Nosocomial Infections Due To Multidrug Resistant Pseudomonas Aeruginosa

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Abstract: *Pseudomonas aeruginosa is a leading cause of nosocomial infections. The rise of emergence of antibiotic resistance may vary with different antibiotic treatments. To compare the risks of emergence of resistance associated with panel of antibiotics used in the treatment of nosocomial infection, a case study was conducted. A total of 120 P. aeruginosa isolates were recovered from lower respiratory tract (38.0%), blood stream (13.0%), infected wounds (22.0%), and urinary tract infections (27.0%). The clinical isolates were tested for antibiotics susceptibility and showed variation in their sensitivity, 8.0% of the isolates were resistant to Imipenem, 11.0% to Amikacin, 16.0% to Ciprofloxacin, 20.0% to Gentamycin, 25.0% for both Ceftazidim and Aztreonam, 33.0% to Piperacillin, 35.0% to Tobramycin, 75.0% to Ceftriaxon, 80.0% to Amoxicillin/clavulanic acid, and 100% of the isolates were resistant to Ampicillin. The multidrug resistance test (resistance to at least three of the drugs Ceftazidim, Ciprofloxacin, Gentamycin, and Imipenem) showed that 40.0% of the isolates were considered as multidrug resistant. These strains distributed as 39.0% of the isolates recovered from lower respiratory tract, 44.0% from blood stream, 37.0% from wound infections, and 42.0% of the strains isolated from urinary tract infections. Greater decreases in susceptibility rates were, however, observed for Fuoroquinolones and Ceftazidim among P. aeruginosa isolates.*

Key ward*: Pseudomonas aeruginosa, Nosocomial infection, Multidrug resistance*

Introduction

Pseudomonas aeruginosa is a nonfermentative Gram negative bacteria that have minimal nutritional requirements and can survive on a wide variety of surfaces and in aqueous environments. *P. aeruginosa* rarely cause serious infections in otherwise healthy persons and is infrequently identified as normal microbial flora in healthy individuals **[1].** Infections with *P. aeruginosa* is of great concern for hospitalized patients particularly those in intensive care units where these opportunistic pathogens are capable of causing severe invasive infections in critically ill and immunocompromised patients**[2,3].** *P. aeruginosa* is commonly responsible for urinary tract infections, pneumonia and blood stream infection **[4,5].** In particular this organism is responsible for 3-7% of blood stream infection cases **[6,7]**; notably high morbidity and mortality rates (range 27 to 48%) have

been observed in critically ill patients **[8,9]**. Resistance to multiple drugs is a common feature of hospital acquired *P. aeruginosa* strains **[10]**.

Among 70067 isolates obtained from patients admitted to hospitals in five different geographic areas and evaluated by SENTRY antimicrobial resistance surveillance program, the prevalence rates of *P. aeruginosa* infections were higher in Latin American and the Asia-Pacific regions (11.4% of total isolates in each region) than in Europe 9.3%, the United States and Canada (8.7%) **[11,12].** The reasons for such differences are unclear and may be related to the use of suboptimal infection control practices in some of these regions. Infections due to this pathogen are becoming difficult to treat because of limited choices of effective antimicrobial agents **[13].**

Resistance to multiple drug is a common feature of hospital acquired *P. aeruginosa* strains **[14].** Low permeability of the Outer Membrane Proteins (OMPs), production of the inducible AMPC chromosomal β-lactamase and multidrug efflux system contribute to the intrinsic resistance of this species **[15]**. Thus drugs suitable against *P. aeruginosa* infections are limited to aminoglycosides e.g.; Gentamycin, Amikacin, Fluoroquinolones, Ciprofloxacin remains the most active, selected β-lactamase e.g.; Ceftazidim, Carbapenems and one β-lactam/lactamase inhibitor combination (Pipracillin, Tazobactam) **[10,7,14].** Unfortunately, acquired resistance to different categories of the antipseudomonal agents is also possible and has been widely illustrated; in particular resistance to β-lactamase is very common and is due to mutations amplifying intrinsic resistance mechanisms (i.e.; AMC) and/or acquisition of additional β-lactamase genes by horizontal transfer **[16,17].** Acquired β-lactamases found in *P. aeruginosa* isolates can be classified into three different groups, (i) narrow spectrum enzymes (e,g.; PSE114) that efficiently degrade Penicillins and Cefoperazone, (ii) extended spectrum β-lactamase (ESBL) e.g.; PER-1, VEB-1, Ges112) that also degrade Cephems and Monobactam, (iii) Metalo β-lactamases (MBL) e. g.; IMP, V1M type that efficiently degrade all anti-pseudomonas β-lactamase with the exception of Monobactams **[18,19].**

Risk factors, empirical treatment and treatment outcome of pseudomonal infection have been investigated by different authors **[20,21],** possible emphasis has been posed on clinical differences observed between multidrug resistant and susceptible *P. aeruginosa* isolates **[9].**

