Staphylococci sp.*, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella sp.*, *Proteus sp.* and Methicillin resistant *Staphylococcus aureus*.

Among immunocompromised burn patients, *Pseudomonas aeruginosa* is one of the opportunistic pathogen that causes a wide range of infections (Church *et al.*, 2006). Since *P. aeruginosa* is resistant to all effective antibiotics, the infection caused by organism is a complicated situation for patients (Salimi *et al.*, 2010). It is related with variety of hospital acquired and community acquired infections (Nwankwo and Shuaibu, 2010). In the past years, *P. aeruginosa* has become an important nosocomial pathogen (Greta *et al.*, 2007). *Pseudomonas aeruginosa* a commensal of human microflora in normal people. The rate of commensalism gradually rises with the increased exposure of hospital stay (Altopark *et al.*, 2004).

P. aeruginosa is a gamma proteobacteria belongs to family of Pseudomonads. The characteristics of genus are: rod shaped, polar flagella providing motility, aerobic and non-spore forming. *Pseudomonas* is gram negative bacillus characterized by the production of peculiar pyocyanin, which is a water soluble pigment. It exhibits a great potential to colonize a wide range of habitats (Madigan and Martinko, 2005). *Pseudomonas sp.* is responsible for various nosocomial infections, especially in intensive care units, various instruments and ventilator- associated pneumonia, therefore, a lot of attention is now being paid to it as a potential pathogen in hospitals.

There has been a rapid increase in number of multiple drug resistant strains of *P.aeruginosa*. Due to the prevalence of multiple drug resistant strains, infection with *P. aeruginosa* leads to higher mortality rate and antibiotic costs (Ullah *et al.*, 2009). The resistance of bacterium to multiple antibiotic ranges is because of its permeability barrier and its ability to form biofilms which makes the cells impervious to therapeutic antibiotics.

Several studies regarding the antibiotic sensitivity pattern of *P. aeruginosa* have been performed over the past years. Organisms isolated from wound swabs were more resistant to azithromycin followed by ceftazidime, ceftriaxone and ciprofloxacin. A quite similar study was performed at Burn unit of Fauji Foundation Hospital, Rawalpindi. *P. aeruginosa* showed low sensitivity to penicillin and macrolides

which may be due to chromosome mediated type – 1 beta lactamase and higher sensitivity to co-amoxiclav (78.46%) and aztreonam (73.84%) (Khan *et al.*, 2008).

One of the most important features of *P. aeruginosa* is its capability to produce enzymes called AmpC Beta-Lactamases. This group of enzymes confers the bacterium with the ability to render penicillin and cephalosporin inactive (Ullah *et al.*, 2009). The inactivation of β - lactams, including third generation cephalosporin, penicillin, etc is done by hydrolization.

The present study was planned to study the presence of *P. aeruginosa* in burn patients and to study their antibiotic susceptibility with different antimicrobial agents.

Material and Methods

Sample collection and Isolation of *Pseudomonas aeruginosa*

Samples were collected from the depth of burn lesion using a sterile cotton swab from Burn unit of Post Graduate Institute of Medical Education and Research, (PGIMER), Chandigarh and was transported to the Microbiology lab of SUS College of Research and Technology, Tangori in Stuarts' transport medium (HiMedia Mumbai). Isolation of bacteria was done using a selective media i.e. Cefrimide agar (CA) (HiMedia Mumbai) and incubating the plates at 37°C. After 24-48 hrs, the plates were checked for colony characteristics. The predominant colonies appeared on the plate were purified by streak plating on fresh CA plates. The purified cultures were preserved on Nutrient Agar slants for further characterization and antibiotic susceptibility studies.

Characterization of isolated bacteria – The bacterial isolates were characterized for their biochemical and morphological characteristics as given in Bergey's manual of determinative bacteriology (1957) and Cappuccino and Sherman (1999).

