ANTIBIOGRAM PROFILE AND BIOFILM FORMING POTENTIAL OF PSEUDOMONAS SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMENS

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ABSTRACT

Objective: The present study aimed at finding the resistance pattern of Pseudomonas aeruginosa and other Pseudomonas species isolated from various clinical specimens in the laboratory.

Methods: A total of 150 isolates of different species of Pseudomonas obtained from various clinical specimens processed at the Microbiology laboratory of Kasturba Medical College, Manipal Academy of Higher Education, were taken for this study. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted according to the CLSI guidelines. Biofilm assay was performed by modified O'Toole and Kolter method. The results were analyzed using SPSS 17.0 and Student's unpaired t-test, Kruskal-Wallis, Mann-Whitney, ANOVA, and Chi-square test. p<0.05 was considered statistically significant.

Results: Increased resistance was observed by P. aeruginosa to cefotaxime, cotrimoxazole, levofloxacin, ofloxacin, and ticarcillin clavulanate. There was also a good correlation between antibiotic resistance to aztreonam, netilmicin, and ceftazidime and biofilm production. Results of the present study, therefore, demonstrated the occurrence of resistance to various antipseudomonal agents among the biofilm-producing P. aeruginosa isolates.

Conclusion: The present study may help in assessing the seriousness of drug resistance caused by biofilm formation in P. aeruginosa and devise strategies through antibiotic policies to minimize such problems.

Keywords: Biofilm, Pseudomonas aeruginosa, Antibiogram, Antipseudomonal agents.

INTRODUCTION

Pseudomonas is a large group of aerobic, non-sporing Gram-negative motile rods, which are pervasive in nature. Pseudomonas aeruginosa is a major opportunistic pathogen responsible for acute and chronic infections mainly in hospital settings, especially in patients with compromised host defense mechanism and also with serious underlying disease conditions [1]. According to the CDC, approximately 8% of all health care-associated infections reported to the National Healthcare Safety Network are caused by P. aeruginosa.

The survival of Pseudomonas within the host in the initial stages of infection is aided by the secretion of various toxins and virulence factors including pyocyanin, proteases, and elastases [2]. P. aeruginosa possesses various intrinsic and acquired mechanisms of drug resistance [3]. Risk factors associated with the emergence of drug-resistant strains include previous antipseudomonal drug treatment and prolonged use of antibiotics. Besides, prolonged hospital stay and increased susceptibility of patients to secondary bacteremia lead to the acquisition of resistant strains [4].

P. aeruginosa forms microcolonies enclosed in extracellular polymeric substances (EPSs) termed as biofilms [5]. Biofilm formation leads to persistent and chronic infection by resisting the action of antimicrobial agents [6]. It has the ability to resist the suppression of the organism by various physical and chemical treatments [5]. Biofilm forms the major response mechanism to external stress factors by inducing many additional phenotypic alterations such as loss of motility, reduced growth rate, and altered susceptibility to host response [6,7].

The present study aimed at correlating biofilm production by P. aeruginosa and other species of Pseudomonas isolated from various clinical samples with their antibiogram pattern.

METHODS

Collection and Identification of bacterial isolates
A total of 150 isolates of Pseudomonas (P. aeruginosa -75 and other Pseudomonas species-75) obtained from various clinical specimens processed at the Microbiology laboratory of Kasturba Medical College, Manipal Academy of Higher Education, were taken for this study (with a 95% confidence level and 80% power, the sample size came up to 75 each). The Institutional Ethics Committee clearance was obtained for the study. The isolates were identified by standard biochemical methods [8] or by VITEK 2 system. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted according to the CLSI guidelines [9]. For isolates identified by VITEK 2 system, sensitivity was recorded from it.

Biofilm assay
This was done by modified O'Toole and Kolter method [10-13]. The bacterial colony was inoculated in brain heart infusion (BHI) broth and incubated at 37°C for 18 h. It was then diluted with fresh BHI broth, and the turbidity was adjusted to 0.5 McFarland standard. 200 µl of the suspension was dispensed into microtiter plate wells in duplicate. The plate was incubated at 37°C for 24 h. The contents were aspirated and washed with phosphate-buffered saline (pH 7.4) following which 100 µl of the suspension was dispensed into microtiter plate wells in duplicate. The plate was incubated at 25°C for 10 min. The contents were discarded, and the wells were stained with 1% crystal violet. After 1 min, the excess stain was rinsed off by placing the plate under running tap water. Then, 33% glacial acetic acid was added to each well and optical densities of stained adherent bacterial films were read with Micro ELISA plate reader at 570 nm. Mean reading from two wells were calculated. P. aeruginosa ATCC 27853 was included as control.
Statistical analysis: All experiments were performed in duplicate. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and using Student’s unpaired t-test, Kruskal-Wallis, Mann-Whitney, ANOVA, and Chi-square test. p<0.05 was considered statistically significant.

