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Analysis of *Proteus mirabilis* Distribution in Multi-Species Biofilms on Urinary Catheters and Determination of Bacteria Resistance to Antimicrobial Agents

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Abstract

The objectives of the investigation presented in this paper were: to examine the frequency of *P. mirabilis* isolation from catheters and assess the complexity of multi-species biofilms which these bacteria form, as well as to determine the vulnerability of planktonic and sessile *P. mirabilis* populations to popular antibiotics and compare it to the susceptibility of other Gram-negative bacteria isolated as associated flora from multi-species biofilm. 88 urological catheters, collected from long-term catheterized patients were examined. Uropathogens were recovered from the catheter surface by sonication, and identified on standard diagnostic media. The broth-microdilution method and the MBEC High-throughput Screening assay were used to determine the bacterial resistance to antibiotics. 279 microorganisms were isolated from 88 urinary catheter biofilms. The *Enterobacteriaceae* family were the most frequently detected bacteria (53.2% of isolates), whereas *Proteus* spp. isolation accounted for 17.9%, which placed these bacilli on the third position in the *Enterobacteraceae* family. Among all the tested drugs, amikacin and cephalosporins (ceftriaxone, cefotaxime and cefaclor) exhibited the highest activity against *P. mirabilis* planktonic cells, 86% and 73% of strains were susceptible to these antibiotics, respectively. 100% of *P. mirabilis* sessile forms were resistant to cefepime, ciprofloxacin, gatifloxacin, and norfloxacin. Amikacin and ceftriaxone affected only 5% of sessile forms. The planktonic cells of the other studied uropathogens were mostly vulnerable to the all tested drugs (exception *P. aeruginosa* strains), the most effective of which occurred to be amikacin and cefepime. Obtained MBECs values were 2-512-fold higher than MICs assessed for planktonic forms.

K e y w o r d s : *Proteus mirabilis*, antibiotic resistance, associated flora, biofilm, urinary catheter

Introduction

Complicated urinary tract infections (UTIs) are associated with structural or functional abnormalities in the urinary tract such as a catheterization, calculi, immunosupression and others (Jacobsen, 2008). Probability of developing the infection is similar in women and men and in all age groups (Pallet and Hand, 2010). Devices, *e.g.* indwelling catheters, which are inserted in the urinary tract facilitate microbial colonization. Bacteria attach to outer and inner surfaces of a catheter and form biofilm, which is responsible for 65–85% cather associated UTIs. After a few days of catheterization multi-species biofilm, consisting of several organisms, is often isolated (Muzzi-Bjornson and Macera, 2011). Species like: *Enterococcus faecalis, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* are most frequently detected on the catheter surface (Niveditha *et al.*, 2012). The structure of multi-species biofilm is different from biofilm formed by one species of bacteria. The interactions between microorganisms which form this structure change biofilm physiology and function, facilitating the horizontal gene transfer. Consequently, in multi – species biofilms resistance to antimicrobial agents and host defense is observed more frequently (Yang *et al.*, 2011). Biofilm age, its density and the extent of the surface which it covers are the main factors affecting its susceptibility to antimicrobials. It is possible that microorganisms in biofilm use a number of mechanisms responsible for their resistance. The first one is slow or incomplete penetration of antimicrobial agents into biofilm. A significant role in this process is played by a sticky matrix which covers bacterial cells and prevents the diffusion of drugs to the target by binding them or influencing the rate of

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molecules transport to the biofilm interior. An explanation for high biofilm resistance could be the reduction of the metabolic activity of sessile bacterial cells. A significant role in biofilm resistance to antibiotics is played by persisters: a spore-like cells, dormant and incapable of growth (Hoiby *et al.*, 2010, Lazar and Chifiriuc, 2010). The main mechanism is still undetermined, recognition of this process will help to develop new methods of preventing biofilm formation and matriculation processes. Numerous mechanisms of biofilm resistance cause that concentrations of antibacterial agents needed for biofilm eradication are 1000–1500 times higher than concentrations needed to eradicate planktonic bacteria of the same species (Chen and Wen, 2011). Urea-splitting bacteria: *Proteus* spp., *Providencia stuartii* and *Morganella morganii* are often detected on indwelling catheter surfaces. The presence of these microorganisms leads to many complications such as formation of biofilm encrusted with calcium and magnesium phosphates crystals, which results in catheter blockage (Pallett and Hand, 2010). High ability of urease synthesis is the main reason why *Proteus* infections cause more cytological damage in renal tissue, than those developed by *E. coli* (Wilde *et al.*, 2013). This dangerous pathogen, despite the fact that it is often isolated from urological catheters, still remains inadequately characterized. Therefore, the first objective of the investigations presented in this paper was to examine the frequency of *P. mirabilis* isolation from catheters and assess the complexity of multi-species biofilm formed by these bacteria. The next aim was to determine the susceptibility of *P. mirabilis* (planktonic and biofilm populations) to antibiotics. Finally, the vulnerability of sessile and planktonic cells of other Gram-negative bacteria (*K. pneumoniae, E. coli, Enterobacter cloacae, P. stuartii, M. morganii, P. aeruginosa*) was compared to that of *P. mirabilis*.

