



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 inpatients 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 $\mu\text{g/mL}$ (28.57%) MIC for piperacillin-tazobactam and 16 $\mu\text{g/mL}$ (28.57%) for the outpatients. The most common MICs for ciprofloxacin were 0.19 $\mu\text{g/mL}$ (31.81%) for the nosocomial isolates, and 0.25 $\mu\text{g/mL}$ (28.57%) for the outpatients' ones. The most common MICs for amikacin of the nosocomial isolates were 6 $\mu\text{g/mL}$ (40.9%), and for the outpatients ones 4 $\mu\text{g/mL}$ (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 highest 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 serum (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 $\mu\text{g/mL}$ (28,57%), a ambulantnog 16 $\mu\text{g/mL}$ (28,57%). Najčešći MIC za ciprofloksacin kod bolničkih izolata bio je 0,19 $\mu\text{g/mL}$ (31,81%), a ambulantnih 0,25 $\mu\text{g/mL}$ (28,57%). Najčešći MIC za amikacin kod izolata bolničkog porekla bio je 6 $\mu\text{g/mL}$ (40,9%), a ambulantnog 4 $\mu\text{g/mL}$ (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.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common bacteria colonizing the hospital environment¹⁻⁵. High genetic variability, flexible physiology, adaptability, metabolic potential, production of broad capsules⁶, biofilm forming⁷⁻⁹, control of external membrane permeability¹⁰ and resistance to antibiotics and disinfectants allow the bacillus to be widely dispersed¹¹.

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 burns¹²⁻¹⁴. 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 calves¹⁶.

There are multiple mechanisms of *P. aeruginosa* antibiotic resistance¹⁷. β -lactamases production is among the most important mechanisms of resistance. *P. aeruginosa* produces over 100 β -lactamases¹⁸. 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 Ambler class B, subclass B1, group 3 by Bush and Sykes¹⁹. 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-1¹⁷.

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 resistance³.

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 wound swabs were cultivated in the Healthcare Centre "Aleksinac" Microbiology Department, in

Aleksinac, Serbia. A total of 90 outpatient swabs and 55 inpatient 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 standards²¹. 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 μ g), imipenem (10 μ g), meropenem (10 μ g), colistin (10 μ g), ceftazidime (30 μ g), cefepime (30 μ g), amikacin (30 μ g), gentamicin (10 μ g), netilmicin (30 μ g), ofloxacin (5 μ g) and ciprofloxacin (5 μ g) (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 mm²¹.

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 agar²².

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%) inpatient 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 ($\chi^2 = 0.67$, $p = 0.67$; $C = 0.089$) and the out-

patients 17 females (51.51%) and 16 (48.49%) males ($\chi^2 = 1.46$, $p = 0.226$, $C = 0.152$).

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%), ($\chi^2 = 91$; $p < 0.001$; $C = 0.99$) (Table 1).

The respondents were most frequently (78.62%) diagnosed with ulcer cruris: 74.54% of inpatients and 81.11% of outpatients (Table 2). A total of 72.44% respondents with ulcer cruris also had diabetes mellitus. The patients with isolated *P. aeruginosa* were frequently (77.35%) diagnosed with ulcer cruris (74.54% inpatients and 26.66% outpatients) and burns (16.98%). *P. aeruginosa* was isolated from 74.6% patients with ulcer 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 <i>P. aeruginosa</i> was cultivated							
Sex	Patients' age (years), n (%)						Total
	31-40	41-50	51-60	61-70	71-80	81-90	
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)	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 <i>P. aeruginosa</i> was cultivated				
Diagnosis	Inpatients, n (%)		Outpatients, n (%)	
	Total	Positive	Total	Positive
Burns	3 (5.45)	2 (10)	7 (7.77)	7 (21.21)
Ulcer 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 <i>P. aeruginosa</i> 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 (27.27)	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 – piperacillin-tazobactam; I – imipenem; M – meropenem; CAZ – ceftazidime; 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, ofloxacin, netilmicin and gentamicin. The largest manifested resistance was to ceftazidime. 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 ($\chi^2 = 17.09$; $p < 0.001$; C 0.92). 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%).