RESULTS

Of 75 isolates of *P. aeruginosa*, 64 (35.2%) were isolated from males, while 11 (14.7%) were isolated from females. With regard to the other *Pseudomonas* spp., of 75 isolates, 54 (72.0%) were from males and 21 (28.0%) were from females. As shown in Fig. 1, the highest rate of isolation was from swabs and exudate material from wound infections (21.3%). Similarly, isolation rate of other species of *Pseudomonas* also accounted to 24.0% as shown in Fig. 2.

Antibiotic resistance of the isolates

Among the *P. aeruginosa* strains, 80% were resistant to cefotaxime, 75% to cotrimoxazole, and 68.2% to ticarcillin/clavulanic acid. All the isolates were sensitive to colistin and polymyxin-B (Fig. 3). Resistance to levofloxacin was 62.5%. Similarly, the other species of *Pseudomonas* also showed maximum resistance to cefotaxime (75%) and cotrimoxazole (75%), while levofloxacin was third in the list (66.7%) as shown in Fig. 4.

Resistance to aminoglycosides

Among the aminoglycosides, the percentage resistance of *P. aeruginosa* revealed 38.4% resistance to amikacin, 41.3% resistance to gentamicin, and also a percentage as high as 46.2% for netilmicin. However, the percentage of resistance to amikacin was lower in other species of *Pseudomonas*, being 20.8% for amikacin, while it was 36.2% for gentamicin and 12.5% for netilmicin.

Carbapenem resistance

*P. aeruginosa* strains exhibited 24.7% resistance to imipenem while it was 19.2% for meropenem which was almost on par with other species of *Pseudomonas*, and the resistance to imipenem and meropenem being 27% and 21.1%, respectively.

Resistance to antipseudomonal penicillins

Most of the strains were sensitive to piperacillin and piperacillin-tazobactam. Resistance to piperacillin and piperacillin-tazobactam by *P. aeruginosa* was 16.7% and 12.1%, respectively, while for other species, the percentage of resistance to piperacillin and piperacillin-tazobactam was 19.6% and 17.8%, respectively. However, 68.2% resistance was shown by *P. aeruginosa* against ticarcillin-clavulanic acid, a carboxypenicillin.

Resistance to quinolones

Both *P. aeruginosa* and the other species showed high level of resistance to the quinolones with higher resistance to levofloxacin (62.5% and 66.7%, respectively), while for ciprofloxacin, the resistance exhibited by *P. aeruginosa* and the other species was 45.8% and 34.2%, respectively.

Resistance to minocycline

There was minimal resistance to minocycline, a tetracycline group of drug by *P. aeruginosa* strains, while other species showed 100% susceptibility to this drug.

Resistance to cephalosporins

The isolates also exhibited resistance to ceftazidime, a third-generation antipseudomonal drug (28.4% by *P. aeruginosa* and 23.2% by the other...
species). There was a very high percentage of resistance to cefotaxime by \textit{P. aeruginosa} and the other species of \textit{Pseudomonas} (80% and 75%, respectively). The percentage resistance of \textit{P. aeruginosa} and the other species of \textit{Pseudomonas} to cefoperazone-sulbactam was 30.4% and 23.4%, respectively, while the resistance to cefoperazone was 16.2% and 20.6%, respectively.

**Resistance to monobactams**
There was 20% resistance to aztreonam, a monobactam antibiotic by \textit{P. aeruginosa}, while 24% resistance was shown by other species of \textit{Pseudomonas}.

**Results of biofilm formation by the isolates**
\(OD_{152}\) of biofilm produced by the isolates is depicted in Tables 1 and 2, and the comparison between the biofilm produced by \textit{P. aeruginosa} and the other species of \textit{Pseudomonas} is shown in Fig. 5 and Table 3.

**Aminoglycosides**
Among the three aminoglycosides, gentamicin- and netilmicin-resistant strains of \textit{P. aeruginosa} showed more biofilm production while amikacin sensitive strains produced more biofilm. In case of other \textit{Pseudomonas} spp., more biofilm production was shown by aminoglycoside-sensitive strains.