Experimental

Materials and Methods

88 urological catheters, obtained from long-term catheterized patients – mostly men aged 41–91 years and three women, were examined in this study. Patients had been catheterized for the periods of a few months to a few years, however, each catheter was replaced every two weeks. Most frequent indication for catheterization in older patients was benign prostatic hypertrophy, in younger ones: stroke, multiple sclerosis or spine damage. Patients were treated in two outpatient clinics in Łódź. In the case of urinary tract infection (30% of patients), the patients were subjected to treatment with antibiotics: ciprofloxacin, norfloxacin,

nitrofurantoin, sometimes antifungal therapy (nystatin, fluconazole) was implemented. Information about patients from whom studied strains were collected is presented in Table I.

Strains used in this research were stored at –80°C in Tryptone Soya Broth (TSB) and in dimethyl sulfoxide (DMSO), and cultivated on TSA plates for 24 h at 37°C. *E. coli* ATCC 25922 was used as the reference strain.

Bacteria from the catheter surface were recovered by sonication of the catheter fragments. Obtained sus-

Table I
Characteristics of patients from whom bacterial strains were isolated.

Bacteria	Strains no.	Gender/patient age	Disease
Proteus mirabilis	5	M/72	BPH
	6	M/92	BPH
	7	M/77	BPH
	8	M/83	diabetes
	9	M/77	BPH
	11	M/89	BPH
	12	M/73	BPH
	15	M/82	BPH
	20	M/86	stroke
	24	M/41	multiple sclerosis
	31	M/57	spine damage
	32	M/80	stroke
	33	M/88	BPH, stroke
	34	W/73	stroke, diabetes
	41	M/71	BPH
	44	M/81	stroke
	46	M/81	BPH, stroke
	57	M/71	renal failure
	70	M/80	BPH
	71	M/81	stroke
	77	M/67	BPH
	84	M/71	urethral structure
Morganella morganii	42	M/72	BPH
	67	M/79	BPH, stroke
Enterobacter cloacae	31	M/57	spine damage
	36	W/72	stroke
Providencia stuartii	9	M/73	BPH
	71	M/81	stroke
Klebsiella pneumoniae	46	M/81	BPH, stroke
	56	M/80	retention of urine
Pseudomonas aeruginosa	11	M/89	BPH
	46	M/81	BPH, stroke
Escherichia coli	9	M/73	BPH
	71	M/81	stroke

BPH – benign prostatic hypertrophy; M – man; W – woman

pensions were plated on blood and McConkey agars. Next, the identification of morphologically different colonies was performed. The microscopic slide and culturing the bacteria in different identification media were carried out using traditional diagnostic media and API 20 E and NE tests. Antimicrobial sensitivity of *P. mirabilis* planktonic cells was examined by the standard broth-microdilution method by Clinical and Laboratory Standards Institute (CLSI). The MICs (minimum inhibitory concentrations) and MBCs (minimum bactericidal concentrations) were determined in Mueller Hinton broth, by reading the turbidity in wells. Antimicrobials: amoxicillin and clavulanic acid AMC (Polfa), cefazolin CFZ, cephalexin LEX, cefuroxime CXM, cefotaxime CTX, cefaclor CEC, ceftriaxone CRO, gentamicin GM, amikacin AMK (Sigma), ciprofloxacin CIP (Fluca), ofloxacin OFX, norfloxacin NOR (Sigma) and cotrimoxazole SXT (Polfa) were used in the test.

Biofilms susceptibility to antimicrobial agents was studied using the MBEC (minimum biofilm eradication concentration) High-throughput Screening assay (Calgary Alberta, Canada). Antibiotics with the highest activity toward *P. mirabilis* planktonic cells (in the broth-microdilution method) were chosen: amikacin, cephalexin, ceftriaxone; and ciprofloxacin – fluoroquinolone often used in Poland to treat urinary tract infections and the drugs which are not used in the Polish medical practice: cefepime FEP and gatifloxacin GAT (Bristol Squibb Myers).

The test was performed according to the previously described method by Ceri *et al.* (1999), which consists of three stages: 1. Biofilm formation; 2. MIC values determination, 3. MBEC values determination.

Results

279 microorganisms were isolated from 88 urinary catheter biofilms and classified into 28 species. Over 90% of the isolates were classified into three groups: *Enterobacteriaceae* family – the most frequently isolated bacteria – 53.2%, *Pseudomonadaceae* family – 21.7% and *Streptococcus* group D – 21.3% (Fig. 1A). Distribution of different genera from the *Enterobacteriaceae* family is summarized in Fig. 1B. *E. coli* was the most often isolated species, whereas *Proteus* spp., (17.9% of isolates) was ranked in the third position. Among *Proteus* isolates, *P. mirabilis* species was detected most frequently (88% – 22 strains), mostly from multi-species biofilms (21 cases), composed of 2–7 microorganisms (Table II). Most common *P. mirabilis* associated flora involved: *Streptococcus* group D – in 63% of biofilms and *Pseudomonas* spp. – in 59% of biofilms. *P. stuartii* was often isolated together with *P. mirabilis* strains – almost 50% of *P. stuartii* isolates were acquired from biofilms mixed with *Proteus*.