Determining antibiotic sensitivity –For determining the sensitivity or resistivity of *P. aeruginosa* against various antibiotics, qualitative method of susceptibility by Kirby Bauer method (Bauer *et al.*, 1966) was used. Freshly prepared solidified Muller-Hinton agar plates were seeded with the culture. Inoculation was done with the help of sterile cotton swab. Culture was then allowed to dry on the plate for 5 to 10 minutes at room temperature. Antibiotic disks were dispensed on the cultured plate by gently pressing the disk with sterile forceps. Plates were then incubated for 16 to 18 hrs at 37°C. Four antibiotic disks dispensed on the plates were: Ampicillin disk, bacitracin disk, ciprofloxacin disk and gentamicin disk. On the completion of incubation period, zone of inhibition around the disk was measured by using

ruler. For each antibiotic, resistance or susceptibility was determined by the parameters as shown in Table 1:

Table 1: Antibiotic susceptibility or resistance criteria

	Zone of inhibition (mm)
Resistant	10mm or less
Intermediate	11mm -15mm
Susceptible	16 mm or more

Result and Discussion

Pseudomonas aeruginosa is commonly isolated nosocomial pathogens. Infections caused by *P. aeruginosa* are difficult to treat because of its increasing resistance towards antibiotics (Emori and Gaynes, 1993). This situation has become a global health concern and requires combined efforts of microbiologists, epidemiologists, surgeons and other health professionals to devise a strategy that would be able to counter the continuous evolution of resistance mechanisms upon which *P. aeruginosa* thrives. It is known to cause increased mortality and morbidity in critically ill and immunocompromised patients during long period of hospitalization (Zorgani *et al.*, 2002).

For present study, 20 samples were collected from burn patients of age 1- 50 yrs. The incidence of burn injuries was higher in males (70%) than females (30%). The samples were inoculated on selective media i.e Cetrimide agar and nutrient agar. The growth of *P. aeruginosa* on nutrient agar plate is indicated by the change of colour from yellow to light green due to pigmentation. The predominant colonies were picked up for further morphological and biochemical characterization. The growth of isolated *P. aeruginosa* on nutrient agar showing pigmentation is shown in Figure 1.

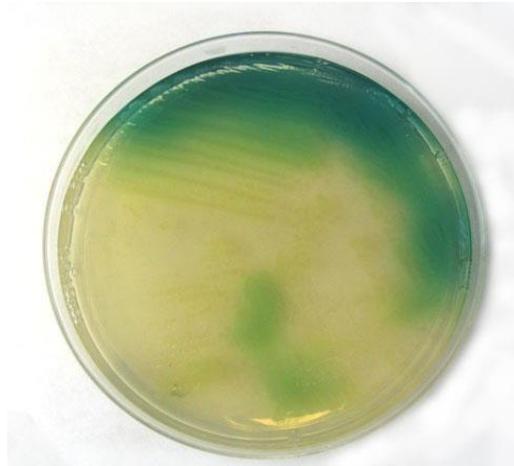


Fig. 1: Growth of *P. aeruginosa* on nutrient agar showing fluorescence

Morphological and biochemical characterization was performed for the of *P. aeruginosa* using various tests viz. Indole test, Methyl Red test, Citrate test, Nitrate reduction , Litmus milk test, Triple Sugar Iron agar test, and Gelatin liquefaction test. The prevalence of various isolates in burn patients are shown in Figure 2. Of 20 samples, 13 samples (65%) showed presence of *P. aeruginosa*. Other isolates obtained were *Proteus sp.* (15%), *Staphylococcus sp.* (15%) and *Klebsiella sp.* (5%). This agrees with the study conducted in a total of 100 patients by Rajput and coworkers (2008), the most prevalent bacteria was *P. aeruginosa*, followed by *S. aureus* , *Klebsiella*, *Acinetobacter*, *Proteus* and *E. coli*. The maximum number of burn patients was found to be between 20 and 30 years age (Ansari and Askarian, 2003). The results of present study indicated higher incidence in males (70%) than females (30%) which is contradictory with results of Khan *et al.* (2008) where the incidence was higher in females (63%) than males (37%).

