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RESISTANCE PATTERN OF INPATIENT AND OUTPATIENT ISOLATES OF PSEUDOMONAS AERUGINOSA IN THE CITY OF NIS, 2004 TO 2006

SUMMARY

Pseudomonas aeruginosa is widerspread in nature and one of the most common pathogens involved in hospital infections. The resaearch was undertaken in order to determine the current level of antibiotic susceptibility of isolates of Pseudomonas aeruginosa obtained from inpatients and outpatients and multidrug resistance. The study was carried out in the laboratory for biocultures of the Public Health Institute Nis, from November 2004 to October 2006. A total of 994 isolates of Pseudomonas aeruginosa were tested on antibiotic susceptibility using disc diffusion method, according to CLSI. These isolates were from the samples of hospitalized patients (408) and from outpatients (586). The larges number of Pseudomonas aeruginosa isolates were from surgery clinic. All isolates were tested for their sensitivity to the following antibiotics: piperacillin/tazobactam, meropenem, imipenem, ceftriaxone, ceftazidime, netilmicin, gentamicin, amikacin, ciprofloxacin, ofloxacin (Bioanalyse, Turkey between November 2004 to May 2006 and between May to October 2006 Rosco, Danmark). Susceptibility rates of outpatient isolates were higher than those from inpatient ones. Isolates showed the highest susceptibility rate to carbapenems: meropenem 87.17% and imipenem 9.25% for isolates from inpatients, meropenem 96.13% and imipenem 95.13% for isolates from outpatients. Susceptibility to piperacillin/tazobactam was 80.63% for hospital isolates and 89.83% for isolates from outpatients. The lowest susceptibility was to ceftriaxone 23.53% for hospital and 38.97% for isolates from outpatients. In our study, the multidrug resistance was in 18.14% for isolates from inpatients and 8,19% for isolates from outpatients.

Key words: **Pseudomonas aeruginosa, antibiotic susceptibility, multi-drug resistance**

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacillus that is not an obligate parasite. It may be found in moist enviroments. Community-acquired Pseudomonas aeruginosa infections may occur after exposure to the organism in whirpools, or swimming pools, or by the use of contaminated sponges (1). Keratititis due to Pseudomonas aeruginosa is associated with the use of contact lenses stored in contaminated contact lens solution (2). This organisms may be found in the patient's own flora. It colonizes the intestine of up to 40% of healthy people, but in inpatients this percentage increases with increasing duration of hospitalization. Pseudomonas aeruginosa needs minimal nutritional conditions, and exhibit natural resistance to many antibiotics and antiseptics in which may survive (3). It is widely distributed in the hospital enviroment and it is difficult to eradicate.

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Pseudomonas aeruginosa is one of the most common nosocomial pathogens responsible for infections in immunocompromised hosts. It can infect almost any external site or organ, and the spectrum of infections ranges from superficial skin infections to more serious infections such as fulminant sepsis (4). The rate of antibotic resistance to Pseudomonas aeruginosa is increasing and most studies have found a higher prevalence of antimicrobial resistance in hospitals than in community (5-7). Local monitoring of prevalence and and antimicrobial susceptibility patterns may help to establish preventive and therapeutic guidelines for the empirical antimicrobial therapy.

The aim of this study was to evaluate and compare the prevalence and resistance of inpatient and outpatient isolates of Pseudomonas aeruginosa isolated in the Public Health Institute Nis, Serbia.

