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Pseudomonas aeruginosa serotypes and resistance to antibiotics from wound swabs

Serotipovi i rezistencija na antibiotike *Pseudomonas aeruginosa* iz briseva rana

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Abstract

Introduction/Aim. *Pseudomonas aeruginosa (P. aeruginosa*) is the most common cause of wound infections, following the disruption of the skin or mucous membranes integrity. The aim of this study was to analyze of the presence *P. aeruginosa* in wound swabs, antibiotics susceptibility testing, determination of the minimum inhibitory concentrations (MICs) of antibiotics, testing of the metallo-β-lactamases (MBLs) production, isolates serotyping and analysis of the most common serotypes resistance. **Methods.** A total of 90 outpatients and 55 intpatients wound swabs were cultivated. Wound swabs were taken from the patients with wound infections symptoms. Antibiotics susceptibility testing was performed to: meropenem, imipenem, piperacillin-tazobactam, ceftazidime, cefepime, amikacin, gentamicin, netilmicin, ofloxacin, ciprofloxacin and colistin (HiMedia). Polyvalent and monovalent antisera for agglutination (Biorad) were used in *P. aeruginosa* agglutination. **Results.** *P. aeruginosa* was isolated from 36.55% wound swabs (36.66% of the inpatients wounds and 36.36% of the outpatients). The analyzed isolates showed the highest degree of sensitivity to colistin (100%) and meropenem (93.44%) and the lowest to cefepime (19.54%). The majority of the inpatients isolates had 12 μg/mL (28.57%) MIC for piperacillin-tazobactam and 16 μg/mL (28.57%) for the outpatients. The most common MICs for ciprofloxacin were 0.19 μg/mL (31.81%) for the nosocomial isolates, and 0.25 μg/mL (28.57%) for the outpatients' ones. The most common MICs for amikacin of the nosocomial isolates were 6 μg/ml (40.9%), and for the outpatients ones 4 μg/mL (33.33%). Five (9.43%) isolates produced MBLs. The most common serotypes were P11 (22.64%), P6 (15.09%) and P1 (11.32%). **Conclusion.** Neither the increased presence of *P. aeruginosa* was noticed in wounds swabs, nor the antibiotic resistance in the nosocomial isolates compared to those from outpatients. The analyzed isolates had the higest sensitivity to colistin and meropenem, and the lowest to cefepime.

Key words:
pseudomonas aeruginosa; wounds infection; serotyping; anti-bacterial agents; drug resistance.

Apstrakt

Uvod/Cilj. *Pseudomonas aeruginosa* (*P. aeruginosa*) spada među najčešće uzročnike infekcija rana nakon narušavanja integriteta kože ili sluzokože. Cilj ispitivanja bila je analiza prisustva P. aeruginosa u brisevima rana, zatim ispitivanje osetljivosti na antibiotike, određivanje minimalne inhibitorne koncentracije (MIC) antibiotika, ispitivanje produkcije metalo-β-laktamaza (MBL), serotipizacija izolata i analiza rezistencije najčešćih serotipova. **Metode.** Kultivisana su 90 ambulantno i 55 bolnički uzorkovanih briseva rana. Brisevi su uzimani kod bolesnika sa simptomima infekcije rana. Ispitivanje osetljivosti *P. aeruginosa* vršeno je na: meropenem, imipenem, piperacilin-tazobaktam, ceftazidim, cefepim, amikacin, gentamicin, netilmicin, ofloksacin, ciprofloksacin i kolistin (Himedia). U aglutinaciji *P. aeruginosa* korišćeni su polivalentni i monovalentni serumi (Biorad). **Rezultati.** *P. aeruginosa* izolovan je iz 36,55% briseva rana (36,66% rana bolničkog porekla i 36,36% ambulantnog). Izolati su pokazali najveći stepen osetljivosti na kolistin (100%) i meropenem (93,44%), a najmanji na cefepim (19,54%). Najveći broj izolata bolničkog porekla imao je MIC za piperacilin-tazobaktam 12 μg/mL (28,57%), a ambulantnog 16 μg/mL (28,57%). Najčešći MIC za ciprofloksacin kod bolničkih izolata bio je 0,19 μg/mL (31,81%), a ambulantnih 0,25 μg/mL (28,57%). Najčešći MIC za amikacin kod izolata bolničkog porekla bio je 6 μg/mL (40,9%), a ambulantnog 4 μg/mL (33,33%). Pet (9,43%) izolata proizvodilo je MBL. Najprisutniji bili su serotipovi P11 (22,64%), P6 (15,09%) i P1 (11,32%). **Zaključak.** Nije uočena češća kultivacija *P. aeruginosa* u brisevima rana, niti rezistencija na antibiotike kod izolata bolničkog porekla u odnosu na ambulantne. Analizirani izolati pokazali su najveći stepen osetljivosti na kolistin i meropenem, a najmanji na cefepim.

Ključne reči:
pseudomonas aeruginosa; rana, infekcija; serotipizacija; antibiotici; lekovi, rezistencija.