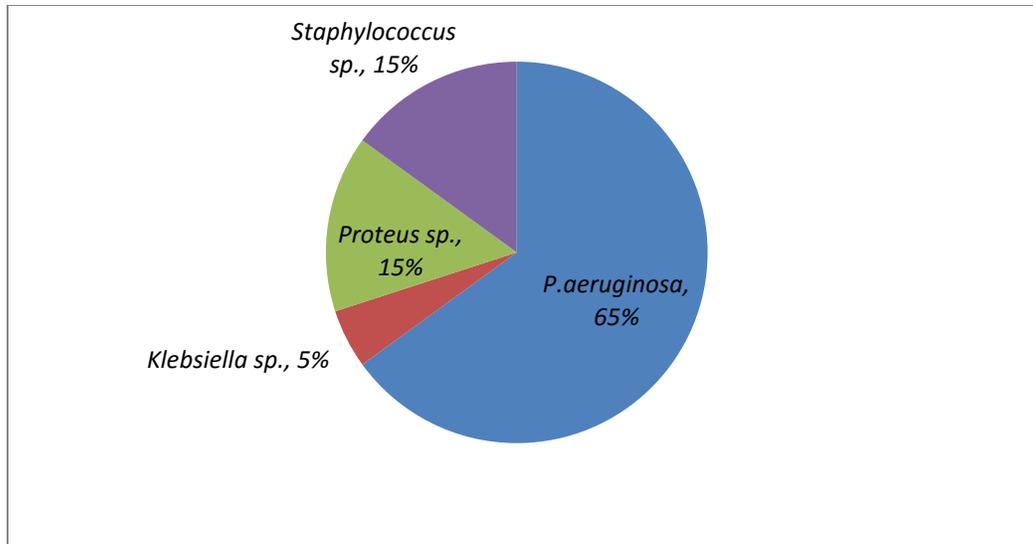


Fig. 2: Prevalence of various microorganisms in burn patients

Similar results were obtained from the study in burn patients where 32% *P. aeruginosa* was found in wound infections (Anuprabha *et al.*, 2006). However, some other researchers reported increased percentage of *S. aureus* (35%) followed by *P. aeruginosa* (32.5%), *Proteus sp.* (18.5%), *Klebsiella sp.* (6.5%) and *Streptococcus sp.* (0.5%) (Khan *et al.*, 2008). The ecology of organisms cultured showed that commonly isolated organisms are the same in different centers with one predominating the other in prevalence. In the present study, Gram +ve cocci were found in 15% and Gram –ve bacilli in 85% of the cases (Figure 3). Similarly, Khan *et al* (2008) found Gram positive cocci in 36.40% and Gram negative bacilli in 63.60% cases and Nasser *et al* (2003) found 40.3% gram positive cocci and 55.7% cases of gram negative bacilli.

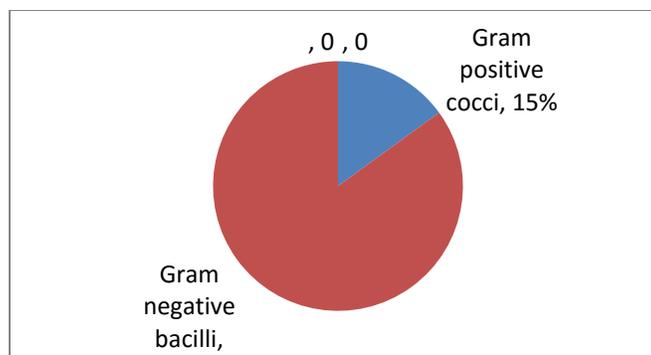


Fig. 3: Gram positive cocci and Gram negative bacilli in burn patients

Assessment for Antibiotic susceptibility

Antibiotic susceptibility of *P. aeruginosa* against different antibiotics was determined by the method described by Bauer *et al.*, (1966). Different antibiotics used were Ampicillin, Bacitracin, Ciprofloxacin and Gentamicin (Table 2).