P. aeruginosa infection in burn units is a common infection and different reports are present in the records of hospitals. An environmental survey conducted by **[22]** showed that an outbreak of infections in burn unit caused by a serotype O:11 and a multidrug resistant serotype O:12. *P. aeruginosa*. **[23]** reported an outbreak of infection in a burn unit due to *P. aeruginosa* originating from contamination of tubing used for irrigation of patients. In Pakistan (2006) a study conducted in institute of medical services showed that 27.3% of infected patients under study was caused by *P. aeruginosa.*

Materials and Methods

Bacterial isolates and identification procedures

P. aeruginosa isolates were recovered from 200 patients admitted to different wards of Shifa hospital and European Gaza Hospital. The collection period was from October 2004 to July 2005. Isolates in each hospital were consecutive, meaning that all *P. aeruginosa* isolates that fitted the inclusion criteria were from inpatients with a nosocomially acquired infection.

Nosocomial infections were defined as infections diagnosed >72hr after the start of hospitalization **[24].** Sample collection strain isolation and identification were carried out according to the standard procedures in each participating hospital. The isolates were obtained from different clinical specimens, including urine specimens 32 (27.0%), respiratory tract secretion 46 (38%) wound infection 26 (22%) and blood stream infection 16 (13%). The clinical specimens were collected in sterile wide mouth container for urine and sputum and in swabs for wound, blood specimens collected by drawing 5ml venous blood and inoculated into blood bottles containing 50ml trypticase broth.

Microbiological analysis

The bacteria were isolated from clinical specimens by standard microbiology procedure recommended by **[25,26]**

The collected clinical specimens were inoculated into blood agar and MacConkey plates which incubated at 37^{o}C for 18-24hr. Blood bottles were incubated for 24hr at 37^{o}C and subcultured at MacConkey and blood agar plates. If negative the blood bottles will be kept in incubator for at least 5 days before discarded as negative.

Bacterial isolates were identified with API system (Biomerieux) and the conventional biochemical tests like cytochrome oxidase test,

pigment production, glucose oxidation, arginine dihydrolase activity and growth at 42°C.

P. aeruginosa were stored in Todd Hewitt broth supplemented with 20% glycerol for additional confirmation tests

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of the bacterial isolates was performed by the disk diffusion method as descried by the National Committee for Clinical Laboratory Standards (NCCLS) **[27].** The Mueller Hinton agar plates were inoculated with bacterial isolates inoculums calibrated with 0.5 McFarland standard. The susceptibility of the bacterial isolates to 11 antimicrobial agents (Amikacin, Aztreonam, Ceftazidim, Ciprofloxacin, Gentamycin, Imipenem, Piperacillin, Amoxicillin/clavulanic acid, Ceftriaxone, Ampicillin, and Tobramycin), was recorded.

Interpretation of inhibition zone diameter obtained following the manufacturer instructions. The results were expressed as resistant or susceptible.

Multiple resistant strains

The strains will be considered as multiple resistant in case of resistance to at least three of the drugs (Ceftazidim, Ciprofloxacin, Gentamycin, and Imipenem) **[28,29].**

Results

A total of 120 *P. aeruginosa* strains isolated from patient specimens complied with the criteria for entry in the study. At least one isolate from each site of patient was selected, the number of clinical samples included in the study were present in table (1). 38.0% of *P. aeruginosa* isolates were isolated from lower respiratory tract, 13.0% from blood stream, 27.0% form urinary tract, and 22.0% from infected wound.

Table (1) Distribution of *P. aeruginosa* isolates from different sites of infection

Site of infection	Number of isolates	Percentage
Lower respiratory tract infection	46	38
Blood stream infection	16	13
Urinary tract infection	32	27
Wound infection	26	22
Total	120	100

Table (2) Distribution of antimicrobial resistance among *P. aeruginosa* isolates (N=120)

Antimicrobial agents	Number of resistant isolates	Percentage of resistant
Imipenem	9	8
Amikacin	13	11
Ciprofloxacin	19	16
Gentamycin	24	20
Aztreonam	30	25
Ceftazidim	30	25
Piperacillin	39	33
Tobramycin	42	35
Ceftriaxone	90	75
Amoxicillin/clavulanic acid	96	80
Ampicillin	120	100

Antimicrobial susceptibility testing carried out for 120 isolates showed variation in the rate of resistance to each antimicrobial agents tested. The results of antimicrobial resistance among *P. aeruginosa* present in Table (2).

Imipenem has the lowest resistant rate (18.0%), followed by Amikacin (11.0%, Ciprofloxacin (16.0%), Gentamycin (20.0%), both Aztreonam and Ceftazidim showed equal resistance (25.0%), Piperacillin (33.0%), Tobramycin (35.0%), Ceftriaxone (75.0%), Amoxicillin/clavulanic acid (80.0%), and Ampicillin (100%)

The frequency of resistance at different sites of infection are presented in table (3). The results expressed as number of resistant strains at each site of infection.