**Carbapenems**
\textit{P. aeruginosa} which was resistant to imipenem produced more amount of biofilm, whereas in other species production, it was more in case of sensitive isolates. With regard to meropenem, immediately susceptible strains produced more biofilm in case of both \textit{P. aeruginosa} and \textit{Pseudomonas} spp.

**Antipseudomonal penicillins**
Biofilm production was more by piperacillin susceptible strains of both \textit{P. aeruginosa} and \textit{Pseudomonas} spp. The strains which were immediately susceptible to piperacillin-tazobactam isolates showed more biofilm in case of \textit{P. aeruginosa}. In other species, piperacillin-tazobactam resistant strains produced more biofilm.

**Quinolones**
\textit{P. aeruginosa} which was resistant to ciprofloxacin produced more amount of biofilm, whereas in \textit{Pseudomonas} spp. strains that were intermediately susceptible to ciprofloxacin produced more biofilm. \textit{P. aeruginosa} sensitive to levofloxacin showed more biofilm production, while in case of other species, levofloxacin-resistant ones produced more biofilm.

**Cephalosporins**
Isolates of both \textit{P. aeruginosa} and \textit{Pseudomonas} spp. were resistant to ceftazidime sulbactam produced more biofilm. In case of ceftazidime, more biofilm production was shown by \textit{P. aeruginosa} intermediately susceptible isolates and ceftazidime-resistant isolates of other \textit{Pseudomonas} spp. isolates of both \textit{P. aeruginosa} and \textit{Pseudomonas} spp. which were resistant to cefotaxime and cefoperazone produced more biofilm.

**Monobactams**
\textit{P. aeruginosa} which was resistant to aztreonam produced more amount of biofilm, whereas in \textit{Pseudomonas} spp., more biofilm production was in sensitive strains.

Minimum inhibitory concentration (MIC) of certain antibiotics (ceftazidime, ciprofloxacin, and piperacillin-tazobactam) against biofilms also demonstrated that when MIC values increase, there was a significant increase in biofilm.

**DISCUSSION**
Antibiotic resistance is a major problem in \textit{P. aeruginosa}. The organism exhibits intrinsic resistance to several beta-lactam antibiotics and may also acquire additional resistance mechanisms either due to mutational events or due to the acquisition of transferable genetic elements [14].

\textit{P. aeruginosa} also shows intrinsic resistance by the expression of chromosomally encoded inducible AmpC beta-lactamase and also by several important efflux pump systems that export antibiotics, biocides, dyes, detergents, metabolic inhibitors, organic solvents, and molecules involved in bacterial cell-to-cell communication [15].

Carbapenem resistance mechanisms have emerged under the pressure of carbapenem use in clinical settings and may be classified as enzymatic, which include carbapenemases, aminoglycoside-modifying enzymes, and 16S RNA methylases or nonenzymatic involving decreased transcription of OprD gene and overproduction of MEXAB-OprM efflux system [16]. Carbapenem resistance, however,

**Table 1: OD\(_{152}\) of biofilm produced by \textit{P. aeruginosa} isolated from various clinical specimens**

| Clinical specimen          | Mean number of isolates±SD | OD\(_{152}\)±SD |
|---------------------------|-----------------------------|-----------------|
| Sputum                    | 5±1.023                     | 0.847           |
| Bronchoalveolar lavage    | 3±0.135                     | 0.971           |
| Et and suction tip        | 11±0.473                    | 0.825           |
| Urine                     | 13±0.484                    | 0.965           |
| Blood                     | 9±0.512                     | 1.069           |
| Catheter tip              | 2±0.520                     | 1.062           |
| Wound swab                | 16±0.439                    | 0.839           |
| Ear swab                  | 5±0.554                     | 1.226           |
| Pus                       | 4±0.446                     | 0.807           |
| Bone tissue               | 3±0.690                     | 1.434           |
| Deep tissue               | 4±0.323                     | 1.067           |
| Placental membrane        | 0                           | 0               |

\textit{P. aeruginosa}: \textit{Pseudomonas aeruginosa}

**Table 2: OD\(_{152}\) of biofilm produced by \textit{Pseudomonas} spp. isolated from various clinical specimens**

| Clinical specimen          | Mean number of isolates±SD | OD\(_{152}\)±SD |
|---------------------------|-----------------------------|-----------------|
| Sputum                    | 11±0.524                    | 0.989           |
| Bal                       | 3±0.593                     | 0.631           |
| Et and suction tip        | 6±0.517                     | 0.925           |
| Urine                     | 15±0.387                    | 0.988           |
| Blood                     | 2±0.517                     | 1.077           |
| Catheter tip              | 3±0.581                     | 1.202           |
| Wound swab                | 18±0.480                    | 0.866           |
| Ear swab                  | 2±0.941                     | 1.339           |
| Pus                       | 11±0.499                    | 0.854           |
| Bone tissue               | 1±                          | 0.531           |
| Deep tissue               | 1±                          | 0.756           |
| Placental membrane        | 2±0.684                     | 2.187           |