MATERIALS AND METHODS

All samples received from the patients hospitalized in the Clinical Center Nis (inpatients) and from outpatients between November 2004 to October 2006 were processed using the standard microbiological procedures. Samples for microbiological culture comprised sputum, wound swabs, pus, peritoneal dialysates, bronchial aspirates, blood. Pseudomonas aeruginosa isolates were characterised and identified using a combination of colonial morphology, Gram stain characteristics, fermentation test, citrate and oxidase tests and pyocyanin production.The sensitivity of Pseudomonas aeruginosa was determinated using the Kirby-Bauer disk diffusion method according to CLSI criteria. Before the discs were placed, the Muller-Hinton plates were inoculated with swabs submerged in the final inoculum concentration and streaced over the entire surface of the plates. All isolates were tested for their sensitivity to the following antibiotics: piperacillin/tazobactam, meropenem, imipenem, ceftriaxone, ceftazidime, netilmicin, gentamicin, amikacin, ciprofloxcin, ofloxacin (Bioanalyse, Turkey between November 2004 to May 2006 and between May to October 2006 Rosco, Danmark). Multidrug resistance of isolates of P.aeruginosa was defined as resistance to three or more classes of antibiotics. Statistical analysis was performed using the χ^2 test and $p<0,05$ was considered statistically significant.

RESULTS

During the 49-month period from November 2004 to October 2006, a total of 13928 specimens were obtained from inpatients and outpatients. There were 994 isolates of Pseudomonas aeruginosa; 586 (58.95%) were obtained from outpatients and 408 (41.04%) from inpatients *(Table 1)*.

Table 1. Distribution of isolates of Pseudomonas aeruginosa

No. of isolates	outpatients		inpatients	
	No.	%	No.	%
994	586	58.95	408	41.04

From 408 inpatient isolates of Pseudomonas aeruginosa, the hightes isolation rates were among the following departments: surgical 18.63% (76), pediatric 17.40% (71**),** Otorhinolaryngology Clinic 12.50% (51), Clinic of Orthopaedic Surgery and Traumatology 10.05% (41), Nephrology Clinic 7.06% (31), Pulmonary Clinic 5.39% (22) *(Table 2)*.

Table 2. Isolation rates of Pseudomonas aeruginosa according to departments

Department	No. of isolates	%
Surgery Clinic	76	18.63
Pediatric Internal Clinic	71	17.40
Otorhinolaryngology Clinic	51	12.50
Clinic of Orthopaedic Surgery and Traumatology	41	10.05
Nephrology Clinic		
Pulmonary Clinic	22	5.39
Infectious Diseases Clinic	21	5.15
Clinic of Dermatovenerology	20	4.90
Urology Clinic	14	3.43
Other clinics	13	3.19
Neurosurgery	11	2.70
Psychiatric Hospital	10	2.45
Neurology Clinic	9	2.21
Hematology	5	1.23
Ophthalmology Clinic	4	0.98
Clinic of Gynecology and Obstetrics	4	0.98
Endocrinology	3	0.74
Cardiology	1	0.25
Pediatric Surgery Clinic	1	0.25

The antibiotic resistance pattern of Pseudomonas aeruginosa isolates is shown in Table 3 (inpatients) and in Table 4 (outpatients).

Among isolates from inpatients, Pseudomonas aeruginosa isolates were the most sensitive to carbapenems: imipenem (90.25%), and mero-

Table 3. Resistance rates of Pseudomonas aeruginosa isolates from inpatients

Antibiotic	No.of isolates	susceptibile		resistant	
		No.	%	No.	%
Piperacillin/tazobactam	351	283	80.63	68	19.37
Meropenem	382	333	87.17	49	12.83
Imipenem	400	361	90.25	39	9.75
Ceftriaxone	408	96	23.53	312	76.47
Ceftazidime	406	218	53.69	188	46.31
Netilmicin	291	132	45.36	159	54.64
Gentamicin	406	149	36.70	257	63.30
Amikacin	408	243	59.56	165	40.44
Ciprofloxacin	403	227	56.33	176	43.67
Ofloxacin	404	203	50.25	201	49.75

