Research Article

Antibiotic resistance pattern of *Pseudomonas aeruginosa* strains isolated from clinical specimens in a tertiary care hospital

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Abstract

Introduction: *Pseudomonas aeruginosa* (Ps.aeruginosa) is one of the important bacterial pathogens isolated from various samples. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents against having anti-pseudomonal activities, life threatening infection caused by *Ps.aeruginosa* continues to cause complications in hospital acquired infections. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates. **Material and Method:** This study was conducted during June 2013 to June 2014. During this period total of 920 samples were tested, in which 581 samples showed growth of bacteria. Out of 5811 samples, 100 clinical isolates of *Pseudomonas aeruginosa* were isolated. The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose Non-fermenting pale colonies which were oxidase test positive and on Nutrient agar pigmented and non-pigmented colonies with oxidase positive. Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Baur disc diffusion method) according to CLSIs guidelines. **Result**: In present study, maximum isolates of *Ps. aeruginosa* isolated from various samples are highly resistant to cefipime (86%) followed by piperacillin (80%), and ceftriaxone (66%). Combination of pipercillin-tazobactam showed low level resiatance (50%) compared to pipercillin alone. **Conclusion**: To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented.
Keywords: Antibiotic Resistance, Cefepime, *Pseudomonas aeruginosa*.

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INTRODUCTION

Pseudomonas aeruginosa (Ps.aeruginosa) is one of the important bacterial pathogens isolated from various samples. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents against having anti-pseudomonal activities, life threatening infection caused by *Ps.aeruginosa* continues to cause complications in hospital acquired infections. *Ps.aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patient particularly in burn patients, orthopaedic related infections, respiratory diseases, immunosup pressed and catheterized patients. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates[1]. Being gram-negative bacteria, most pseudomonas spp. are naturally resistant to penicillin and majority of related beta-lactum antibiotics, but a number are sensitive to piperacillin, imipenem, tobramycin or ciprofloxacin. Nowadays more and more resistance of *Ps.aeruginosa* are encountered in routine clinical practice, a serious problem, increase morbidity and mortality and also cost of treatment. Its general resistance is due to a

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combination of factors. It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages. In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P.aeruginosa* has been noticed, which is related to high morbidity and mortality. Regional variations in the antibiotic resistance exist for different organisms, including *P.aeruginosa* and this may be related to the difference in the antibiotic prescribing habits[2]. The periodic testing and analysis of antibiotic resistance would enable the physicians to detect the trends in the resistance pattern to the commonly prescribed antibiotics in a given organism. So, we aimed in the present study, to determine the status of antimicrobial resistance to individual anti pseudomonal agents and the magnitude of the multidrug resistance in these organisms.

MATERIALS AND METHODS

This study was conducted at the Department of Microbiology in a tertiary care hospital, Shimoga. It is tertiary care center, referral and teaching hospital. This study was conducted from June 2013 to June 2014. The present study comprises 100 Pseudomonas aeruginosa positive samples obtained from pus/swab, urine, sputum, and blood samples submitted for microbiological diagnosis to the Microbiology Department. All these samples were obtained from various wards of hospital. The clinical data was obtained from the respective units and wards of the patients.

Sample processing

The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose Non-fermenting pale colonies which were oxidase test positive and on Nutrient agar pigmented and nonpigmented colonies with oxidase positive.

Confirmation of pseudomonas spp

After obtaining the pure strains, the strains were subjected to biochemical identification tests to identify Pseudomonas spp. For this purpose samples were inoculated in Triple Sugar Iron media (TSI), Citrate media, Peptone water, Urease media and kept in an incubator for 18 hrs at 37°C. Next day the results were noted on TSI, Citrate media and Urease media. Part of growth on Peptone water was subjected to Indole test with Kovac's Reagent and part for motility test by 'Hanging drop' method. A strain of Pseudomonas in the TSI medium showed alkaline slant, no reaction in butt. It showed negative reaction for indole test, negative urease test and positive citrate test. Glucose is utilised oxidatively, forming acid only[3].