\textit{P. aeruginosa}: \textit{Pseudomonas aeruginosa}

**Fig. 5: Comparison of biofilm production in \textit{Pseudomonas aeruginosa} and \textit{Pseudomonas} spp.**
Significant induction was shown to occur with pharmacokinetically relevant concentrations of clavulenate. Besides, the induction of AmpC by clavulenate was shown to significantly antagonize or substantially diminish the antibacterial activity of ticarcillin. The study also suggested that in the selection of an antipseudomonal β-lactam for the treatment of P. aeruginosa infections, the combination of ticarcillin-clavulanate should be avoided, especially with immunocompromised patients, for whom bacterial killing is required to ensure clinical success.

It was interesting to note the correlation between antibiotic resistance and biofilm production. Among the aminoglycosides, netilmicin resistance and biofilm production demonstrated good correlation (p=0.044). Aminoglycosides which constitute a vital component of antipseudomonal therapy have been showing resistance in the recent past. Hence, it is important to remember that prolonged therapy may lead to persisters by forming biofilms [29].

Table 3: Correlation between antibiotic sensitivity pattern and biofilm production

| Antimicrobial agents | Organism                | Mean OD (isolates±SD) | Mean OD (isolates±SD) | Mean OD (isolates±SD) | p value |
|----------------------|-------------------------|-----------------------|-----------------------|-----------------------|---------|
|                      | P. aeruginosa           |                       |                       |                       |         |
| Amikacin             | P. aeruginosa           | 0.97±0.471            | 0.96±0.58             | 0.94±0.41             | 0.091   |
| Ceftazidime          | Pseudomonas spp.        | 1.03±0.53             | 0.75±0.34             | 0.80±0.089            | 0.166   |
| Cefoperazone          | P. aeruginosa           | 0.97±0.057            | 0.92±0.551            | 1.49±0.492            | 0.015   |
| Ciprofloxacin        | Pseudomonas spp.        | 0.94±0.466            | 1.13±0.614            | 0.70±0.410            | 0.242   |
| Gentamicin           | P. aeruginosa           | 0.93±0.492            | 1.05±0.592            | 0.87±0.603            | 0.901   |
| Imipenem             | P. aeruginosa           | 0.90±0.445            | 1.05±0.586            | 0.92±0.553            | 0.209   |
| Meropenem            | P. aeruginosa           | 0.96±0.489            | 0.93±0.539            | 2.06±1                | 0.093   |
| Piperacillin         | P. aeruginosa           | 0.88±0.454            | 1.05±0.598            | 0.97±0.505            | 0.367   |
| Piperacillin-tazobactam | P. aeruginosa     | 1.04±0.550            | 0.78±0.331            | 1.39±0.968            | 0.183   |
| Netilmicin           | P. aeruginosa           | 0.89±0.454            | 0.97±0.432            | 0.92±0.432            | 0.647   |
| Cotrimoxazole        | P. aeruginosa           | 0.94±0.494            | 1.05±0.764            | 0.95±1                | 0.337   |
| Aztreonam            | P. aeruginosa           | 0.96±0.524            | 0.78±0.398            | 1.46±1                | 0.395   |
| Cefotaxime           | P. aeruginosa           | 0.89±0.190            | 1.29±0.529            | 0.33±0.331            | 0.641   |
| Cefoperazone         | P. aeruginosa           | 0.89±0.482            | 1.04±0.677            | 1.05±0.948            | 0.328   |
| Levofloxacin         | P. aeruginosa           | 0.776±0.337           | 1.63±0.783            | 0.97±0.094            | 0.444   |
| Cotrimoxazole        | P. aeruginosa           | 0.70±0.050            | 1.17±0.951            | 0.13±0.161            | -       |
| Aztreonam            | P. aeruginosa           | 0.10±0.532            | 0.87±0.043            | 0.94±1                | -       |
| Ceftazidime          | P. aeruginosa           | 0.738±1               | 0.74±0.674            | 0.93±0.441            | -       |
| Cefoperazone         | P. aeruginosa           | 0.89±0.443            | 1.09±0.681            | 0.39±0.081            | 0.211   |
| Levofloxacin         | P. aeruginosa           | 0.90±0.460            | 1.05±0.681            | 0.89±0.612            | 0.709   |
| Aztreonam            | P. aeruginosa           | 1.47±0.538            | 1.25±0.668            | 0.77±0.804            | 0.451   |
| Levofloxacin         | P. aeruginosa           | 0.53±1                | 0.84±0.213            | 0.90±0.331            | 0.996   |