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Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common bacteria colonizing the hospital environment [1–5]. High genetic variability, flexible physiology, adaptability, metabolic potential, production of broad capsules [6], biofilm forming [7–9], control of external membrane permeability [10] and resistance to antibiotics and disinfectants allow the bacillus to be widely dispersed [11].

P. aeruginosa is a common cause of inflammation, if there is a disruption of the body's defense forces for any reason: malignant disease, chemotherapy, neutropenia, diabetes mellitus, cardiovascular diseases, alcoholism, smoking and obesity. It mainly causes infections of urinary and respiratory tract and wounds, especially burns [12–14]. It mostly causes inflammation of the skin and subcutaneous tissue if the integrity of the skin and mucous membranes is damaged. *P. aeruginosa* causes wound infections after surgical procedures, various types of injuries, burns and dermatitis [15, 16]. Primary wound colonization occurs after its contact with the external environment, and often during the surgical wound treatments in hospital environments. Infections are more common in diabetes mellitus and peripheral circulatory disorders, which cause the formation of chronic ulcers on calves [16].

There are multiple mechanisms of *P. aeruginosa* antibiotic resistance [17]. β-lactamases production is among the most important mechanisms of resistance. *P. aeruginosa* produces over 100 β-lactamases [18]. Metallo β-lactamases (MBLs) are clinically the most important β-lactamases. Among the Gram negative bacteria, *P. aeruginosa* most often produces MBLs. MBLs belong to the Amblers class B, subclass B1, group 3 by Bush and Sykes [19]. The main difference between MBLs and serine β-lactamases is that MBLs have a metal cofactor, unlike serine, they are sensitive to ethylenediaminetetraacetic acid (EDTA), but not to inhibitors of serine β-lactamases. MBLs have a broad spectrum of activities against many antibiotics, including all β-lactam antibiotics and carbapenems. The most important MBLs produced by *P. aeruginosa* are: IMP, VIM, GIM, SPM and AIM-1 [17].

Serological examination of *P. aeruginosa* serotypes is important for epidemiological analyses. Facts about serotypes facilitate the analysis of the prevalence of certain serotypes and locating sources of infection in hospitals. Sensitivity to antibiotics is easier to follow if there are data about the serotypes present in the specific area. Different serotypes are predominant in certain regions and have different clinical and epidemiological significance, primarily due to different antibiotic resistance [3].

The aim of the study was to analyze the presence of *P. aeruginosa* in wound swabs of inpatients and outpatients, antibiotic susceptibility testing, determination of minimal inhibitory concentrations (MICs) for piperacillin-tazobactam, amikacin and ciprofloxacin, production of MBLs, determination of the presence of certain serotypes and the most common serotypes resistance analysis.

Method

Isolation and identification of P. aeruginosa

During 2012, 145 wounds swabs were cultivated in the Healthcare Centre "Aleksinac" Microbiology Department, in Aleksinac, Serbia. A total of 90 outpatients swabs and 55 intpatients swabs were taken. Swabs were taken from inpatients in the Surgery, Gynecology and Obstetrics and Internal Medicine Departments. The patient's data were collected from medical records kept in the Healthcare Centre "Aleksinac" computer system. Outpatient subjects were patients who came ambulatory to the Microbiological Laboratory. Wound swabs were taken from the patients with signs of wound infection: redness, the presence of pus, pain or fever.

Wound swabs were put on blood agar plates and MacConkey plates, nutrient broth and thioglycollate broth (HiMedia). The inoculated plates were incubated aerobically for 24 hours. Nutrient broth and thioglycollate broth were recultivated on blood agar and MacConkey plates. Identification of *P. aeruginosa* was done on the basis of their microscopic, cultural and physiological-biochemical characteristics [20, 21]. Isolated strains of *P. aeruginosa* were recultured on the trypticase soy agar (Torlak).

Antibiotic susceptibility testing

P. aeruginosa sensitivity testing to antibiotics was performed using the disc diffusion method according to CLSI standards [21]. Bacillus suspension of 0.5 McFarland density was poured onto the Müller-Hinton agar and dried. Then the commercial discs were placed on the agar surfaces: piperacillin-tazobactam (100/10 μg), imipenem (10 μg), meropenem (10 μg), colistin (10 μg), ceftazidime (30 μg), cefepime (30 μg), amikacin (30 μg), gentamicin (10 μg), netilmicin (30 μg), ofloxacin (5 μg) and ciprofloxacin (5 μg) (HiMedia). The resistance to antibiotics was read after 24 hours, based on the zone of inhibition around the disk. Susceptibility was marked as sensitive, intermediate and resistant. MICs for piperacillin-tazobactam, amikacin and ciprofloxacin were determined according to antibiotics tape manufacturer's instructions (Liofilmchem).

MBL production ability was tested by imipenem and imipenem-EDTA discs. Test was marked as positive if the difference in growth inhibition zone around the discs was bigger than 6 mm [21].