Thirteen antibiotics were tested against 22 *P. mirabilis* strains. The bacteria were considered sensitive or resistant on the basis of CLSI breakpoints. The reference strain – *E. coli* ATCC 25922 was sensitive to all the tested drugs. The resistance of *P. mirabilis* strains to antibiotics is summarized in Fig. 2.

Amikacin had the highest inhibitory and bactericidal activity against *P. mirabilis* strains. 19 out of 22 strains (86%) were susceptible to this drug. High effectiveness of cephalosporins was also detected – 73% (16 strains) of *P. mirabilis* were vulnerable to ceftriaxone, cefotaxime and cefaclor. Fluoroquinolones are
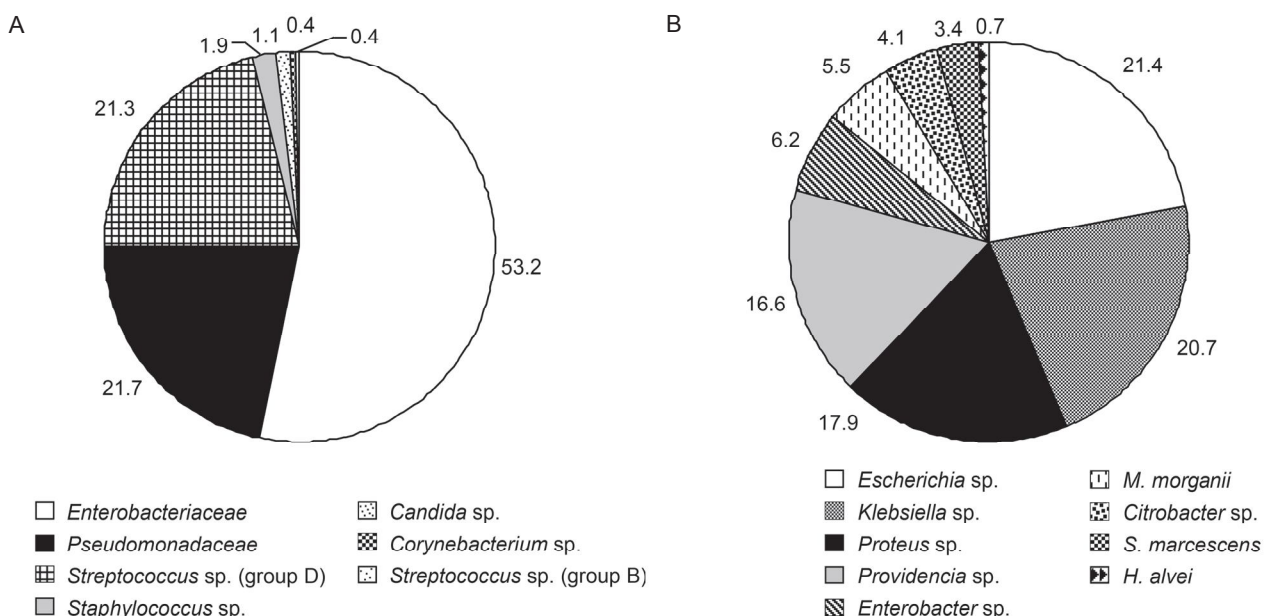

Fig. 1. Percentage of microorganisms isolated from urinary catheter biofilms (A – main groups of microorganisms, B – *Enterobacteriaceae* family).

Table II
Composition of multi-species biofilms, from which *Proteus mirabilis* strains were isolated.

No.	Catheter no.	*Proteus mirabilis* associated flora
1	5	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca*
2	6	*Pseudomonas aeruginosa, Enterococcus* spp.
3	7	*Providencia stuartii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus* spp.
4	8	*Citrobacter diversus, Escherichia coli, Morganella morganii, Enterococcus* spp.
5	9	*Escherichia coli, Pseudomonas aeruginosa*
6	11	*Escherichia coli, Pseudomonas aeruginosa, Providencia stuartii, Enterococcus faecalis*
7	12	*Providencia stuartii, Pseudomonas aeruginosa, Enterococcus* spp.
8	15	*Providencia stuartii, Streptococcus* (group D)
9	20	*Klebsiella pneumoniae, Pseudomonas* spp. *Enterococcus* spp.
10	24	*Providencia stuartii, Pseudomonas aeruginosa*
11	31	*Providencia stuartii, Enterococcus* spp. *Enterobacter cloacae*
12	32	*Streptococcus* (group B)
13	33	*Klebsiella oxytoca, Morganella morganii, Providencia stuartii, Pseudomonas aeruginosa*
14	34	*Pseudomonas aeruginosa*
15	41	*Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Streptococcus* (group D)
16	44	*Escherichia coli, Providencia stuartii, Enterococcus* spp.
17	46	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus* spp.
18	57	–
19	70	*Serratia marcescens, Enterococcus* spp., non-fermentative OX- rods
20	71	*Providencia stuartii, Klebsiella pneumoniae, Escherichia coli, Pseudomonas* sp., *Enterococcus* spp. non-fermentative OX- rods
21	77	*Escherichia coli*, non-fermentative OX+ rods
22	84	*Escherichia coli, Klebsiella pneumoniae, Enterococcus* spp.