Table (4) showed the frequency of multidrug resistant strains isolated from different sites of infection. Multidrug resistant was defined as the resistance to at least three of the following four drugs**:** Ceftazidim, Imipenem, Ciprofloxacin, and Gentamycin.

Discussion

Resistance to antimicrobial agents is an increasing clinical problem and is recognized as a public health threat. *P. aeruginosa* shows a particular propensity for the development of resistance.

Table (3) Distribution of antimicrobial resistance patterns among *P. aeruginosa* at different sites of infection

Antimicrobial agents	Blood stream infection	Urinary tract infection	Lower respirator tract infection	Wound infection
Imipenem	4	3	1	1
Amikacin	5	3	2	3
Ciprofloxacin	7	3	6	3
Gentamycin	14	3	3	24
Aztreonam	14	6	6	4
Ceftazidim	15	7	3	5
Piperacillin	15	11	4	9
Tobramycin	17	12	11	2
Ceftriaxone	40	30	10	10
Amoxicillin/clavulanic acid	40	32	10	14
Ampicillin	50	40	20	10

Table (4) Distribution of multidrug resistant *P. aeruginosa* according to the site of infection

Site of infection	Number of multidrug resistant isolates	Total number of isolates	Percentage of multidrug resistant
Lower respiratory tract infection	18	46	39
Blood stream infection	7	16	44
Wound infection	12	32	37
Urinary tract infection	11	26	42

The emergence of resistance in *P. aeruginosa* also limits future therapeutic choices and is associated with increased rates of mortality and morbidity and higher treatment costs. Therefore we conducted this study to assess the resistance arising during treatment with different antibiotics first by examining the overall effect of each antibiotic on emergence of resistance to individual agents. We found that

emergence of resistance to at least one antibiotic occurred in 4.3% of the patients (table 2). This proportion should be considered a minimum estimate of the risk of emergence of resistance during antipseudomonas therapy since it is based solely on clinical cultures and includes follow-up only during hospitalization, resistance emerged during treatment with each class of antibiotic and did not appear to be significantly prevented by the use of combination therapy with aminoglycosides. However the latter issue needs to be further examined in studies that include larger number of patients on aminoglycosides.

Important differences between antibiotics were significant against *P. aeruginosa*. In terms of percentage susceptibility and Amikacin scored best. Full resistance to Imipenem was however lower than Amikacin and Gentamycin. In case of resistance to other antibiotics including the β-lactam antibiotics Imipenem again remained the most active compound, moreover Imipenem resistance in *P. aeruginosa* become widespread in some hospital, soon after the introduction of this agent **[30].** Although treatment with Imipenem could result more often in the emergence of resistant *P. aeruginosa* than treatments with other antipseudomonal agents this tendency may not translate into a higher prevalence of Imipenem resistance among hospital isolates. Ciprofloxacin resistance for instance, is more common that Imipenem resistance in *P. aeruginosa* isolated from patients at our hospitals. This apparent discrepancy might be related to the differences in the frequency of use of various agents and to the different likelihoods of persistence of resistant strains.

Carbapenems are resistant to hydrolysis by most β-lactamases and therefore are effective agents against a broad range of nosocomial pathogens **[31]** compared with earlier surveys that have examined susceptibility to various antibiotics in *P. aeruginosa* isolates from Gaza Strip hospitals **[32]**. There is a clear tendency towards decreased susceptibility for all groups of antibiotics, the rates at which this loss of activity proceeds.

Multidrug resistant isolates of *P. aeruginosa* were considered as multidrug resistant if these isolates were resistant to at least three drugs of the following four drugs (Ceftazidim, Ciprofloxacin, Gentamycin, and Imipenem). Multidrug *P. aeruginosa* isolates are usually reported to be responsible for outbreaks of nosocomial infections mainly in intensive care units **[33].** In such studies certain strains of drug resistant *P. aeruginosa* may be better adapted to spread among susceptibility hosts on nosocomial settings **[34].**

Conclusion

In Conclusion the present study is important, as it is one of few studies conducted in this field. We believe that the use of Imipenem for treatment of *P. aeruginosa* should be reserved for situations where the infection is polymicrobial particularly when anaerobic bacteria are present or for multidrug resistant *P. aeruginosa* isolates. In cases where Imipenem is selected as the antipseudomonas antibiotics the potential for emergence of resistance should be anticipated and in appropriate circumstances routine culturing and susceptibility testing should be performed to detect the emergence of resistance *P. aeruginosa* as soon as possible. For other antibiotics was concluded that resistance of *P. aeruginosa* to Penicillins, Cephalosporins, Fluoroquinolones and Aminoglucosides varies between various isolates but is increasing.

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