Agglutination

Polyvalent and monovalent antisera (Biorad) were used in agglutination. Agglutination kit contains 4 polyvalent and 16 monovalent antisera. Polyvalent antisera are PMA, PMF, PMC and PME. The PMA group includes the following serotypes: P1, P3, P4 and P6, the PME P2, P5, P15 and P16. The PMC group includes P9, P10, P13 and P14, while PMF includes P11, P12, P7 and P8. Agglutination was described as positive if it caused a positive slide agglutination reaction. Some isolates were polyagglutinative, while some agglutinated only by polyvalent, but not monovalent antisera. Some isolates were not agglutinated with any antisera. Such isolates were described as non-typical. Isolates of *P. aeruginosa* were agglutinated from trypticase soy agar [22].

Statistical data processing

The files were created in the SPSS 12.0 package, where data analysis was done. For the results analysis we used χ^2-

test, C contingency test and coefficient of parametric and nonparametric small and large samples.

Results

The study group consisted of 145 patients, 79 (54.48%) male and 66 (45.52%) female, of which there were 55 inpatients, 30 (66.66%) male and 25 (45.45%) female. Swabs of inpatients were collected mainly from the Surgery Department, 53 (96.36%), one swab (1.81%) was collected in the Gynecology and Obstetrics Department and one in the Department of Internal Medicine. There were 90 outpatients, 49 (54.44%) male and 41 (45.56%) female.

P. aeruginosa was cultured from 53 (36.55%) wound swabs. It was present in 33 (36.66%) cultured outpatient isolates, and in 20 (36.36%) intpatient isolates (p = 0.99), of which 29 (54.72%) were males and 24 (45.28%) females (t = 0.97; p = 0,01). The inpatients included 12 (60%) males and 8 (40%) females (χ^2 = 0.67, p = 0.67; C = 0,089) and the outpatients 17 females (51.51%) and 16 (48.49%) males (χ^2 = 1,46, p = 0.226, C = 0.152).

The average age of patients from whose wound swabs *P. aeruginosa* was isolated was 67.32 ± 24.22 years, median (Me) 67 years, of males 66.65 ± 24.22 years (Me 70 years) and females 67.95 ± 24.35 years (Me 69 years). The largest number of patients was in the sixth to eighth decade of age (84.89%), (χ^2 = 91; p < 0.001; C = 0.99) (Table 1).

The respondents were most frequently (78.62%) diagnosed with ulcus cruris: 74.54% of inpatients and 81.11% of outpatients (Table 2). A total of 72.44% respondents with ulcus cruris also had diabetes mellitus. The patients with isolated *P. aeruginosa* were frequently (77.35%) diagnosed with ulcus cruris (74.54% inpatients and 26.66% outpatients) and burns (16.98%). *P. aeruginosa* was isolated from 74.6% patients with ulcus cruris and diabetes mellitus.

Antibiotic resistance of *P. aeruginosa* inpatients and outpatients isolates is shown in Table 3.

The analyzed isolates of the whole group, as well as of the

Table 1

Demographic characteristics of the patients from whose wound swabs *P. aeruginosa* was cultivated

Sex	Patients' age (years), n (%)						
	31–40	41–50	51–60	61–70	71–80	81–90	Total
Male	1 (1.88)	1 (1.88)	3 (5.66)	3 (5.66)	18 (5.66)	3 (5.66)	29 (54.7)
Female	- -	2 (3.77)	5 (9.43)	10 (18.86)	6 (11.32)	1 (1.88)	24 (45.3)
Total	1 (1.88)	3 (5.66)	8 (15.09)	13 (24.52)	24 (45.28)	4 (7.54)	53 (100)

Table 2

Clinical diagnosis of the patients from whose wound swabs *P.aeruginosa* was cultivated

Diagnosis	Inpatients, n (%)		Outpatients, n (%)	
	Total	Positive	Total	Positive
Burns	3 (5.45)	2 (10)	7 (7.77)	7 (21.21)
Ulcus cruris	41 (74.54)	17 (85)	73 (81.11)	24 (72.72)
Postoperative wound	4 (7.27)	1 (5)	-	-
Posttraumatic wound	4 (7.27)	0 -	8 (8.88)	2 (6.26)
Other	3(5.45)	0 -	2 (2.22)	-
Total	55 (100)	20 (100)	90 (100)	33 (100)

Table 3

The inpatients and outpatients *P. aeruginosa* isolates' resistance to antibiotics

Antibiotics	Inpatients, n (%)			Outpatients, n (%)		
	S	I	R	S	I	R
TAZ	28 (84.9)	1 (3.03)	4 (12.1)	14 (70)	-	6 (30)
I	30 (90.09)	1 (3.03)	2 (6.06)	16 (80)	1 (5)	3 (15)
M	31 (93.93)	-	2 (6.06)	19 (95)	-	1 (5)
CAZ	25 (75.75)	-	8 (24.24)	14 (70)	-	6 (30)
CP	3 (9.09)	1 (3.03)	29 (87.87)	6 (30)	2 (10)	12 (60)
G	19 (57.57)	1 (3.03)	13 (39.39)	9 (40)	1 (5)	10 (50)
A	22 (66.66)	2 (6.06)	9 (27.279	12 (60)	1 (5)	7 (35)
NM	16 (48.48)	3 (9.09)	14 (42.42)	13 (65)	-	7 (35)
OF	20 (60.6)	-	13 (39.39)	7 (35)	-	13 (65)
CC	22 (66.66)	-	11(33.33)	6 (30)	-	4 (70)
C	33 (100)	-	-	20 (100)	-	-