Table 2. Antibiotic susceptibility of different isolates

Sample	Ampicillin	Bacitracin	Ciprofloxacin	Gentamicin
PA I	0 (R)	8 (R)	22.5 (S)	30 (S)
PA II	0 (R)	10.5 (I)	22.5 (S)	27.5 (S)
PA IV	32.5 (S)	0 (R)	22.5 (S)	30 (S)
PA VII	10 (R)	10 (R)	25 (S)	35 (S)
PA VIII	26 (S)	0 (R)	28 (S)	37.5 (S)
PA IX	20 (S)	10.5 (I)	22.5 (S)	0 (R)
PA X	30 (S)	10 (R)	26 (S)	32 (S)
PA XIII	0 (R)	10.5 (I)	24 (S)	31 (S)
PA XIV	10 (R)	0 (R)	22 (S)	32 (S)
PA XV	20 (S)	10 (R)	20 (S)	36 (S)
PA XVI	0 (R)	10.5 (R)	22.5 (S)	27.5 (S)
PA XVIII	10 (R)	10 (R)	25 (S)	30 (S)
PA XX	26 (S)	0 (R)	26 (S)	37.5 (S)

mm- millimeter, **R**- resistant, **S**- sensitive

In the present study, the frequency of susceptibility or sensitivity (Table 3) was highest to Ciprofloxacin (100%) followed by Gentamicin (92%), Ampicillin (46.15%). While the frequency of resistance was highest to Bacitracin (70%) followed by Ampicillin (53.84%), Gentamicin (8%). These results are in accordance with the study conducted by Karlowsky *et al.* (2003) showing 70-80% of isolates being

susceptible to gentamicin and ciprofloxacin. Similarly, a study by Nwankwo and Shuaibu (2010) showed a similar pattern of susceptibility of *P. aeruginosa*. *P. aeruginosa* showed a very high percentage of susceptibility to Ciprofloxacin (82.5%). Commonly used antibiotics like Ampicillin and tetracycline showed high level of resistance.

However, some other studies showed contrasting results to the present study. *P. aeruginosa* strains showed better sensitivity to Penicillin and cephalosporin (Khan *et al.*, 2008). Similar contradictory results were given by Shahid *et al* (2003) who concluded that about 63.3% of isolates were resistant to seven different drugs including tetracycline, amikacin and chloramphenicol.

Table 3. Antimicrobial sensitivity and resistance of *P. aeruginosa*

Antibiotic	Resistance (%)	Sensitive (%)
Ampicillin	53.84%	46.15%
Bacitracin	70%	0%
Ciprofloxacin	0%	100%
Gentamicin	8%	92.30%