very common antibiotics, which are used to treat UTIs, and ciprofloxacin is administered most frequently. In our study only 8 *P. mirabilis* strains were sensitive to ciprofloxacin and 5 strains were intermediate. Within the fluoroquinolones group – norfloxacin had the best efficiency (70% strains were vulnerable). Cotrimoxazole is usually recommended to treat bacteriuria, but most of the investigated strains were resistant (86%) to this drug.
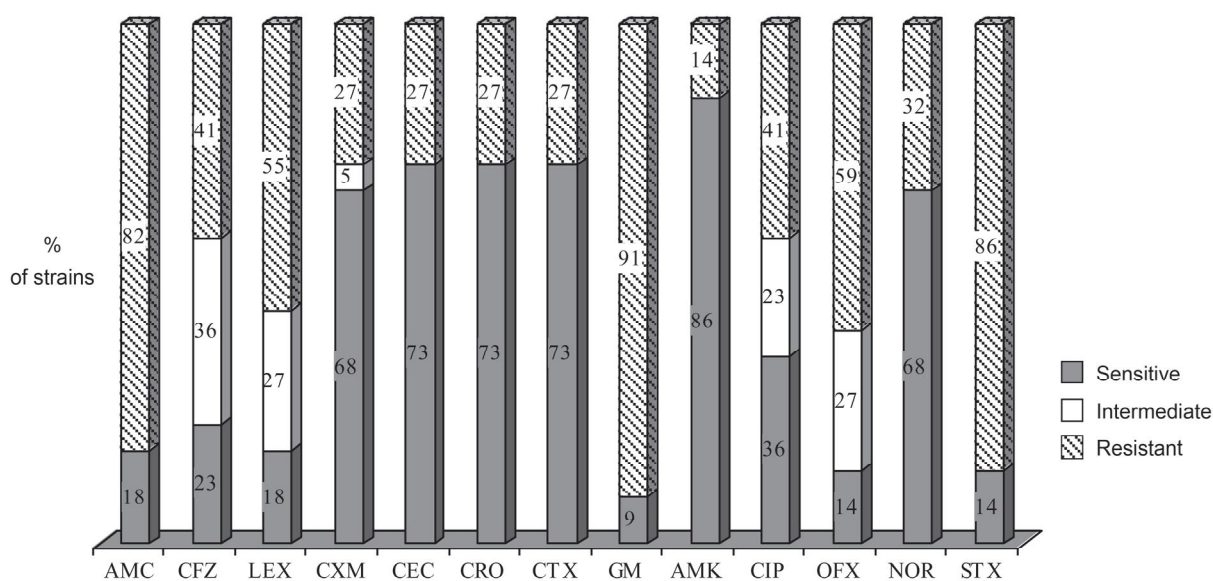

Fig. 2. *Proteus mirabilis* planktonic strains sensitivity to antibiotics: amoxicillin and clavulanic acid (AMC) cefazolin (CFZ), cephalexin (LEX), cefuroxime (CXM), cefotaxime (CTX), cefaclor (CEC), ceftriaxone (CRO), gentamicin (GM), amikacin (AMK), ciprofloxacin (CIP), ofloxacin (OFX), norfloxacin (NOR) and cotrimoxazole (SXT).

Table III
Resistance of *P. mirabilis* planktonic and sessile populations to antibiotics.

P. mirabilis strains	Inoculum ×10⁷ CFU/ml	Biofilm ×10⁶ CFU/ml	Resistance of planktonic population (MIC)						Biofilm resistance (MBEC)					
			NOR	CIP	AMK	CRO	GAT	FEP	NOR	CIP	AMK	CRO	GAT	FEP
5	3.7	1.77	32	8	8	<1	8	<1	>1024	>1024	1024	32	1024	512
6	7.5	0.62	2	<1	4	<1	16	<1	1024	>1024	256	64	1024	512
7	5.3	0.23	4	<1	4	<1	2	<1	1024	1024	1024	32	64	512
8	4.8	2.68	32	8	16	<1	8	<1	>1024	>1024	1024	32	1024	512
9	11.7	0.59	8	8	16	<1	4	<1	1024	1024	1024	32	256	1024
11	4.5	3.05	16	8	8	<1	4	2	1024	1024	128	64	1024	1024
12	6.5	3.30	64	8	8	<1	16	<1	1024	1024	64	32	>1024	1024
15	6.1	3.33	64	8	16	<1	4	<1	1024	1024	32	32	1024	512
20	8.45	1.00	<1	<1	32	<1	<1	8	512	512	1024	32	256	512
24	6.45	2.83	16	8	8	<1	8	<1	1024	1024	64	32	1024	512
31	14.0	2.01	16	8	16	<1	16	2	1024	1024	64	8	1024	256
32	6.1	1.57	8	4	8	>1024	16	1024	1024	1024	16	>1024	1024	>1024
33	10.3	3.91	128	128	16	8	64	16	>1024	>1024	1024	32	>1024	512
34	6.8	1.79	16	4	1024	>1024	8	512	>1024	1024	>1024	>1024	1024	>1024
41	8.2	1.20	512	512	16	>1024	16	128	>1024	1024	1024	>1024	64	>1024
44	7.9	6.40	64	64	64	8	32	64	>1024	1024	1024	32	512	512
46	6.95	9.35	64	32	16	>1024	>1024	>1024	>1024	>1024	512	>1024	>1024	>1024
57	2.9	0.97	32	8	16	<1	16	512	>1024	1024	64	1024	>1024	>1024
70	6.0	9.91	128	128	1024	>1024	16	256	1024	1024	>1024	>1024	1024	>1024
71	3.6	9.65	128	128	64	<1	256	64	>1024	>1024	1024	32	1024	1024
77	9.7	3.63	16	4	8	<1	8	<1	1024	>1024	64	16	1204	32
84	3.0	0.04	64	16	512	1024	8	256	128	128	1024	>1024	8	512