S – sensitive; I – intermediate sensitive; R – resistant; N – number; TAZ – pipercillin-tazobactam; I – imipenem; M – meropenem; CAZ – cefatzidime; CP – cefepime; G – gentamicin; A – amikacin; NM – netilmicin; OF – ofloxacin; CC – ciprofloxacin; C – colistin.

inpatient and outpatient origin respectively, showed the highest degree of sensitivity to colistin (100%), meropenem (93.44%), imipenem (86.7%) and piperacillin-tazobactam (79.24%). There is a slightly lower degree of sensitivity to ciprofloxacin, ofloxsacin, netilmicin and gentamicin. The largest manifested resistance was to cefepime. Nine (16.98%) (5 inpatient and 4 outpatients) isolates were sensitive or intermediately sensitive to all antibiotics, while 3 isolates (5.66%) were resistant to all antibiotics, except to colistin.

The values of MICs for piperacillin-tazobactam are shown in Table 4.

Table 5 shows the MIC values for ciprofloxacin, while MIC values of amikacin are shown in Table 6.

Two inpatients and three outpatients' isolates produced MBLs (9.43%) (t 32.28, *p* 0.05). However, the percentage of outpatients wounds isolates (10%) was higher than the inpatients wounds (9.09%). One nosocomial isolate was atypical and was sensitive to piperacillin-tazobactam and ceftazidime. Another isolate agglutinated only with PME polyvalent antisera and was sensitive to piperacillin-tazobactam, ceftazidime, ciprofloxacin, amikacin, gentamicin, and colistin. Two outpatients isolates were P11, and one was atypical. All the three isolates were resistant to all the tested antibiotics, except to colistin.

Serologically identified *P. aeruginosa* isolates belonged to all serogroups: PMA, PME, PMC and PMF ($\chi^2 = 17.09$; $p < 0.001$; C0.92). The largest number of isolates belonged to PMA (33.94%) and PMF (24.52%) serogroups. The largest number of nosocomial isolates belonged to the PMA group (20.75%), while outpatients ones to the PMF (15.09%) group.

The following serotypes of *P. aeruginosa* were identified: P1, P3, P4, P6, P10 and P11. The most frequent serotypes were P11 (22.64%), P6 (15.09%) and P1 (11.32%) (Table 7), ($\chi^2 = 39.65$; $p < 0.001$; C = 0.98). Fifteen (28.3%) isolates were atypical. Isolates that showed a positive agglutination reaction only with a polyvalent serum reacted mostly with the PME group (16%).

Resistence to antibiotics of the most common serotypes P11, P1, P6 and of atypical isolates (non-typable – NT) is shown on Figure 1.

Table 4

Distribution of piperacillin-tazobactam minimum inhibitory concentration (MIC) values of inpatients and outpatients *P. aeruginosa* isolates

MIC (μmol/mL)	Inpatients isolates	Outpatients isolates
	n (%)	
64	-	1 (7.14)
32	1 (3.57)	1 (7.14)
24	2 (7.14)	1 (7.14)
16	2 (7.14)	4 (28.57)
12	8 (28.57)	2 (14.28)
10	-	1 (7.14)
8	3 (10.71)	1 (7.14)
4	7 (25)	1 (7.14)
3	3 (10.71)	1 (7.14)
2	2 (7.14)	1 (7.14)

Table 5

Distribution of ciprofloxacin minimum inhibitory concentration (MIC) values of inpatients and outpatients *P. aeruginosa* isolates

MIC (μmol/mL)	Inpatients isolates	Outpatients isolates
	n (%)	
0.75	-	-
0.50	-	1 (12.5)
0.38	2 (9.09)	-
0.25	5 (22.72)	3 (28.57)
0.125	6 (27.27)	1 (12.5)
0.19	7 (31.81)	1 (12.5)
0.094	1 (4.54)	-
0.032	-	-
0.018	-	-
0.016	1 (4.54)	-

Table 6

Distribution of amikacin minimum inhibitory concentration (MIC) values of inpatient and outpatient *P. aeruginosa* isolates

MIC (μmol/mL)	Inpatients isolates	Outpatients isolates
	n (%)	
16	2 (9.09)	1 (8.33)
12	1 (4.54)	- -
8	5 (22.72)	3 (25)
6	9 (40.9)	1 (8.339
4	4 (18.189	4 (33.33)
3	1 (4.54)	3 (25)

Table 7

***P. aeruginosa* serotypes present in the wound swabs**

Serotype	Inpatients isolates	Outpatients isolates	Total
	n (%)		
P11	6 (11.32)	6 (11.32)	12 (22.64)
P6	4 (7.54)	4 (7.54)	8 (15.09)
P1	3 (5.66)	3 (5.66)	6 (11.32)
P10	2 (3.77)	-	2 (3.77)
P4	-	2 (3.77)	2 (5.66)
P3	1 (1.88)	-	1 (1.88)
NT	11 (20.75)	4 (7.54)	15 (28.2)
PMA	1 (1.88)	-	1 (1.88)
PMF	1 (1.88)	-	1(1.88)
PME	4 (7.54)	1 (1.88)	5 (9.43)
Total	33 (62.26)	20 (37.73)	53 (100)

N – number; NT – non-typable (atypical) isolates.