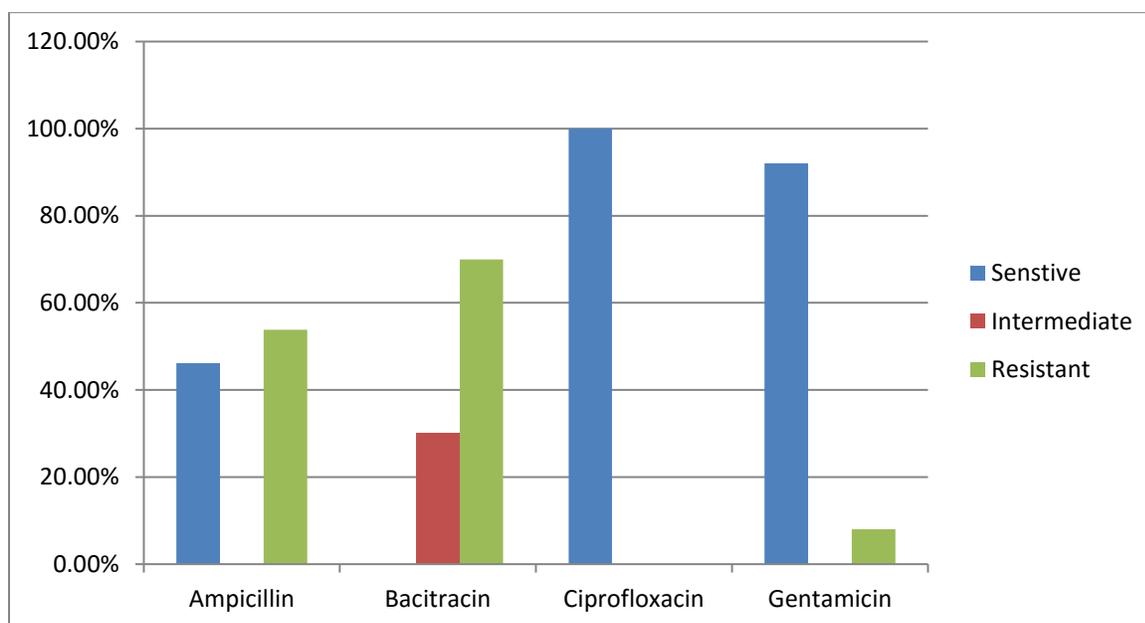


Fig. 4: Antimicrobial sensitivity and resistance of *P. aeruginosa*

Amutha and coworkers (2006) after subjecting isolated *P. aeruginosa* strains to antibiotic sensitivity test found that the strains exhibited highest resistance to ampicillin, followed by amikacin, norfloxacin and ciprofloxacin. Antibiotic susceptibility of the isolates was determined by Anuprabha *et al* (2006) who showed that cefoperazone is the most effective followed by ciprofloxacin and ceftazidime. The susceptibility pattern of *P.aeruginosa* isolates to a variety of antimicrobial agents like Ciprofloxacin, Ceftazidime, Ofloxacin, Gentamicin, Augmentin, Ampicillin, Amoxycilin, Chloramphenicol and Tetracycline by Nwankwo and Shuaibu, 2010 were observed as follows: 93.5%, 13.8%, 75.4%, 75.6%, 13.8%, 82.5%, 78.9%, 0%, 0%, 2.3% and 0%. The bacterium showed highest susceptibility to ceftazidime followed by ciprofloxacin.

In the present study, ampicillin showed zone of inhibition (ZOI) with a diameter ranging from 0-32.5 mm with a median of 10 mm as shown in Table 4. Also, from the table 3, it can be analyzed that for ampicillin 53.84% isolates were resistant and 46.15% isolates were susceptible (Figure 4).

In the present study, it was observed that Bacitracin showed ZOI with a diameter ranging from 0-10.5 mm with a median of 10 mm. Also, the table 4 shows that 70% of the isolates were resistant and 30% isolates were having intermediate ZOI. In this case, no isolate was found to be susceptible as sensitivity percentage was 0%. From this observation, it can be stated that Bacitracin is not an effective drug for the treatment of *P. aeruginosa* infections. Bacitracin is a mixture of cyclic polypeptides. It works by inhibiting the dephosphorylation of the C55-isoprenyl pyrophosphate (Murphy *et al.*, 2007).

Table 4. Range and Median of different antibiotics

ANTIBIOTICS	RANGE	MEDIAN
Ampicillin	0-32.5 mm	10 mm
Bacitracin	0-10.5 mm	10 mm
Ciprofloxacin	20-28 mm	22.5 mm
Gentamicin	0-37.5 mm	31 mm

Similarly, table 6 shows that ciprofloxacin showed the ZOI with a diameter ranging from 20-28 mm with a median of 22.5 mm. In this case, all the isolates were found to be susceptible as sensitivity was found to be 100%. So, from all the observations, it can be concluded that ciprofloxacin (a second generation antibiotic) is the most trenchant and competent antibiotic to be used for the remediation of *P.*

aeruginosa infections in burn patients. Ciprofloxacin is a broad spectrum second generation, synthetic chemotherapeutic antibiotic (Nelson *et al.*, 2007), which inhibit DNA gyrase, a type II topoisomerase and topoisomerase IV (Drlica *et al.*, 1997).

Gentamicin shows the ZOI in the diameter ranging from 0-37.5 mm with a median of 31 mm. Percentage resistance in this case came out to be 8%. This low resistance percentage indicates a high percentage of gentamicin sensitive isolates (92%). Gentamicin is an aminoglycoside antibiotic which acts by interfering with the proofreading process thereby causing increased rate of error in synthesis with premature termination. They can disrupt the integrity of bacterial cell membrane (Shakil *et al.*, 2007).

In the present study, it was found that 62% (8) isolates were sensitive to 2 antibiotics out of 4 antibiotics used. While 38% (5) isolates were sensitive to 3 antibiotics out of 4 antibiotics used (Figure 5). These isolates were resistant to mainly two antibiotics named Ampicillin and Bacitracin. On the contrary, a study by Shahid and coworkers (2003) on the antibiotic resistance studies of *P. aeruginosa* revealed that 83.3% of bacterial isolates were resistant to more than seven antibiotics.