Table 4. Resistance rates of Pseudomonas aeruginosa isolates from outpatients

Antibiotic	No.of isolates	susceptibile		resistant	
		No.	%	No.	%
Piperacilin/tazobactam	521	468	89.83	53	10.17
Meropenem	543	522	96.13	21	3.87
Imipenem	584	557	95.38	27	4.62
Ceftriakson	585	228	38.97	357	61.03
Ceftazidim	586	438	74.74	148	25.26
Netilmicin	451	334	74.06	117	25.94
Gentamicin	586	352	60.07	234	39.93
Amikacin	586	446	76.11	140	23.89
Ciprofloksacin	582	459	78.87	123	21.13
Ofloksacin	586	388	66.21	198	33.79

penem (87.17%); a large proportion of isolates was susceptible to piperacillin/tazobactam (83.63%); about half of Pseudomonas aeruginosa isolates were susceptible to ceftazidime, netilmicin, amikacin, ciprofloxacin i ofloxacin (53.69%, 45.36%, 59.56%, 56.33%, 50.25%), respectively. Pseudomonas aeruginosa isolates were the most resistant to gentamicin among aminoglycosides investigated (63.30%). From caphalosporins investigated, the resistance rate for ceftriaxone was high (76.47%).

More than half of Pseudomonas aeruginosa isolates were obtained from outpatients. More than 90% of the outpatient isolates were susceptible to imipenem and meropenem (95.38%, 96.13%, respectively). High susceptibility rate was to piperacillin/tazobactam (89.83%); 70% to 80% of isolates were susceptible to amikacin, netilmicin, ceftazidime and ciprofloxacin; 60% to 70% of isolates were susceptible to gentamicin and ofloxacin. Pseudomonas aeruginosa isolates were the most resistant to ceftriaxone among all antibiotics investigated (61.03%).

The outpatient isolates of Pseudomonas aeruginosa presented higher rate of antimicrobial susceptibility than the inpatient isolates.

The difference between these isolates in susceptibility rates was significant for carbapenems and quinolones ($p<0.05$).

The prevalence of multidrug resistance of isolates of Pseudomonas aeruginosa was investigated. Multidrug resistance occurred in 12.27% of all isolates. The rate of multidrug resistance was 18.14% (74 of 408) for inpatient isolates, and 8.19% (48 of 586) for outpatient isolates. Multidrug resistance was significantly higher for inpatient isolates than for outpatient isolates ($p<0.05$).

DISCUSSION AND CONCLUSION

Pseudomonas aeruginosa is naturally resistant to many antimicrobial agents and susceptible to the antipseudomonal antibiotics, including antipseudomonal penicillins, carbapenems, cephalosporins, aminoglycosides, quinolones. However, Pseudomonas aeruginosa has the ability to develop resistance to each of the antipseudomonal antibiotics. Monitoring antibiotic resistance is very important for the choice of antibiotics for empirical therapy in critically ill patients.

In this study, 994 isolates of Pseudomonas aeruginosa obtained from inpatients and outpatients were studied. Most isolates were obtained from outpatients (58,95%), but there was no data if any of the patients was previously hospitalized and was a home-nursing patient. In this study, community-acquired infections by the definition were not evaluated, but Pseudomonas aeruginosa as a pathogen outside the hospital. Similar studies evaluated resistance rates for inpatient and outpatient Pseudomonas aeruginosa isolates. In the study performed by Bouza et al. (8) 30.5% of isolates were obtained from outpatients; in the study performed by Al-Tawfig et al. (7) outpatient isolates constituted 48% of the total, and the total number of outpatient isolates collected in 1999 to 2002 in the study of Flamm et al. (5) was 15 994 of 50 332 (31.77%). Low resistance rate of outpatient isolates to ciprofloxacin (3%) was reported in the study of Al-Tawfig et al. (7). In the study of Bouza et al. (8) 24.5% of outpatient isolates were resistant to quinolones. Similar resistance rate of outpatient isolates were reported by Flamm et al. (5) and resistance rate to ciprofloxacin was 26.9% in the period from from 1999 to 2002. In the present study resistance to ciprofloxacin and to ofloxacin was 21.13% and 33.79%, respectively. Flamm et al. (5) showed that resistance rates of outpatient isolates to amikacin, caftazidime, gentamicin, imipenem, and piperacillin/tazobactam were 9.2%, 8.7%, 19.5%, 9.5%, and 7.7%, respectively. In our study, only the resistance rate to imipenem was lower (4.62%) than that reported in the study performed by Flamm et al. (5).