norfloxacin (NOR); ciprofloxacin (CIP); amikacin (AMK); ceftriaxone (CRO); gatifloxacin (GAT); cefepime (FEP)

Among the tested *P. mirabilis* strains, three multi-drug resistant strains were found (no. 34, 70 and 84), against which none of the tested antibiotics was active.

The MBEC assay was used to examine the ability of the tested bacteria to form biofilm on polystyrene surface (Table III and IV). All studied uropathogens formed biofilm on polystyrene pegs – *P. mirabilis* with density from 0.04×10^6 to 9.91×10^6 cells per peg (the most intensive growth was observed for strains no. 70, 71, 46) and in the case of the other uropathogens the average density was, for example, 6.78×10^6 CFU/peg for *P. stuartii* and 0.47×10^6 CFU/peg for *E. cloacae*.

Six antibiotics were used to study biofilm sensitivity (norfloxacin, ciprofloxacin, amikacin, ceftriaxone, gatifloxacin, cefepime). The distribution of MICs and MBECs is presented in Table III. Significant differences between MICs and MBECs values of most antibiotics were detected. The MBECs were often more than 1000–fold higher than the MICs. The most effective drugs in planktonic cell eradication occurred to be amikacin and ceftriaxone (16 of 22 strains were susceptible). 12 strains were vulnerable to cefepime. Some activity of norfloxacin, ciprofloxacin and gatifloxacin

was also observed. Similarly to the results obtained in the microdilution method, three strains were found to be resistant to all the tested antibiotics (*P. mirabilis* 34, 70 and 84). It was revealed that 100% of *P. mirabilis* sessile forms were resistant to norfloxacin, gatifloxacin, cefepime and ciprofloxacin. Amikacin and ceftriaxone inhibited only two *P. mirabilis* strains (5%). The correlation between *P. mirabilis* sessile forms sensitivity to drugs and biofilm intensity was not observed.

E. coli, *P. aeruginosa*, *K. pneumoniae*, *P. stuartii*, *E. cloacae* and *M. morganii* were isolated from urinary catheters as *P. mirabilis* associated flora. The susceptibility of these rods to the tested drugs was determined and compared to *P. mirabilis* sensitivity. MICs and MBECs values of the studied uropathogens are presented in Table IV.

The planktonic cells of the uropathogens were mostly vulnerable to the tested drugs *e.g. M. morganii* strains were sensitive to all antibiotics. *K. pneumoniae* strains were resistant only to gatifloxacin; *E. cloacae* to ceftriaxone and ciprofloxacin (strain no. 31). A different observation was made for *P. aeruginosa* strains, which in high concentrations were resistant to all the tested

Table IV
Resistance of studied uropathogens to antibiotics.

Strains	Inoculum ×10⁷ CFU/ml	Biofilm ×10⁶ CFU/ml	Resistance of planktonic population (MIC)						Biofilm resistance (MBEC)					
			NOR	CIP	AMK	CRO	GAT	FEP	NOR	CIP	AMK	CRO	GAT	FEP
K. pneumoniae 46	6.30	4.31	<1	<1	4	<1	128	<1	256	128	128	32	256	16
K. pneumoniae 56	8.80	0.50	<1	<1	<1	<1	<1	<1	512	32	<1	32	<1	4
P. aeruginosa 11	3.40	1.50	1024	1024	>1024	>1024	256	>1024	1024	1024	>1024	>1024	1024	>1024
P. aeruginosa 46	5.20	0.94	128	128	512	1024	512	>1024	512	512	1024	>1024	1024	>1024
P. stuartii 9	0.80	2.78	256	128	8	4	512	8	1024	1024	32	256	1024	>1024
P. stuartii 71	4.30	10.78	16	8	8	8	4	<1	1024	1024	1024	128	512	512
M. morganii 67	12.90	0.70	<1	<1	4	4	<1	<1	64	32	32	32	16	16
M. morganii 42	12.30	0.60	<1	<1	4	2	<1	<1	64	8	8	8	<1	32
E. cloacae 31	5.40	0.28	<1	<1	2	256	<1	16	128	32	2	128	32	256
E. cloacae 36	8.40	0.66	4	4	4	128	2	<1	64	4	4	64	2	128
E. coli 9	15.20	1.31	512	512	16	16	8	2	>1024	>1024	1024	512	16	128
E. coli 71	12.0	2.20	<1	<1	2	2	<1	128	128	16	1024	64	16	256

norfloxacin (NOR); ciprofloxacin (CIP); amikacin (AMK); ceftriaxone (CRO); gatifloxacin (GAT); cefepime (FEP)

antibiotics. The most effective drugs against the uropathogens planktonic forms were: amikacin and cefepime.

