Original Research Article

Prevalence and antibiotic susceptibility patterns of pseudomonas aeruginosa in urinary tract infections in a Tertiary care hospital, Central Kerala: A retrospective study over 4 years

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Abstract

Background and Objective: Pseudomons aeruginosa (P.aeruginosa) is an important uropathogenthat has shown varied antibiotic susceptibility patterns. This study aims to findout the changing trends in theprevalenceand antibiotic susceptibility patterns ofurinary isolates of P.aeruginosa over four consecutive years. **Methodology:** A retrospective, record based study was conducted on all culture and sensitivity (C/S) reports ofurine samples obtained in the microbiology lab in a tertiary care centre, Central Kerala (January 2014 -December 2017). The C/S reports which were positive for significant growth of P.aeruginosawere analyzed to findout its prevalence andantibiotic susceptibility patterns. Descriptive statistics was used for data analysisand the results wereexpressed in percentages. **Result:** Out of total 6622 urine samples received (14%) showed significant bacteriuria. P.aeruginosa was the third most common uropathogen isolated with an isolation rate of 3.5%. The antibiotic resistance observedwere Gentamicin (53.1%), Amikacin (28%), Cefipime (28%), Ceftazidime (34.4%), Ciprofloxacin (43.7%), Norfloxacin (40.6%), Ofloxacin (40.6%), Piperacillin (37.5%), Piperacilli-Tazobactam (25%) and Imipenem (28%). The isolation rates of P.aeruginosa were 3.9%, 2.6%, 4.5 % and 2.9% in 2014, 2015, 2016 and 2017 respectively and overthe years it maintained its third position.The year wise analysis of antibiotic resistance showed fluctuating pattern except Amikacin,Cefipime and Fluoroquinoles which displayed a decreasingtrend. The reserve drugs like Piperacillin –tazobactam and Imipenem showed alarming drug resistance, although a hopeful reduction in the resistance was noted in 2017. **Conclusion:** P.aeruginosa remains as a common uropathogen. Drug resistant strains are markedly high in our area. Antibiotic resistance of P.aeruginosado not show a consistent trend over years and vary from region to region. Soeach institution should have an antibiotic policy based on the local antibiogram which is to be renewed regularly.Instead of opting for higher antibioticseach time, strict implementation of restrictive and rotational antibiotic policies and adherence to the concept of 'Reserve drugs" should be followed. This is the only modality to inhibit the emergence of resistance strains of all uropathogens especially opportunistic pathogens like P.aeruginosa.

Key words: Antibiotic resistance patterns,Pseudomonas aeruginosa, Urinary tract infections.

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Introduction

Urinary tract infections (UTI) are the most common infections encountered by clinicians and one of the leading causes of morbidity in human population [1,2]. Although Escherichia coli. is predominantly associated with the etiology of UTI, other organisms such as Klebsiella pneumonia, Proteus mirabilis, Enterobacter, Citrobacter, Staphy lococcus aureus, Enterococci etc account for most of the rest [1,3]. Aerobic non-fermenting gram negative bacilli are now emerging as important uropathogens. Among these non-fermenters, P.aeruginosa is the predominant and most well-known organism [3,4].

P. aeruginosais a ubiquitous, gram-negative bacillus that can survive in myriad of environment such as aquatic and terrestrial [4]. It is a versatile opportunistic pathogen, associated with nosocomial infections along with other serious implications with high rate of morbidity and mortality[5]. According to the report of

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nosocomial infection surveillance system of center for disease control and prevention, P. aeruginosa is the third most common organism causing nosocomial urinary tract infections [5].

Treatment of UTI constitutes a great portion of prescription of antibiotics. Urinary pathogenshave shown a changed pattern of susceptibility to antibiotics, showingan increasedresistance to commonly used antibiotics due to extensive and inappropriate use of antimicrobial agents [6,7]. In P.aeruginosa, increasing resistance towards the available antimicrobials preclude the effectiveness of any antimicrobial regimen.

Because of increasing multidrug resistant (MDR) P. aeruginosa isolates in health care settings, infections are difficult to treat, causing life threatening conditions [5].

MDR mechanism in P. aeruginosa are due to lower outer membrane permeability, acquisition of enzymes like β-lactamases especially extended spectrum enzymes, carbapenemasesoraminogly coside modifying enzymes. Resistance is conferred by the transfer of plasmids which carry genes to produce antimicrobial enzymes [4,5].