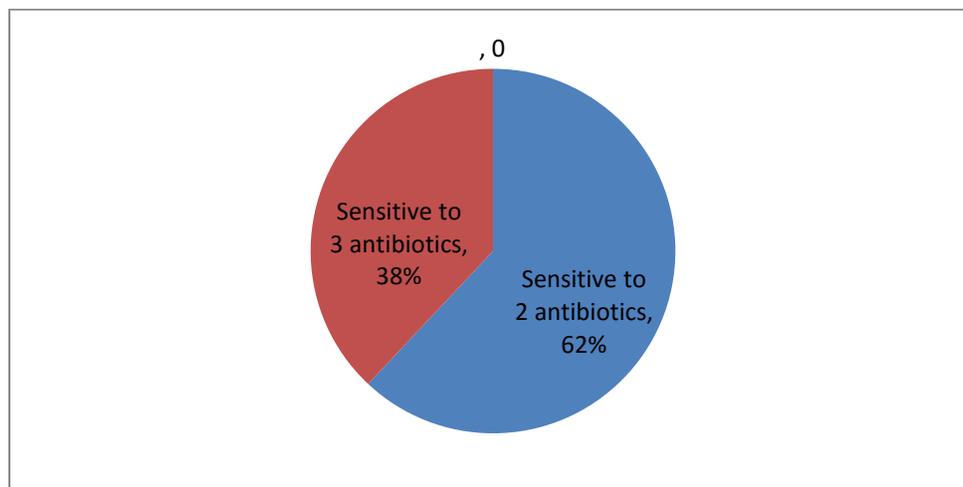


Fig. 5: Prevalence of Sensitivity of *P. aeruginosa* towards various antibiotics

Amutha *et al* (2009), also reported that *P. aeruginosa* was resistant to β -lactams antibiotics, amino glycosides and fluoroquinolones. Poirel *et al* (2002) reported similar results for *P. aeruginosa* showing that isolates were resistant to most of the tested antibiotics.

Presently, *P. aeruginosa* is common nosocomial pathogens responsible for burn infections. These infections are difficult to cure because of increasing antibiotic resistance. Studies suggested that the extensive use of antimicrobial agent is a major reason for the prevalence of antimicrobial resistant strains. The sub optimal concentration of antibiotic concentration present in wounds, inappropriate concentration

of β - lactam antibiotic and regular use of amino glycoside along with beta lactam, provide the good condition for the growth of multidrug resistant *P. aeruginosa* strains.

Presence and disease causing potential of *P. aeruginosa* varies from communities to communities and from patient to patient. Due to these variations, studies on the prevalence of *P. aeruginosa* and its antibiotic resistance pattern should have to be done timely. This will help the clinicians to decide whether which antibiotic should be administrated to the patient for effective treatment.

Conclusion

P. aeruginosa is the major etiological agent for nosocomial infections. These infections are becoming more problematic due to the increasing antibiotic resistance. Studies show that *P. aeruginosa* is naturally resistant towards many antibiotics and has the inherent ability to mutate in more resistant way during therapy. Nosocomial infection due to multidrug resistant *P. aeruginosa* has become a health care problem effecting people throughout the globe, since it prolongs the duration of treatment and also increases the cost of treatment. Studies should be planned with more number of antimicrobial agents at a large scale so as to better understand the resistance pattern of *P. aeruginosa*.

References

1. Altopark, U., Erols, S., Akcay, M. N., Celebi, F., Kandali, A. (2004) Time related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. *Burns*. 30: 660-664.
2. American Society of Health-System Pharmacist (2006) *Ampicillin*: beta-lactam antibiotics-penicillins, Cyclosporin and Lansoprazole. <http://en.wikipedia.org/wiki>.
3. Amutha, R., Padmakrishnan, Murugan, T., Renuga devi, M. P. (2009) Studies on multi drug resistant *Pseudomonas aeruginosa* from pediatric population with special reference to extended spectrum beta lactamase. *Indian Journal of Science and Technology*. 2: 11-13.
4. Anuprabha, S., Bhattacharjee, A., Garg, A., Sen, M. (2006) Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from wound infections. *Indian J Dermatol*. 51: 286-288.
5. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turk, M. (1966) Antibiotic susceptibility testing by a standardised single disc method. *Am. J. Clin. Pathol*. 45: 493-496.
6. Bergey, D. (Seventh ed.) (1957) *Manual of Determinative Bacteriology*. American Society for Microbiology. Williams and Willkins Co. Publishers, Baltimore, USA.