Biofilms of the studied uropathogens (similarly to those of *Proteus* strains) were more resistant to the tested antibiotics than planktonic cells (MBECs were 2–512 fold higher than the MIC values), however, the drugs concentrations required for biofilm eradication were lower. Biofilms of *M. morganii*, *E. cloacae* and *K. pneumoniae* strains were more vulnerable to some antibiotics (*e.g. M. morganii* 42 was sensitive to amikacin, ceftriaxone and gatifloxacin, *K. pneumoniae* 56 to amikacin, gatifloxacin and cefepime). *P. aeruginosa*, *P. stuartii* and *E. coli* biofilms were resistant to all the tested drugs. There is no connection between biofilm thickness on the pegs and its susceptibility to antimicrobials.

Discussion

Catheter associated UTIs is very common bacterial disease in humans, specially in long time catheterized patients. The cause of this recurrent infections is bacterial biofilm, which coats the catheter surface and constitutes a reservoir of organisms resistant to host defense and drugs. In our research biofilms from 88 catheters, obtained from long term catheterized persons were isolated. About 50% of the isolates belonged to three species of bacteria: *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *E. coli*. *Klebsiella pneumoniae* and *Proteus mirabilis* were also commonly detected, with the frequency of 20.7% and 17.9%, respectively.

MacLeod and Stickler (2007) examined 106 urinary catheters, collected from hospitalized patients, and isolated a very similar composition of bacterial species (*P. aeruginosa*, *E. faecalis*, *E. coli*, and *P. mirabilis*). *P. mirabilis* isolation frequency was higher – 30.2% and that of *K. pneumoniae* was less frequent (17.9%). When urine specimens collected from long therm catheterized or hospitalized patients were analyzed, a different composition of bacterial species was observed: *E. coli* were the most commonly isolated bacteria (70% of samples), *P. aeruginosa* – 1.7–4% and *Enterococcus* spp. – 2–5% of samples (Ghadiri *et al.*, 2012, Niveditha *et al.*, 2012). *Proteus* isolates were detected with lower frequency (6.7%), which may suggest that *Proteus* forms crystalline biofilms and bacterial cells are not easily released into urine. Our studies showed that *Proteus mirabilis* formed multi-species biofilm – 98% of all tested catheters were coated with biofilm consisting of several microorganisms species (2–7 species), only from two catheters single-species biofilm was recovered. Patients, from whom urinary catheters were isolated had been catheterized for a long period of time (from a few months to a few years), time of catheter replacement was different (usually two weeks) – these factors contributed to a number of bacterial species detected in biofilm. By comparison, in Stickler (2008) studies one species of microorganisms was isolated from about 28% of catheters. The species composition of mixed biofilms was diversified, however, *P. mirabilis* associated flora most often included *E. faecalis* and *P. aeruginosa*. We also observed frequent co-colonization of *P. stuartii* and *P. mirabilis*. These results confirm the studies described

by MacLeod and Stickler (2007), who also detected *Proteus* together with *E. faecalis* and *P. stuartii*. It is probable that these microorganisms interact in a positive way and form mixed biofilm, which facilitates their survival in the unfavorable urinary tract environment.

It is widely known that *P. mirabilis* urinary tract infections are difficult to eradicate because these bacilli produce urease, which catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Urea disintegration results in environment alcalization, ammonia contributes to changes in local environment and it is believed to cause damage to host cells (Jacobsen*et al.*, 2008, Oki *et al.*, 2010). The consequence of this process is an increase in the urine pH and the production of struvite and carbonate apatite crystals. This process in catheterized patients leads to encrustation and blockage of the catheter lumen – a common problem of long term catheterized persons (Stickler and Feneley, 2011). Wilde *et al.* (2013) observed that 24% of studied patients reported a few events of catheter blockage in two months, which increased treatment costs, due to the necessity of additional therapy. Interestingly, bladder irrigating (which is not recommended practice and was done by 42% of patients) contributed to catheter blockage (Wilde *et al.*, 2013).