P. aeruginosa: Pseudomonas aeruginosa

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against different test organisms, and in clinical isolates, similar antibiotics exhibited varied effects in their effective concentration[34,35]. Our study revealed that isolates of both P. aeruginosa and Pseudomonas spp. which were resistant to cefotaxime and ceftazidime produced more biofilm. A particular study has shown the ability of cefoxime to dislodge the biofilm formation in Pseudomonas by 65.57%.

Increase in biofilm with an increase in MIC of antibiotics seen in the present study is in par with other studies reported [10,13,14].

CONCLUSION

Results of the present study demonstrated the occurrence of resistance to various antimicrobial agents among the P. aeruginosa isolates as well as in other species of Pseudomonas. While certain drugs such as pipercillin-tazobactam, carbapenems, and amikacin remain the mainstay of the treatment of pseudomonal infections, it is alarming to note an increase in resistance levels to these antibiotics. Besides this, the organisms also produce biofilms which serve as barriers to effective therapy. Regular antimicrobial susceptibility monitoring would help and guide the physicians in prescriing the right combinations of antimicrobial to limit and prevent the emergence of multidrug-resistant strains of P. aeruginosa. Antibiotics should be used judiciously and at the optimum concentration so as to inhibit biofilm formation and eradicate persistant cells. Studies have postulated that combination of a biofilm inhibitor with a conventional antibiotic to control biofilms thereby permits the drug to reach the cells trapped inside the biofilm. As this is a hospital-based epidemiological data, the present study will help in implementation of better patient management and infection control strategies.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Anvarinejad M, Japoni A, Rafastpour N, Mandaneh J, Abbassi P, Shahidi MA, et al. Burn patients wounds infected with metallo-beta-lactamase-producing Pseudomonas aeruginosa: Multidrug resistant strains. Arch Trauma Res 2014;3:e18182.

2. Van’t Wout E, van Schadewijk A, van Boxtel R, Dalton L, Clarke H, Jamsheera and Suman. E. coli causing urinary tract infection. Ind J Med Microbiol 2007;25:305-6.

3. Suman E, Singh S, Kotian MS. Pseudomonas aeruginosa biofilms in hospital water systems and the effect of sub-inhibitory concentration of chlorhexidine. J Hosp Infect 2008;70:199-201.

4. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia 2012;16:303-7.

5. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa. Expert Rev Antimicrob 2002;3:634-40.

6. Poole K. Pseudomonas aeruginosa: Resistance to the max. Front Microbiol 2011;2:65.

7. Hammeni S, Ghezzi R, Burghoffe B, Arlet G, Redjeb S. Mechanisms of carbapenem resistance in non-metallo-beta-lactamase-producing clinical isolates of Pseudomonas aeruginosa from a Tunisian hospital. Pathol Biol (Paris) 2009;57:530-5.

8. El Amin N, Giske CG, Jalal S, Keijser B, Kronvall G, Wretlind B. Carbapenem resistance mechanisms in Pseudomonas aeruginosa: Alterations of porin OprD and efflux proteins do not fully explain resistance patterns observed in clinical isolates. APMIS 2005;113:187-96.

9. Strateva T, Yordanov D. Pseudomonas aeruginosa—A phenomenon of bacterial resistance. J Med Microbiol 2009;58:1107-24.

10. O'Toole GA, Kolter R. Initiation of biofilm formation in Escherichia coli causing urinary tract infection. Ind J Med Microbiol 2007:25:305-6.

11. Van ‘t Wout E, van Schadewijk A, van Boxtel R, Dalton L, Clarke H, Jamsheera and Suman.

12. Suman E, Singh S, Kotian MS. Pseudomonas aeruginosa biofilms in hospital water systems and the effect of sub-inhibitory concentration of chlorhexidine. J Hosp Infect 2008;70:199-201.

13. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia 2012;16:303-7.

14. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa. Expert Rev Antimicrob 2002;3:634-40.