Knowledge of the local bacterial etiology and susceptibility patterns are required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made. In this study we focused on P. aeruginosa because,thisbacteria have different therapeutic options when compared to other commonly encountered gram negative uropathogens.

Eventhougha number of studies have been done on the prevalence and antimicrobial resistance patterns of uropathogens, no data have been reported from the present study area on P.aeruginosa causing urinary tract infections.

The aim of the study is therefore to determine changing trends in the prevalence of P.aeruginosa from suspected UTI cases and their antibiotic susceptibilitypatterns to the most commonly used antipseudomonal antibiotics.

Materials and Methods

A retrospective, record basedstudy was conducted on allculture and sensitivity (C/S) reports ofurine samples obtained in the microbiology lab at Sree Narayana Institute of Medical Sciences, Ernakulam during four consecutive years (January 2014 -December 2017).

Inclusion criteria: AllUrine C/S reports with positive P.aeruginosa showing $\geq 10^5$ colony forming units/ml.

Exclusion criteria: UrineC/S reports of P.aeruginosa from repeat culture of previously recruited patients.

Method: Mid stream urine samples were collected in sterile containers. The samples were cultured on blood agar and CLED agar (Cystine Lactose Electrolyte Deficient) medium with a standard loop and were incubated at 37^{o}C overnight.

A growth of $\geq 10^5$ colony forming units/ml was considered as significant bacteriuria. The P.aeruginosa isolates were identified by conventional biochemical test[8].

Antibiotic sensitivity testing was done by Kirby- Bauer disc diffusion method on Mueller-Hinton agar and interpretations were done according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [9]. Antibiotics against which susceptibility tested were Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Cefepime (30µg), Ceftazidime (30 µg), Piperacillin (100 µg), Piperazillin –Tazobactam (100/10 µg), Imipenem (10µg).

Quality control was performed using P.aeruginosa ATCC 27853. The data regarding the number of P.aeruginosa and its susceptibility patterns were collected from the Microbiology lab register.

Statistical analysis: Descriptive statistics was used for analysis. Collected data were entered in MS-Excel and statistical analysis was done using SPSS 15 software and were expressed as percentages.

Result

Out of 6622 urine samples received in our lab during the study period of 4 years, we got 925 (14%) culture positive cases with significant bacteriuria. The isolation rate of P.aeruginosa over the four years was 32 (3.5%). The year wise isolation rate of P.aeruginosais shown in (Table:1).

The antibiotic susceptibility patterns of all P.aeruginosa isolates and year wisedrug resistance pattern ratesare shown in (Table: 2) and (Table:3) respectively.

Table-1: Isolation rates of P.aeruginosa from urine samples.

Year	Total number of Urine samples	Significant bacteriuria(%)	Isolation rate ofP.aeruginosa (%)
2014	1353	230 (17)	9 (3.9)
2015	1659	235(14.2)	6 (2.6)
2016	1594	222 (13.9)	10 (4.5)
2017	2016	238 (11.8)	7(2.9)
Total	**6622**	**925 (14)**	**32 (3.5)**

P.aeruginosa was the third most common urinary isolate after E.coli and Klebsiella species during the entire study period and year wise also it maintained its third position. The isolation rate was maximum in 2016.

Table-2: Antibiotic resistance patterns of P.aeruginosa isolates (N=32)

Antimicrobial agents	Number	Resistance (%)
Gentamicin	17	53.1
Amikacin	9	28
Cefipime	9	28
Ceftazidime	11	34.4
Ciprofloxacin	14	43.7
Norfloxacin	13	40.6
Orfloxacin	13	40.6
Piperacillin	12	37.5
Piperacillin -tazobactam	8	25
Imipenem	9	28

The drug resistance was highest against Gentamicin followed by Fluoroquinolones.Reserve drugs like Piperacillin – tazobactam and Imipenem exhibited similar resistance rates.