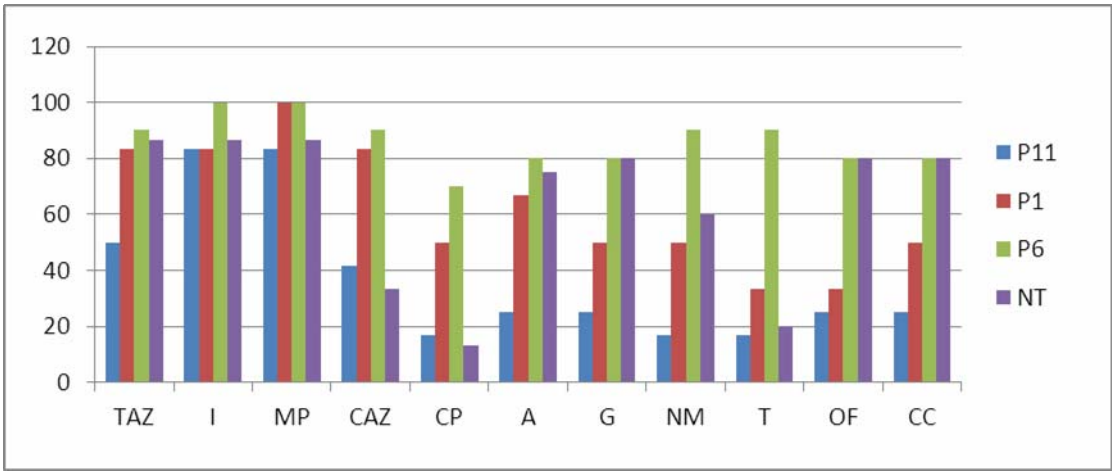

Fig. 1 – Resistance to antibiotics of the most common serotypes of *P. aeruginosa*
TAZ – pipercillin-tazobactam; I – imipenem; M – meropenem; CAZ – ceftazidime; CP – cefepime; G – gentamicin; A – amikacin; NM – netilmicin; OF – ofloxacin; CC – ciprofloxacin; C – colistin; NT – non-typable (atypical) isolates.

Discussion

P. aeruginosa is one of the most common pathogens responsible for wound infections all over the world [22–25]. According to the American Center for Monitoring Nosocomial Infections, multidrug-resistant *Pseudomonas* was given a threat level of serious threat in the Centers for Disease Control (CDC) Antibiotic Resistance (AR) Threat Report [26].

In Serbia, according to data of the Third National Study of the Nosocomial Infections Prevalence of October 2011, 13.3% of nosocomial infections are caused by *P. aeruginosa* [27]. In our study, *P. aeruginosa* with almost similar frequency was present in the inpatients and outpatients wound swabs. There was no significant difference in the isolation of bacilli in men and women. *P. aeruginosa* is the most frequently isolated in elderly patients, men in the eighth and women in the seventh decade; from wound swabs of patients with ulcus cruris and diabetes mellitus. This confirms data in the literature that it is most often present in wound swabs of patients with immune system disorder or skin defects. *P. aeruginosa* is the most common cause of burn infections. Among our respondents, bacillus was present in all outpatients wound swabs and in 66% outpatients ones with burns [28–31].

In studies in the USA 2,039 hospitals reported one or more health-care associated infection (HAIs) during 2009–2010, out of which 1,749 (86%) were general acute care hospitals, and 1,143 (56%) had fewer than 200 beds. These data were compared to data reported from HAIs occurring during 2007–2008. Central line–associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia, and surgical site infections were included. There were 69,475 HAIs and 81,139 pathogens reported. Eight pathogen groups accounted for about 80% of reported pathogens: *Staphylococcus aureus* (16%), *Enterococcus* spp. (14%), *Escherichia coli* (12%), coagulase-negative staphylococci (11%), *Candida* spp. (9%), *Klebsiella pneumoniae* (and Klebsiella oxytoca; 8%), *Pseudomonas aeruginosa* (8%), and *Enterobacter* spp. (5%). The percentage of resistance was similar to that reported in the period of 2007 and 2008 yaers. Carbapenem-resistant *P. aeruginosa* was 2% [32].

In Iran, *P. aeruginosa* causes 73.9% burn infections and is their main cause [33]. By the 34. Gjødsbøl et al. [34] study in Sweden in 2006, *P. aeruginosa* causes 52.2% of *ulcus cruris* infections associated with varices, with tendency to increase.