Isolates of Pseudomonas aeruginosa in this study constituited 41.04% of inpatient isolates. Resistance rates of inpatient isolates were higher than the outpatient isolates. The high rate of antimicrobial resistance of Pseudomonas aeruginosa isolated from hospitalized patients could be explained by the patient exposure to more and broader spectrum antibiotics, wich may result in the developing resistance to several antipseudomonal antibiotics. The resistance rates varies between communities and often between hospitals in the same community. In this study, imipenem was the most active agent against inpatients isolates (resistance rate 9.75%), followed by meropenem (resistance rate 12.83%). A surveillance study of 65 laboratories in the USA from 1998 to 2001 found 11% of isolates from non-intensive care units (ICU) to be resistant to imipenem and 10,5% to meropenem. (9). In Spain, resistance rate (8) to imipenem was 14% and 8% to meropenem; in Turkey, resistance rate to imipenem and meropenem were 15% and 20.4% respectively (10). Lithuanian study showed that 23.9% of isolates were resistant to imipenem and 11.3% to meropenem (11). It was reported that resistance rate of isolates obtained from ICU patients to imipenem and meropenem in USA was 16.7% and 15.8%, respectively (9), and in the study of Flamm et al. (5) 21.7% of isolates were resistant to imipenem. Wroblewska et al.(12) showed that very high percentage of imipenem-resistant isolates was obtained from ICU

patients (66%) in Poland. Obritsch et al.(13) reported that from 1993 to 2002, dramatic increases in antimicrobial resistance of Pseudomonas aeruginosa isolates from ICU patients occurred with imipenem (15% to 23%, p<0.0001). Relatively low resistance rate to imipenem (5.8%) was reported in Saudi Arabia (7).

During our study, 19.37% of inpatient isolates were resistant to piperacillin/tazobactam. This was a higher resistance rate than was observed in two studies in the USA. Karlowsky et al.(9) showed that resistance rate for ICU isolates was 8,8%, and for non-ICU isolates 7,8%; in the study of Flamm et al. (5) resistance rate to piperacillin/tazobactam for ICU isolates was 14.3%, and for non-ICU isolates 12.2%.

Resistance rates to ceftazidime in USA ranged from 8.0% to 14.2% for non-ICU isolates, and from 9.9% to 18.7% for ICU isolates (5.9); in the study performed in Lithuania 12.8% of isolates were resistant ot ceftazidime (11). In our study, high resistance rate to ceftazidime was observed - 46.31%. The prevalence of Pseudomonas aeruginosa inpatient isolates resistant to ceftazidime was similar as in Turkey (48.9%) (10).

Resistance rate to aminoglycoside in our study was high (resistance to netilmicin, gentamicin, amikacin: 54.64%, 63.30%, 40.44%, respectively). Opposite to this, susceptibility of Pseudomonas aeruginosa to amikacin was high in USA (89.4% to 93.1%)(5.9). The MYSTIC study data reported high aminoglycoside susceptibility rates with tobramycin and gentamicin susceptibilities at 92% and 82%, respectively (14). Friedland et al. reported that the agent with the lowest in vitro resistance rate for Pseudomonas aeruginosa was amikacin (15). Resistance rate of the inpatient isolates was similar to that reported from Turkey and Lithuania (10,11). In the present study, about half of Pseudomonas aeruginosa inpatient isolates were resistant to quinolones (ciprofloxacin 43.67%, ofloxacin 49.75%). In Europe, according to the MYSTIC data, percentage of isolates resistant to ciprofloxacin was 36.7% and in USA 37,2% (16, 17).