15. Poole K. Pseudomonas aeruginosa: Resistance to the max. Front Microbiol 2011;2:65.

16. Hammeni S, Ghezzi R, Burghoffe B, Arlet G, Redjeb S. Mechanisms of carbapenem resistance in non-metallo-beta-lactamase-producing clinical isolates of Pseudomonas aeruginosa from a Tunisian hospital. Pathol Biol (Paris) 2009;57:530-5.

17. El Amin N, Giske CG, Jalal S, Keijser B, Kronvall G, Wretlind B. Carbapenem resistance mechanisms in Pseudomonas aeruginosa: Alterations of porin OprD and efflux proteins do not fully explain resistance patterns observed in clinical isolates. APMIS 2005;113:187-96.

18. Strateva T, Yordanov D. Pseudomonas aeruginosa—A phenomenon of bacterial resistance. J Med Microbiol 2009;58:1107-24.

19. Walton MA, Villarreal C, Herndon DN, Heggens JP. The use of aztreonam as an alternate therapy for multi-resistant Pseudomonas aeruginosa. Burns 1997;23:225-7.

20. Khatri B, Basnyat A, Poudel A, Shrestha B. Etiology and antimicrobial susceptibility pattern of bacterial pathogens from urinary tract infection. Nepal Med Coll J 2012;14:129-32.

21. Khatri B, Basnyat A, Poudel A, Shrestha B. Etiology and antimicrobial susceptibility pattern of bacterial pathogens from urinary tract infection. Nepal Med Coll J 2012;14:129-32.

22. Zakaria EA. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza Strip, Palestine. J Biomed Biotechnol 2005;3:238-41.

23. Naeem M, Khan MA, Qin SM. Antibiotic susceptibility pattern of bacterial pathogens causing urinary tract infection in a tertiary care hospital. Ann Pak Inst Med Sci 2010;6:214-8.

24. Hasan AS, Nair D, Kaur J, Bawjwa G, Deb M, Aggarwal P. Resistance patterns of urinary isolate in a tertiary Indian hospital. J Ayub Med Coll Abbottabad 2007;19:39-41.

25. Chikwendu CI, Amadi ES, Obi RK. Prevalence and antimicrobial resistance in Pseudomonas aeruginosa and Klebsiella pneumoniae isolates from non-clinical urine samples. New York Sci J 2010;3:194-200.

26. Drago L, De Vecchi E, Mombelli B, Nicola L, Valli M, Gismondo MR. Activity of levofloxacin and ciprofloxacin against urinary pathogens. J Antimicrob Chemother 2001;48:37-45.

27. Prakash D, Saxena RS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut City, India. ISRN Microbiol 2013;2013:749629.

28. Lister P D, Gardner V M, Sanders CC. Clavulinate induces expression of the Pseudomonas aeruginosa AmpC cephalosporinase at physiologically relevant concentrations and antagonizes the antibacterial activity of ticarcillin. Antimicrob Agents Chemother 1999;4:882-9.

29. Poole K. Aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2005;49:479-87.

30. Bagge N, Schister M, Cabot G, Moya B, Rojo-Molinero E, Macia M, Rubio R, Mombaerts N, Schister M, Hentzer M, Ciofu O, Givskov M, Greenberg EP, et al. Pseudomonas aeruginosa biofilms exposed to imipenem exhibit changes in global gene expression and β-Lactamase and alginate production. Antimicrobial Agents Chemother 2016;60:2912-22.

31. Rojo-Molinero E, Macia M, Rubio R, Moya B, Cabot G, Lopez-Casaspe C. Sequential treatment for biofilms with aztreonam and tobramycin is a novel strategy for combating Pseudomonas aeruginosa chronic respiratory infections. Antimicrobial Agents Chemother 2016;60:2912-22.

32. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa. Clin Infect Dis 1990;27:Suppl 1:S93-9.

33. Yu Q, Griffin EF, Moreau-Maugnis S, Shwartzman JD, Stanton BA, O’Toole GA. In vitro evaluation of tobramycin and aztreonam versus Pseudomonas aeruginosa biofilms oncystic fibrosis derived human airway epithelial cells. J Antimicrob Chemother 2012;67:2673-81.

34. Mary RN, Banu N. Screening of anti-biofilm and anti quorum sensing potential of Vitis trifolia in Pseudomonas aeruginosa. Int J Pharm Sci Res 2015;7:242-5.

35. Rathinam P, Viswanathan P. Effects of antibiotics upon quorum sensing regulated characters: A propitious scheme against device associated infections. Int J Pharm Pharm Sci 2014;6:95-90.