The consequences of *P. mirabilis* infections are dangerous and it is necessary to devise new, efficient methods, which can help control biofilm related infections. Unfortunately, antibiotics applied in therapy are often ineffective. The National Working Group for Rational Therapy and Prevention of Infections in Poland recommended to treat UTIs in the first place with fluoroquinolones and trimethoprim – sulfamethoxazole. To treat complicated urinary tract infections fluoroquinolones, aminopenicilins with a β-lactamases inhibitor are administered. Aminopenicilins with a β-lactamases inhibitor and cephalosporins are the first drugs prescribed to pregnant women with UTIs (Kupilas, 2006). The studied *P. mirabilis* strains were resistant to cotrimoxazole, amoxicillin with clavulanic acid (more than 80% of strains). 41% of the studied *P. mirabilis* strains were also resistant to ciprofloxacin. Ciprofloxacin is characterized by broad activity and reaches a high concentration in blood and UT tissues (Mierzynska and Niemir, 2009). All fluoroquinolones are commonly used to treat UTIs. They could penetrate the biofilm matrix *e.g.* ciprofloxacin penetrates into the biofilm structure within 20 minutes and obtains the MIC (Anderl *et al.*, 2000). Furthermore, these antibiotics are active against slow growth cells so they are recommended to treat biofilm related infections (Passerini de Rossi *et al.*, 2009).

Cephalosporins, very often recommended to treat UTIs, have great bactericidal activity with the exception of strains producing ESBL (Extended-Spectrum Beta-

Lactamases). 14.4% of *P. mirabilis* strains in Poland produce ESBL (Kwiecinska-Piróg *et al.*, 2010). It has been proved that *Proteus* strains, synthesizing these enzymes and forming strong biofilm, are particularly dangerous and infections caused by them are persistent and difficult to treat (Nucleo *et al.*, 2010). Most studied strains (planktonic populations) were vulnerable to these drugs, but their biofilms were resistant. Explanation for biofilm resistance could be the presence of β-lactamases, which are accumulated in the biofilm matrix – β-lactams antibiotics could be hydrolyzed before their penetration into biofilm (Hoiby *et al.*, 2010). Spoering and Lewis (2001) suggest that biofilm resistance could result from the fact that microorganisms in biofilm have slow metabolism, similarly to bacteria in the stationary growth phase, while β-lactams are antibiotics which bind to fast growing cells. It is possible that cephalosporins (*e.g.* cefuroxime) activity relies only on delaying biofilm formation (Koseoglu *et al.*, 2006, Spoering and Lewis 2001). Cefepime is characterized by stability against ESBL and AmpC enzymes and it is recommended to treat UTIs caused by ESBL – producing organisms (da Silva Nogueira *et al.*, 2011). Bantar *et al.* (2004) suggest that substituting ceftriaxone with cefepime in UTIs caused by *P. mirabilis* results in a decrease in bacterial resistance to cephalosporins.

Aminoglycosides are recommended as an alternative to fluoroquinolones and cephalosporins to treat especially complicated UTIs (Kupilas, 2006). However, amikacin is not often used to treat UTIs because of dangerous side effects, despite the fact that its concentration in kidneys is high. The examined *P. mirabilis* planktonic cultures occurred to be sensitive to amikacin. The rare administration of this drug (usually in hospitals) in UTIs could be the reason for its high efficacy in *P. mirabilis* eradication. *P. mirabilis* biofilms were more resistant to this antibiotic contrary to biofilms of the other studied uropathogens (*M. morganii, E. cloacae, K. pneumoniae*). The extracellular matrix binds the drugs and inhibits penetration to deeper biofilm layers, which could be the reason why biofilms are resistant to aminoglycosides (Spoering and Lewis 2001). It is also probable that the oxygen limitation and the metabolic rate play an important role in this process (Hoiby *et al.*, 2010).

Because of a higher rate of bacterial resistance to popular drugs, many urologists increasingly suggest a change of bad antibiotic-prescription behaviour. Trimethoprim/sulfomethoxazol and fluoroquinolones should not be recommended for therapy in the case of complicated UTIs (Mazzulli, 2012). Fosfomycin/trometamol, nitrofurantoin, pivmecillinam should be current drugs of first choice (Wagenlehner and Naber, 2012). Pallett and Hand, 2010 also suggest alternative treatment *e.g.* combination of cefepime or cefpirome with clavulanate or fosfomycin.

In conclusion, *P. mirabilis* is very often isolated from urinary catheters, where it forms encrusted, multi-species biofilm, resistant to the majority of drugs. Biofilm related infections are persistant and difficult to treat. It is important to administer antibiotics with care (*e.g.* appropriate drugs in adequate doses), because the consequence of badly conducted treatment could be the extension of bacterial resistance to antibiotics.

Literature

Anderl J., M. Franklin and P. Stewart. 2000. Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicilin and ciprofloxacin. *Antimicrob. Agents. Chemother.* 44: 1818–1824.

Bantar C., E. Vesco, C. Heft, F. Salamone, M. Krayeski, H. Gomez, M.A. Coassolo, A. Fiorillo, D. Franco, C. Arango and others. 2004. Replacement of broad spectrum cephalosporins by piperacillin-tazobactam: impact on sustained high rates of bacterial resistance. *Antimicrob. Agents. Chemother.* 48: 392–395.

Ceri H., M. Olson, C. Stremick, R. Read, D. Morck and A. Buret. 1999. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol.* 37: 1771–1776.