Most of our isolates were susceptible to colistin (100%), meropenem (93.93%), imipenem (90.09%) and piperacillin-tazobactam (84.9%). Colistin has been recently re-

gistered in Serbia, and very rarly used therapeutically, which explains absolute sensitivity to this antibiotic. According the data from Iran, isolates from burns are resistant to colistin [35]. Our isolates had the highest resistance to cefepime (77.35%), despite it being the fourth-generation of cephalosporins and not often used in our hospital. Contrary to expectation, the resistance to piperacillin-tasobactam and imipenem was higher in outpatients than in inpatients isolates. This, however, does not apply to meropenem. Our test results show that carbapenems are important antibiotics that can be used in therapy of infections caused by *P. aeruginosa*.

Our isolates MIC values for piperacillin-tazobactam are similar for inpatients and outpatients isolates. Distribution of MIC values is wide, but most isolates MIC values were from 4 μg/mL to 16 μg/mL. MIC values for amikacin differ for inpatients and outpatients isolates. The largest number of hospital isolates (40.9%) had MICs 6 μg/mL, 81.8% of the isolates had the value of 4 μg/mL to 8 μg/mL. Most inpatients isolates had MIC values of 4 μg/mL, 8 μg/mL and 3 μg/mL. Our results confirm the hypothesis that amikacin is very efficient against *P. aeruginosa*, even against multi-resistant isolates. The MIC value we obtained was lower than in data recently published in Croatia. In a study that included 662 isolates, 90% of them had MIC 32 μg/mL [17]. In the Higgins et al. [36] study, which included imipenem resistant isolates, there were 70% isolates sensitive to amikacin, with MIC_{90} > 64 μg/mL. The largest number of inpatients isolates had MIC values 0.19 μg/mL, 0.125 μg/mL and 0.50 μg/mL [36]. The outpatients isolates had much higher MIC values of which none was significantly higher. Data from Croatia indicate that the MIC_{90} value of 32 μg/mL was the most often, but the analyzed isolates were imipenem resistant [17]. The resistance to ciprofloxacin of isolates from Switzerland was lower than ours [37].

In the Healthcare Centre "Aleksinac" the first *P. aeruginosa* isolates producing MBL were detected in 2011. The recent data indicate that the frequency of the MBL production was similar in the outpatients and inpatients isolates. In a Lepšanović et al. [38] study an isolate producing VIM-1 MBL was detected. MBL production was higher in the study at the Institute for Health Protection of Mother and Child "Dr.Vukan Čupić" Serbia in Belgrade (36.5%) [39]. Testing in Croatia in 2009 identified 3.6% *P. aeruginosa* strains producing MBL [40]. VIM-1 and VIM-2 MBLs are present in the most European countries [41–43]. A total of 1.3% of strains in Japan [17] and 30% in Canada [44] produce MBL.

The following serotypes *P.aeruginosa* were serologicaly identified in our studies: P1, P3, P4, P6, P10 and P11. The most frequent serotypes were P11 (22.64%), P6 (15,09%) and P1 (11.32%), while others were present in the smaller percentages. Totally 28.3% of strains were atypical (NT). Serotypes P11 (22.64%), P6 (11.32%) and P1 (11.32%) were most common in inpatients swabs. In outpatients samples P11 (15.09%), P6 (7.54%) and P1 (5.66%) serotypes were the most frequent ones. Similar results were obtained in the Tomanovic's et al. [45] study. P1 (21%), P6 (18%) and P12 (16%) were the most common serotypes. Testing in Slovenia included 208 clinical *P. aeruginosa* isolates on which serotyping and susceptibility testing was performed. The most often serotypes were P11 (36%) and P6 (14.4%), 25.6% of the isolates belonged to other serotypes, and 20.2% were poly-agglutinative [46]. The results from Croatia in 2009, after analysis of isolates mainly from respiratory tract samples, indicate that most common serotypes were P12 (58.6%) and P11 (17.1%), while other serotypes were less frequent, while 10.65% of the isolates were atypical [17].

The most common serotype P11 was the most resistant to almost all tested antibiotics, except to colistin. Atypical isolates were next according to their sensibility to antibiotics, and then P1 and P6. The highest resistance to antibiotics allows P11 serotype to be widely spread.

Conclusion

The data we obtained in the study on the *P. aeruginosa* presence in inpatients and outpatients wounds, antibiotic resistance, minimum inhibitory concentration value and metallo-β-lactamases production are different from what we expected. *P. aeruginosa* is present with similar frequency in inpatients and outpatients isolates, their resistance to antibiotics is similar, as well as minimum inhibitory concentration values and metallo-β-lactamases production. Contrary to expectations, isolates from outpatient's swabs produced more MBL than inpatients isolates. The spatial correlation of the surgical department and the surgical clinic explains it. The lack of health personnel and inadequate organization contributes to the spread of resistant strains.