7. Cappucino, J. G., Sherman, N. (Seventh ed.) (1999) *Microbiology: A Laboratory Manual*. Pearson Education, Inc.
8. Church, D., Elsayed, S., Reid, O. (2006) Burn wound infections. *Clin Microbiol Rev.* 19: 403-434.
9. Drlica, K., Zhao, X., K. (1997) DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev.*61: 377-92.
10. Emori, T. G., Gaynes, R. P. (1993) An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin. Microbiol. Rev.* 6: 428-442.
11. Greta, G., Alvydas, P., Violeta, K. (2007) The peculiarities of *Pseudomonas aeruginosa* resistance to antibiotics and prevalence of serogroups. *Medicina(Kaunas)*. 43: 36-42.
12. Gupta, M., Gupta, O. K., Yaduvanshi, R. K., Upadhayaya, J. (1993) Burn epidemiology: The pink city scene. *Burns.* 19: 47-51.
13. Karlowsky, J. A., Draghi, D. C., Jones, M. E., Thornsherry, C., Friedland, I. R., Saham, D. F. (2003) Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States. *Antimicrob Agents Chemother.* 47: 1681-1688.
14. Khan, A. R., Fatima, N., Afridi, Z. U. D., Khan, B. A. (2008) Prevalence of Various Pathogens and their Sensitivity Pattern in Patients with Burns at a tertiary care hospital. *J. Med. Sci.* 16: 64-67.
15. Madigan, M., Martinko, J. (Eleventh ed.) (2005). *Brock Biology of Microorganisms*. Prentice Hall.
16. Murphy, T., Roy, I., Harrop, A., Dixon, K., Keshavarz, T. (2007) Effect of oligosaccharide elicitors on bacitracin a production and evidence of transcriptional level control. *Journal of Biotechnology.*131: 397-403.
17. Nasser, S., Mabrouk, A., Maher, A. (2003) Colonization of burn wounds in Ain Shams University burn unit. *Burns.* 29: 229-233.

18. Nelson, J. M., Chiller, T. M., Powers, J. H., Angulo, F. J. (2007) Fluoroquinolone-resistant *Campylobacter species* and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clin Infect Dis.*44: 977–80.
19. Nwankwo, E.O.K., 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.* p. 37-40.
20. Poirel, L., Weldhagen, G. F., Champs, C., Nordmann, P. (2002) A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended spectrum beta-lactamase GES-2 in South Africa. *J. Antimicrobial.Chemotherapy.* 49: 561-565.
21. Rajput, A., Singh, K. P., Kumar, V., Saxena, R., Singh, R. K. (2008) Antibacterial resistance pattern of aerobic bacteria from burn patients in tertiary hospital. *Biomedical Research.* Vol. 19.
22. Salimi, H., Owlia, P., Yakhchali, B., Lari, A. R. (2010) Characterization of *Pseudomonas aeruginosa* in Burn patients Using PCR-Restriction Fragment Length Polymorphism and Random Amplified Polymorphic DNA Analysis. *Iran J Med Sci.* 35: 236-241.
23. Shahid, M., Malik, A., Sheeba. (2003) Multidrug-resistant *Pseudomonas aeruginosa* strains harbouring R-plasmids and AmpC β -lactamases isolated from hospitalized burn patients in a tertiary care hospital of North India. *FEMS Microbiology Letters.* 228: 181-186.
24. Shakil, S., Khan, R., Zarrilli, R., Khan, A. U. (2007) Aminoglycosides versus bacteria – a description of the action, resistance mechanism, and nosocomial battleground. *Journal of Biomedical Science.*15: 5–14.
25. Ullah, F., Malik, S. A., Ahmad, J. (2009) Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. *Burns.* 35: 1020-1025.
26. Zorgani, A., Zaidi, M., Ranka, R., Shahan, A. (2002) The pattern and outcome of septicaemia in a burns intensive care unit. *Ann. Burns Diasters.* 15: 179-182.