Table-3: Year wise antibiotic resistance (%) of P.aeruginosa

Antimicrobial agents	2014	2015	2016	2017
Gentamicin	44	66	50	57
Amikacin	22	50	30	14.3
Cefipime	33	50	20	14.3
Ceftazidime	44	33	20	42.9
Ciprofloxacin	44	83	40	14.3
Norfloxacin	33	83	40	14.3
Orfloxacin	33	83	40	14.3
Piperacillin	44.4	66	20	28.6
Piperacillin-tazobactam	33.3	33	20	14.3
Imipenem	44.4	50	10	14.3

Amikacin, Cefipime and Fluoroquinolones exhibit a decreasing trend over years but Gentamicin, Ceftazidime and Piperacillin shows an increasing pattern of drug resistance. When compared to 2015, Piperacillin- tazobatam and Imipenem shows a tremendous reduction in the drug resistance.

Discussion

P.aeruginosa has established itself as a significant uropathogen which may cause dreaded complications if not treated properly. Antibiotic resistance is a major clinical problem in treatinginfections caused by this organism. The resistance patterns and isolation rates of P.aeruginosa varies regionally. Hence, increasing importance has been placed on the careful monitoring of antimicrobial resistance patterns of P.aeruginosa isolates for appropriate empirical as well as targeted treatment of the same. The study shows the prevalence and the antibiotic susceptibility patterns of urinary isolates of P.aeruginosa in a tertiary care hospital, Central Kerala.

In the present study P.aeruginosa was the third most common urinary isolate after E,coli and Klebsiella species Otherauthors also observed P.aeruginosa asthe third most frequent urinary isolate [10-13]. In some studies it acquiredsecond positionbut in a recent study from Pakisthan it rankedonly fifth [2,4]. The isolation rates in the present study is compared to some recent studies from India and other countries (Table:4).Eventhough there is a slight variation in the prevalence, P.aeruginosa continue to be an important uropathogen in majorities of the studies.

Table-4 : Prevalence of P.aeruginosa from urine samples in various recent studies

Various studies	Isolation rateof P.aeruginosa (%)
Present study	3.5
Bency JAT et al ; 2017; Kerala [10]	3.8
Singh VP et al; 2017;UP[2]	6.7
Sangeeta et al; 2017;Maharashtra [13]	9.85
Shah DA et al ;2015;Karachi [4]	5.4
Jain et al;2014;Patiala [14]	9
Prakash D et al; 2013;Meerut [12]	12.9
Syed MA et al; 2012;Kerala [11]	2.74

The antibiotic resistanceof P.aeruginosa is compared with various Indian and international studies in (Table:5).

Table-5 : Comparison of Antibiotic resistance patternsof P.aeruginosawith various recent studies

	Percentage of resistance (%)									
Various studies	Gentamicin	Amikacin	Cefipime	Ceftazidime	Ciprofloxacin	Norfloxacin	Ofloxacin	Piperacillin	Piperazillin-Tazobactam	Imipenem
Present study	53.1	28	28	34.4	43.7	40.6	40.6	37.5	25	28
Singh VP et al[2].	-	-	-	-	20	14	-	-	10	
Shah DA et al[4]	35.3	25.3	63.9	56.1	50	-	49	-	19.6	10.4
BencyJATet al [10]	30	20	-	90	90	-	-	30	10	-
Syed MA et al [11]	49	12.5	58	71	21	25	-	-	0	0
Sangeeta et al[13]	85.7	85.7	-	100	-	42.85	-	-	71.4	14.28
Oladeinde BH et al [15].	100	-	-	-	40	-	60	-	-	-
Thomas ss et al [16]	47	47	33	33	60	-	-	-	33	20
Juayang C et al [17]	15.8	6.7	-	-	-	-	-	-	9	11.1

In the presentstudy, 53.1% and 28% of the isolates were resistant to Gentamicin and Amikacin respectively. Over the 4 years, the resistance to Gentamicin was fluctuating but Amikacin showed a decreasing trend. The Aminoglycosides

inhibit protein synthesis by binding to the 30 S subunit of the ribosome and the inactivation of the aminoglycosides occurs through the production of enzymes whichtransfer acetyl, phosphate or adenyl groups to the amino acidhydroxyl substituents on the antibiotics [16]. The resistance to Amikacin was highest during the year 2015 (50%), but it was lowerthan resistance to Gentamicin. This shows the growing resistance of P.aeruginosa to Gentamicin. Amikacin has been used sparingly only in severe forms of diseases owing to high cost and the intravenous nature of administration. Therefore, drug resistance has been slow to emerge.In our study, Amikacinis noted to be a comparativelyeffective drug. However, because of itsnumerous side effects including renal toxicity, blurred vision, hearing loss, Bartter-like syndromes neuromuscular blockade, arthralgia, apnoea and many more, it is not commonly used[17].