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R E F E R E N C E S

1. *Severino P, Magalhães VD*. The role of integrons in the dissemination of antibiotic resistance among clinical isolates of Pseudomonas aeruginosa from an intensive care unit in Brazil. Res Microbiol 2002; 153(4): 221−6.
2. *Loughlin MF, Jones MV, Lambert PA*. Pseudomonas aeruginosa cells adapted to benzalkonium chloride show resistance to other membrane-active agents but not to clinically relevant antibiotics. J Antimicrob Chemother 2002; 49(4): 631−9.
3. *Li L, Ledizet M, Kar K, Koski RA, Kazmierczak BI*. An indirect enzyme-linked immunosorbent assay for rapid and quantitative assessment of Type III virulence phenotypes of Pseudomonas aeruginosa isolates. Ann Clin Microbiol Antimicrob 2005; 4: 22.
4. *Worgall S, Krause A, Qiu J, Joh J, Hackett NR, Crystal RG*. Protective immunity to pseudomonas aeruginosa induced with a capsid-modified adenovirus expressing P. aeruginosa OprF. J Virol 2007; 81(24): 13801−8.

5. *Perumal D, Lim CS, Chow VT, Sakharkar KR, Sakharkar MK.* A combined computational-experimental analyses of selected metabolic enzymes in Pseudomonas species. Int J Biol Sci 2008; 4(5): 309−17.
6. *Ramsey DM, Wozniak DJ.* Understanding the control of Pseudomonas aeruginosa alginate synthesis and the prospects for management of chronic infection in cystic fibrosa. Mol Microbiol 2005;56(2): 309−22.
7. *Stoodley P, Sauer K, Davies DG, Costerton JW.* Biofilms as complex differentiated communities. Annu Rev Microbiol 2002; 56: 187−209.
8. *Sugawara E, Nestorovich EM, Bezrukov SM, Nikaido H.* Pseudomonas aeruginosa porin OprF exists in two different conformations. J Biol Chem 2006; 281(24): 16220−9.
9. *O'May CY, Sanderson K, Roddam LF, Kirov SM, Reid DW.* Iron-binding compounds impair Pseudomonas aeruginosa biofilm formation, especially under anaerobic conditions. J Med Microbiol 2009; 58(Pt 6): 765−73.
10. *Hancock RE.* The Pseudomonas aeruginosa outer membrane permeability barrier and how to overcome it. Antibiot Chemother (1971) 1985; 36: 95−102.
11. *Ninane G, Harper PB.* The in vitro activity of ceftazidime against a multi-resistant serotype 12 Pseudomonas aeruginosa. Infection 1983; 11 Suppl 1: S16−9.
12. *Jamasbi J.* Frequency and Distribution of Pseudomonas. J Bacteriol 2002; 184(13): 3614−22.
13. *Al-Dujaili AH, Harris DM.* Evaluation of commercially available antisera for serotyping of Pseudomonas aeruginosa. J Clin Pathol 1974; 27(7): 569−71.
14. *van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK,* et al. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect 2007; 13 Suppl 3: 1−46.
15. *Smith S, Ganiyu O, John R, Fowora M, Akinsinde K, Odeigah P.* Antimicrobial resistance and molecular typing of pseudomonas aeruginosa isolated from surgical wounds in Lagos, Nigeria. Acta Med Iran 2012; 50(6): 433−8.
16. *Adediran SG, Dauplaise DJ, Kasten KR, Tschöp J, Dattilo J, Goetzman HS*, et al. Early infection during burn-induced inflammatory response results in increased mortality and p38-mediated neutrophil dysfunction. Am J Physiol Regul Integr Comp Physiol 2010; 299(3): R918−25.
17. *Sardelić S* Metalo-β -lactamases in carbapenem-resis tant clinical isolates of Pseudomonas aeruginosa [dissertation]. Zagreb: University of Zagreb Medical School Repository; 2010. (Croatian)
18. *Gužvinec M, Butić I, Jelić M, Bukovski S, Lučić S, Tumbić-Andrašević A.* Antimicrobial resistance in Pseudomonas aeruginosa. Infektol Glas 2012; 32(2): 71−80. (Croatian)
19. *Bush K, Sykes RB.* Characterization and epidemiology of β-lactamases. Philadelphia, PA: Elsevier Science Publishers BV; 1987.
20. *Karakašević B.* Manual of Standard Methods for microbiolo routine work. Beograd: Medicinska knjiga; 1967. (Serbian).
21. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Suspectibility tests for bacteria That Grow Aerobicaly. Wayne: Clinical and Laboratory Standards Institute; 2012.
22. *Al-Dujaili AH, Harris DM.* Evaluation of commercially available antisera for serotyping of Pseudomonas aeruginosa. J Clin Pathol 1974; 27(7): 569−71.
23. *Blanc DS, Petignat C, Janin B, Bille J, Francioli P.* Frequency and molecular diversity of Pseudomonas aeruginosa upon admission and during hospitalization: a prospective epidemiologic study. Clin Microbiol Infect 1998; 4(5): 242−7.
24. *Navon-Venezia S, Ben-Ami R, Carmeli Y.* Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr Opin Infect Dis 2005; 18(4): 306−13.
25. Q&A: What is Pseudomonas aeruginosa infection? [updated 2012 October 9]. Available from: http://www.bbc.co.uk/news/health-16645957