Antimicrobial susceptibility testing

Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Baur disc diffusion method) according to CLSIs guidelines. The following antibiotics were tested by disc diffusion method, Ceftazidime(30 µg), Piperacillin (100 µg), Piperacillin-tazobactam(100/10 µg), Cefipime(30 µg), Imepenam(10 µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacillin (5 µg), Levofloxacillin(5 µg), Ceftriaxone (30 µg),,

RESULTS

During a period of 1 year a total of 920 samples were tested, out of 920 samples, 581 samples were showing growth on culture and out of 581samples, 100 Ps.aeruginosa were isolated and tested for antibiotic sensitivity.

Table 1: Sex wise distribution of cases

Sex	Total No	Percentage (%)
Male	61	61
Female	39	39
Total	**100**	**100**

Table 2: Isolation of Pseudomonas aeruginosa from different clinical samples

Name of sample	No. of sample in which pseudomonas aeruginosa Isolated
Pus	58
Blood	22
Urine	12
Sputum	04
Others	04
Total	**100**

Table 3: Antibiotic resistance of Pseudomonas aeruginosa isolated from different clinical Samples

Antibiotic	Resistance (%)
Ceftazidime (CAZ)	60
Piperacillin (PI)	80
Piperacillin tazobactam (PIT)	50
Cefepime (CPM)	86
Cefriaxone (CTR)	66
Ciprofloxacin (CIP)	60
Levofloxacin (LE)	20
Amikacin (AK)	45
Gentamycin (GM)	63
Imepenam (IM)	12

DISCUSSION

Pseudomonas aeruginosa is ranked second among gram-negative bacteria isolated in hospital environment, and leading cause of nosocomial infections responsible for

high morbidity and mortality rate. High prevalence of pseudomonal infections is common among critically ill patients on admission on intensive care unit and those with underlying clinical condition[4]. *Pseudomonas aeruginosa* emerged as an important pathogen and responsible for the nosocomial infections. It is one of the important causes of morbidity among hospital patients. The pre-eminent of pseudomonas aeruginosa in hospital infections is due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in hospitals. Being an extremely adaptable organism, it can survive and multiply even with minimum nutrients, if moisture is available. As *Pseudomonas aeruginosa* causes serious infections, and is one of the leading causes of hospital acquired infections, this study was carried out to detect antibiotic sensitivity pattern for the various drugs available. Such study helps clinicians for the better management of patients. In the present study sex wise prevalence of clinical isolates shows that infections caused by *Pseudomonas aeruginosa* are more common in males (61%) compared to females (39%). This is comparable with study of Javia *et al*[3] and Jamshaid Ali Khan *et al*[5] In present study, the maximum clinical isolates of Ps. aeruginosa were isolated from pus/swab (58%), followed by blood (22%) and urine (12%). In this study, maximum isolates of Ps. aeruginosa were highly resistant to cefipime (86%) pipercillin (80%), followed by other third generation cephalosporines. They were highly sensitive to imepenem and levofloxacin which was comparable to Patel H *et al*[6]. Piperacillin tazobactam combination showed less resistance (50%) as compared to pipercillin alone (80%). Among yhe aminoglycosides gentamycin showed more resistance (63%) compared to amikacin (45%). This is in line with other studies[3,4,7]. This study shows that the clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics. The antimicrobial agents are losing their efficacy because of the spread of resistant organisms due to indiscriminate use of antibiotics, lack of awareness, patient non compliance and unhygienic condition. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented. In the meantime, it is desirable that the antibiotic susceptibility pattern of bacterial pathogens like *Ps. aeruginosa* in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the resistance.

CONCLUSION

Out of 100 clinical isolates of Ps. aeruginosa, maximum isolates (58%) were isolated from pus/swab followed by blood samples (22%) and from urine (12%), 4% from other samples. Out of 100, 61% were males and 39% were females. Most of samples were collected from surgical wards, followed by paediatric ward, medical ward, orthopaedic ward and ICU. Maximum resistant isolates of *Pseudomonas aeruginosa* were isolated from pus/swab samples. In present study, maximum isolates of *Ps. Aeruginosa* isolated from various samples are resistant cefepime (86%) followed by piperacillin (80%) and ceftriaxone (66%). It is the need of the time that antibiotic policies should be formulated and implemented to resist and overcome this emerging problem. Every effort should be made to prevent spread of resistant organisms. The solution can be planned by continuous efforts of microbiologist, Lclinician, pharmacist and community to promote greater understanding of this problem. Frequent hand washing to prevent spread of organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

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