Cefipime and Ceftazidime are the most frequently prescribed third and fourth generation Cephalosporins respectively [18]. In the present study, Ceftazidime showed 34.4% and Cefipimedisplayed 28% of resistance. A recent study from South India showed comparable resistance level but there are some studies showing a very high resistance to both [4,11,16]. In 2017 there was a tremendous decrease in the resistance against Cefipime (14.3%) but Ceftazidime resistance was almost thrice (42.9%) than that of Cefipime. The increased prevalence of Ceftazidime resistant P.aeruginosacan be related to inappropriate use of beta lactam antibiotics.Selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains[18]. It is recommended to restrict use of Ceftazidime for a period of time to bring the developing resistance under control.

Among Fluoroquinolones, Ciprofloxacin, Norfloxacin and Ofloxacin showed almost similar resistance of 43.7%, 40.6% and 40.6% respectively. Different authors observed the resistance of ciprofloxacin as low as zero to as high as 90% [2,4,10,11,15,16,19]. Principal modes of Flouroquinolone resistance in P. aeruginosa is due totarget modifications in DNA gyrase (gyr A) andtopoisomerase IV(par C) or mutations in regulatory genesfor efflux pumps that reduce intracellular concentrationsof the antibiotic [20]. Resistance for Fluoroquinolones decreaseddrastically and reached a promising low level of 14.3% in 2017. Similar study from Punjab also documented a decreasing trend over years [21]. This decreasing trend gives a promising evidenceof rotational antibiotic policy in our institution.

25% of the isolates are Pipereacillin –tazobactam resistant. Similar pattern of resistance to Piperacillin- tazobactamwas observed by various authors butan extremely high resistance was noticed by a study conducted by Sangeeta et al [2,10,13,4,16,17]. Penicillins are highly ineffective against P aeruginosa except for Piperacillin-tazobactam because of the beta lactamase inhibitor in addition to the extended spectrum and scare use of the drug [4]. From 2014 to 2017, Piperacillin- tazobactam resistance remained almost consistent with a hopeful dip in 2017.Piperacillin –tazobactam continueto be a good choice of reserve drug for treating UTI caused by P.aeruginosa.

Resistance to Imipenem (28%) was also noted inthis study. This is very high when compared to other recent studies [4,13,16,17]. This increased resistance is quite alarming, taking into account that Carbapenems are the last line of antibiotics for treating Gram-negative bacilli infections. Resistance to Carbapenems may be due to the result of complex interactions of several mechanisms including production of carbapenemase, over production of efflux system and loss of outer membrane porins. P.aeruginosa isolates that are Carbapenem resistant, specifically carbapenemase producing, are the worst, for the reason that they are associated with ahigher mortality rate [17]. When compared to 2015, the resistance rate (50%), showed a tremendous decrease in 2017 (14.3%). This may be due to the strict implementation of antibiotic policy and infection control practices in our hospital.

Conclusion

P.aeruginosa remains as a common uropathogen. Drug resistant strains are markedly high in our area. The susceptibility pattern of one region differs widely from the other. The resistance of P.aeruginosado not have a consistent trend over years. Irregular resistance pattern is observed except in some antibiotics such as Amikacin, Cefipime and Fluoroquinolones which showed a decreasing trend. Reserved drugs like Piperacillin- tazobactam and Imipenem showed an alarming drug resistance. It is emphasized that each institution should have an antibiotic policy based on the antibiogram which should be renewed yearly. Instead of going for higher options of antibioticseach time, strict implementation of restrictive and rotational antibiotic policies and adherence to the concept of ‘Reserve drugs” should be followed by each institution.

This is the only modality to inhibit the emergence of resistance strains of all uropathogens especially opportunistic pathogens like P.aeruginosa.

What's New in this study: This study provides information regarding the prevalence and anti microbial susceptibility pattern of urinary isolates of P.aeruginosa.

The study stresses onthe importance of C/S reports provided by the microbiology laboratory, so that clinician can select the appropriate antibiotic therapy.

Itemphasizesthe importance of close monitoring of antibiotic susceptibility patterns by preparation of antibiogram and its regular updating.

The study also intends to motivate the strict implementation of restrictive and rotational antibiotic policies and adherence to the concept of reserve drugs.

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