26. *Centers for Disease Control and Prevention.* Pseudomonas aeruginosa in Healthcare Settings. Available from: http://www.cdc.gov/hai/organisms/pseudomonas.htmlCDC
27. *Marković Denić Lj.* National study on the prevalence of hospital infections. VII National Conference "Introduction of the cultture of patients safety in the system of health protection of the Republic of Serbia" Belgrade; 2011 October 17; Belgrade: Ministry of Health, Republic of Serbia; Available from: http://www.minzdravlja.info/downloads/2011/Oktobar/Oktobar2011NacionalnaStudijaPrevalencijeBolnickihInfekcijaProfdrLjiljanaMarkovicDenic.pdf (Serbian)
28. *Persson AE.* Study of Pseudomonas aeruginosa and different wound dressing products. [thesis]. Göteborg, Sweden: Deparment of Chemical and Biological Engineering, Chalmers University of Technology; 2010.
29. *Milic DJ, Zivic SS, Bogdanovic DC, Karanovic ND, Golubovic ZV.* Risk factors related to the failure of venous leg ulcers to heal with compression treatment. J Vasc Surg 2009; 49(5): 1242−7.
30. *Ranjan KP, Ranjan N, Bansal SK, Arora DR.* Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. J Lab Physicians 2010; 2(2): 74−7. Retraction in: J Lab Physicians 2011; 3(2): 129.
31. *Sivanmaliappan TS, Sevanan M.* Antimicrobial Susceptibility Patterns of seudomonas aeruginosa from Diabetes Patients with Foot Ulcers. Int J Microbiol 2011; 2011: 605195.
32. *Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A,* et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol 2013; 34(1): 1−14.
33. *Salimi H, Yakhchali B, Owlia P, Rastegar Lari A.* Molecular epidemiology and drug susceptibility of pseudomonas aeruginosa strains isolated from burn patients. Lab Med 2010; 41(9): 540–4.
34. *Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA.* Multiple bacterial species reside in chronic wounds: a longitudinal study. Int Wound J 2006; 3(3): 225−31.
35. *Yousefi S, Nahaei MR, Farajnia S, Aghazadeh M, Iversen A, Edquist P,* et al. A multiresistant clone of Pseudomonas aeruginosa sequence type 773 spreading in a burn unit in Orumieh, Iran. APMIS 2013; 121(2): 146−52.
36. *Higgins PG, Fluit AC, Milatovic D, Verhoef J, Schmitz FJ.* Antimicrobial susceptibility of imipenem-resistant Pseudomonas aeruginosa. J Antimicrob Chemother 2002; 50(2): 299−301.
37. *Engler K, Mühlemann K, Garzoni C, Pfahler H, Geiser T, von Garnier C.* Colonisation with Pseudomonas aeruginosa and antibiotic resistance patterns in COPD patients. Swiss Med Wkly 2012; 142: w13509.
38. *Lepšanović Z, Libisch B, Tomanović B, Nonković Z, Balogh B, Fuzi M.* Characterisation of the first VIM metallolactamasae-producing Pseudmonas aeruginosa clinical isolates in Serbia. Acta Microbiol Immunol Hung 2008; 55(4): 447−54.
39. *Jovcic B, Vasiljevic Z, Djukic S, Topisirovic Š, Kojic M.* Emergence of VIM-2 metallo-β-lactamase-producing Pseudomonas aeruginosa isolates in a paediatric hospital in Serbia. J Med Microbiol 2011; 60(Pt 6): 868−9.
40. *Bosnjak Z, Bedenić B, Mazzariol A, Jarza-Davila N, Suto S, Kalenić S.* VIM-2 beta-lactamase in Pseudomonas aeruginosa isolates from Zagreb, Croatia. Scand J Infect Dis 2010; 42(3): 193−7.
41. *Gutiérrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P,* et al. Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa isolates from Spanish hospitals. Antimicrob Agents Chemother 2007; 51(12): 4329−35.
42. *Giakkoupi P, Petrikkos G, Tzouvelekis LS, Tsonas S, Legakis NJ, Vatopoulos AC.* Spread of integron-associated VIM-type metallo-

beta-lactamase genes among imipenem-nonsusceptible Pseudomonas aeruginosa strains in Greek hospitals. J Clin Microbiol 2003; 41(2): 822−5.

43. *Pournaras S, Maniati M, Petinaki E, Tzouvelekis LS, Tsakris A, Legakis NJ,* et al. Hospital outbreak of multiple clones of Pseudomonas aeruginosa carrying the unrelated metallo-beta-lactamase gene variants blaVIM-2 and blaVIM-4. J Antimicrob Chemother 2003; 51(6): 1409−14.
44. *Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL.* Molecular epidemiology of metallo-beta-lactamase-producing Pseudomonas aeruginosa in the Calgary Health Region: emergence of VIM-2-producing isolates. J Clin Microbiol 2007; 45(2): 294−8.
45. *Tomanović B, Jokovic B, Tatić M, Mirović V, Nanusević O.* Serotyping and pyocin typing of Pseudomonas aeruginosa in a study of intrahospital infections. Vojnosanit Pregl 1991; 48(1): 31−3. (Serbian)
46. *Müller-Premru M, Gubina M.* Serotype, antimicrobial susceptibility and clone distribution of Pseudomonas aeruginosa in a university hospital. Zentralbl Bakteriol 2000; 289(8): 857−67.

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