Resistance 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 ($\mu\text{mol/mL}$)	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 ($\mu\text{mol/mL}$)	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 ($\mu\text{mol/mL}$)	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 (8.33)
4	4 (18.18)	4 (33.33)
3	1 (4.54)	3 (25)

Table 7

<i>P. aeruginosa</i> 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.

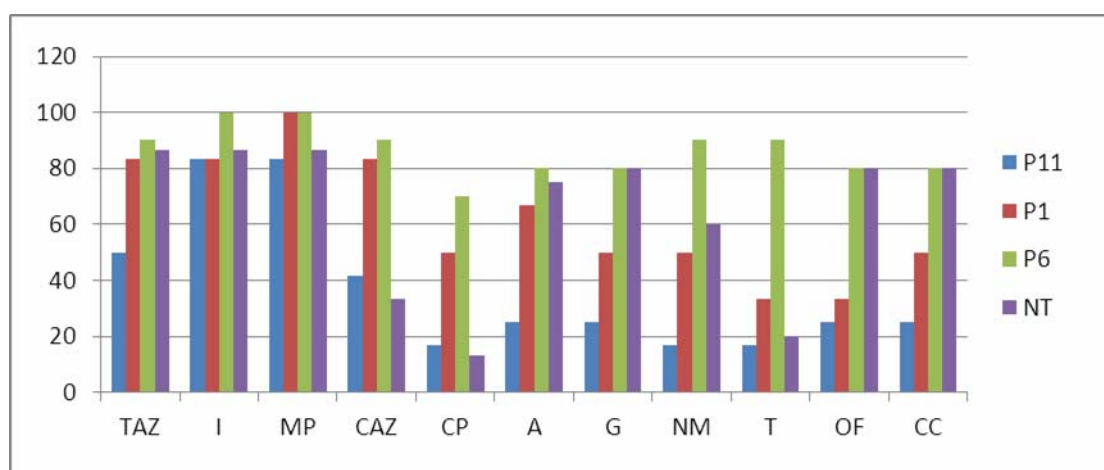


Fig. 1 – Resistance to antibiotics of the most common serotypes of *P. aeruginosa*

TAZ – piperacillin-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 Report²⁶.

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 ulcer 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 years. Carbapenem-resistant *P. aeruginosa* was 2%³².

In Iran, *P. aeruginosa* causes 73.9% burn infections and is their main cause³³. By the 34. Gjødtsbøl et al.³⁴ study in Sweden in 2006, *P. aeruginosa* causes 52.2% of *ulcer 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 rarely used therapeutically, which explains absolute sensitivity to this antibiotic. According to the data from Iran, isolates from burns are resistant to colistin³⁵. Our isolates had the highest resistance to ceftazidime (77.35%), despite it being the fourth-generation of cephalosporins and not often used in our hospital. Contrary to expectation, the resistance to piperacillin-tazobactam 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/mL¹⁷. In the Higgins et al.³⁶ study, which included imipenem resistant isolates, there were 70% isolates sensitive to amikacin, with MIC₉₀ > 64 µg/mL. The largest number of inpatients isolates had MIC values 0.19 µg/mL, 0.125 µg/mL and 0.50 µg/mL³⁶. The outpatients isolates had much higher MIC values of which none was significantly higher. Data from Croatia indicate that the MIC₉₀ value of 32 µg/mL was the most often, but the analyzed isolates were imipenem resistant¹⁷. The resistance to ciprofloxacin of isolates from Switzerland was lower than ours³⁷.

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 Lepšanić 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 MBL⁴⁰. VIM-1 and VIM-2 MBLs are present in the most European countries^{41–43}. A total of 1.3% of strains in Japan¹⁷ and 30% in Canada⁴⁴ produce MBL.

The following serotypes *P. aeruginosa* were serologically 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 Tomanović'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-agglutinative⁴⁶. 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 atypical¹⁷.

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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