Chen L. and Y. Wen. 2011. The role of bacterial biofilm in persistent infections and control strategies. *Int. J. Oral. Sci.* 3: 66–73.

da Silva Nogueira K., A. Vale Daur, I. Taborda de Messias, A.C. Gales and L.M. Dalla Costa. 2011. Cefepime versus extended spectrum β-lactamase-producing *Enterobacteriaceae. Braz. J. Infect. Dis.* 15: 167–169.

Ghadiri H., H. Vaez, S. Khosravi and E. Soleymani. 2012. The antibiotic resistance profiles of bacterial strains isolated from patients with hospital-acquired bloodstream and urinary tract infections. *Crit. Care. Res. Prac.* doi:10.1155/2012/890797.

Hoiby N., T. Bjarnsholt, M. Givskov, S. Molin and O. Ciofu. 2010. Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Ag.* 35: 322–332.

Jacobsen S.M., D.J. Stickler, H.L.T. Mobley and M.E. Shirtliff. 2008. Complicated catheter associated UTI due to *Escherichia coli* and *Proteus mirabilis. Clin. Microbiol. Rev.* 21: 26–57.

Koseoglu H., G. Aslan, N. Esen, B. Sen and H. Coban. 2006. Ultrastructural stages of biofilm development of *Escherichia coli* on urethal catheters and effects of antibiotics on biofilm formation. *Urology* 68: 942–946.

Kupilas A. 2006. Urinary tract infections. *Przegl. Urol.* (in Polish). 4: 50–54.

Kwiecińska-Piróg J., T. Bogiel and E. Gospodarek. 2010. The incidence of extended spectrum beta-lactamases in *Proteus mira-*

bilis strains isolated in 2007–2009. *Przegl. Epidemiol.* (in Polish). 4: 395–398.

Lazar V. and MC. Chifiriuc. 2010. Medical significance and new therapeutical strategies for biofilm associated infections. *Roum. Arch. Microbiol. Immunol.* 69 (3): 125–38.

MacLeod S. and D.J. Stickler. 2007. Species interactions in mixed-community crystalline biofilms on urinary catheters. *J. Med. Microbiol.* 56: 1549–1557.

Mazzulli T. 2012. Diagnosis and management of simple and complicated urinary tract infections (UTIs). *Can. J. Urol.* 19: 42–48.

Mierzynska E. and Z.I. Niemir. 2009. Antibiotics in nephrology (Part II) – principles of treatment of the most frequent diseases and their complications in hospitalized patients. *Contemp. Pharm.* 2: 102–109.

Muzzi-Bjornson L. and L. Macera. 2011. Preventing infection in elders with long-term indwelling urinary catheters. *J. Am. Acad. Nurse. Prac.* 23: 127–134.

Niveditha S., S. Pramodhini, S. Umadevi, K. Shailesh and S. Selvaraj. 2012. The isolation and biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). *J. Clin. Diagn. Res.* 6: 1478–1482.

Nucleo E., G. Fugazza, R. Migliavacca, M. Spalla, M. Comelli, L. Pagani and M. Debiaggi. 2010. Differences in biofilm formation and aggregative adherence between β-lactam susceptible and β-lactamases producing *P. mirabilis* clinical isolates. *New. Microbiol.* 33: 37–45.

Oki K., K. Washio, D. Matsui, S. Kato, Y. Hirata and M. Morikawa. 2010. The role of urease activity on biofilm formation by *Staphylococcus* sp. T-02 isolated from the toilet bowl. *Biosci. Biotechnol. Biochem.* 74: 583–589.

Pallett A. and K. Hand. 2010. Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. *J. Antimicrob. Chemother.* 65 Suppl. 3: 25–33.

Passerini de Rossi B., C. García, M. Calenda, C. Vay and M. Franco. 2009. Activity of levofloxacin and ciprofloxacin on biofilms and planktonic cells of *Stenotrophomonas maltophilia* isolates from patients with device-associated infections. *Int. J. Antimicrob. Ag.* 34: 260–264.

Spoering A. and K. Lewis. 2001. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J. Bacteriol.* 183: 6746–6751.

Stickler D.J. 2008. Bacterial biofilms in patients with indwelling urinary catheters. *Nat. Clin. Pract. Urol.* 5: 598–608.

Stickler D.J. and R.C.L. Feneley. 2011. The encrustation and blockage of long-term indwelling bladder catheters: a way forward in prevention and control. *Spinal. Cord.* 48: 784–790.

Wagenlehner F. and K. Naber. 2012. Prescribing behaviour in urinary tract infection. *Dtsch. Arztebl. Int.* 109: 876–877.

Wilde M., M. McDonald, J. Brasch, J. McMahon, E. Fairbanks, S. Shah, W. Tang and E. Scheid. 2013. Long-term urinary catheter users self-care practices and problems. *J. Clin. Nurs.* 22: 359–367.

Yang L., Y. Liu, H. Wu, N. Hoiby, S. Molin and Z. Song. 2011. Current understanding of multi-species biofilms. *Int. J. Oral. Sci.* 3: 74–81.