Therapeutic options for multidrug resistant isolates of Pseudomonas aeruginosa are limited and it is important to evaluate this phenomenon. In this study, the rate of multidrug resistance was 18.14% (74 of 408) for isolates from inpatients, and 8.19% (48 of 586) for isolates from outpatients. Olayinka et al. (18) reported that 19.6% of the Pseudomonas aeruginosa isolates were resistant to three or more antibiotics tested. In the USA, Friedland et al. (15) reported that 2.2% of Pseudomonas aeruginosa from ICU patients were multiresistant. Percentage of multidrug resistant isolates of Pseudomonas aeruginosa in the study of Flamm et al.(5) was 24,9% for all isolates collected from 1999 to 2002. Rate of multidrug resistant isolates was significantly higher for nursing home patients (29,9%) and ICU patients (29,5%), than for non-ICU patients and outpatients (5).

Monitoring antimicrobial susceptibility patterns provide useful data on the situation in local hospitals and in the community. The analysis of the resistance of Pseudomonas aeruginosa to antibiotics could provide better understending of the trends in antibiotic resistance.

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ISPITIVANJE REZISTENCIJE IZOLATA PSEUDOMONAS AERUGINOSA POREKLOM IZ MATERIJALA AMBULANTNIH I HOSPITALIZOVANIH BOLESNIKA NA ANTIMIKROBNE LEKOVE SPROVEDENO U NIŠU U PERIODU 2004 – 2006

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SAŽETAK

Pseudomonas aeruginosa je široko rasprostranjen Gram negativan bacil i jedan od najčešćih uzročnika nozokomijalnih infekcija. Cilj ovog istraživanja bio je da se ispita osetljivost bolničkih i vanbolničkih izolata Pseudomonas aeruginosa na antipseudomonasne antibiotike i sagleda prisustvo multiple rezistencije. Istraživanje je obavljeno u laboratoriji za biokulture Instituta za javno zdravlje Niš u periodu od novembra 2004. do oktobra 2006. godine. Osetljivost na antibakterijske lekove ispitivana je kod 994 izolata Pseudomonas aeruginosa poreklom iz materijala bolesnika hospitalizovanih na klinikama KC Niš (408) i materijala ambulantnih bolesnika (586). Ispitivanje osetljivosti Pseudomonas aeruginosa na antibiotike izvršeno je primenom disk difuzione metode na Muller-Hinton agaru po standardu CLSI. Osetljivost je ispitivana na: piperacilin/tazobaktam, meropenem, imipenem, ceftriakson, ceftazidim, netilmicin, gentamicin, amikacin, ciprofloksacin, ofloksacin. Rezistencija izolata Pseudomonas aeruginosa dobijenih iz materijala hospitalizovanih bolesnika bila je statistički značajno viša ($p<0,05$) u odnosu na rezistenciju izolata dobijenih iz materijala ambulantnih bolesnika. Najveći procenat osetljivosti bio je prema karbapenemima: kod hospitalnih izolata na meropenem 87,17% i imipenem 90,25% a kod ambulantnih izolata na meropenem 96,13% i imipenem 95,13%. Procenat osetljivosti na piperacilin/tazobaktam bio je nešto niži: 80,63% kod bolničkih, a kod ambulantnih izolata 89,83%. Najmanji procenat osetljivosti bio je na ceftriakson (bolnički 23,53%, ambulantni 38,97%). Multipla rezistencija dokazana je kod 18,14% bolničkih i 8,19% ambulantnih izolata, što predstavlja statistički značajnu razliku ($p<0,05$). Naše istraživanje pokazuje da su antibiotici iz grupe karbapenema najefikasniji antipseudomonasni lekovi, dok je rezistencija prema ceftriaksonu najviša.

Ključne reči: **Pseudomonas aeruginosa, osetljivost na antibiotike, multipla rezistencija**