Primary and Secondary Bacteremia Caused by *Proteus* spp.: Epidemiology, Strains Susceptibility and Biofilm Formation

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A b s t r a c t

Proteus spp. is an etiological factor of urinary tract and bloodstream infections. The aim of this study was the retrospective analysis of susceptibility of *Proteus* spp. strains isolated from bloodstream infections (BSIs) as well as similarity evaluation of the strains isolated from different clinical samples. *Proteus* spp. strains were isolated in 2009–2017 from hospital patients. Identification was based on the colony's morphology and biochemical or MALDI-TOF MS analyzes. The antibiotic susceptibility test was done using the diffusion method. Biofilm formation was evaluated with microplate method using TTC. Bacteremia caused by *Proteus* spp. was found in 97 patients, mainly secondary to urinary tract infection. Most of the strains were susceptible to piperacillin with tazobactam (95.9%) and amikacin (86.7%). Elderly patients have a higher risk of mortality after BSIs caused by *Proteus* spp. A detailed analysis was made for randomly chosen 26 strains isolated from 11 patients with *Proteus mirabilis* bacteremia. Using PFGE, we found that 10 (90.9%) isolates, collected from different clinical specimens of the same patient, were genetically identical.

K e y w o r d s: antimicrobial susceptibility, bacteremia, biofilm, *Proteus* spp.

Introduction

Proteus spp. is widely spread in the environment. It may occur in water, soil, manure or sewage, and it is also a component of human and animal gastrointestinal tract microbiome (O'Hara et al. 2000; Drzewiecka 2016). In the current taxonomy of *Proteus* genus, eight species are included: *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri*, *Proteus cibarius*, *Proteus columbae*, *Proteus incostans*, and *Proteus terrae*. *P. mirabilis* is the most common species of this genus isolated from human infections (Różalski et al. 2007; Armbruster and Mobley 2012).

In addition to be a leading cause of urinary tract infections (UTI) and chronic wound infections (Endimiani et al. 2005), *P. mirabilis* can also cause bloodstream infections. *P. mirabilis* is found in 1–3% of all BSIs (Tumbarello et al. 2012). Other *Proteus* species are rare in BSIs and occur at rates of less than 0.1 per 100 000 (Laupland et al. 2014).

The most common source of bacteria in BSIs caused by *P. mirabilis* is the urinary tract (UT) (Watanakunakorn and Perni 1994). According to Watanakunakorn and Perni (1994) and Kim et al. (2003), UT is a source of 47.6–52.8% of BSIs caused by *P. mirabilis* (Watanakunakorn and Perni 1994; Kim et al. 2003).

Among infectious diseases, BSIs are a major cause of mortality worldwide (Laupland and Church 2014). Endimiani et al. (2005) found that mortality rate attributable to *P. mirabilis* BSIs is higher in BSIs cases caused by strains with extended spectrum beta-lactamases (ESBL). The presence of beta-lactamases may seriously limit BSIs treatment options. *Proteus* spp. is inherently resistant to polymyxins that are used, beside carbapenems, in empiric treatments of BSIs caused by Gram-negative bacilli. *P. mirabilis* might produce likewise, the AmpC-type cephalosporinases and carbapenemases (KPC) (Wang et al. 2014; Di Pilato et al. 2016).

The aims of this study were: a) retrospective susceptibility analysis of *Proteus* spp. strains isolated from BSIs, b) the similarity evaluation of 26 *P. mirabilis* strains isolated from clinical samples collected from randomly chosen 11 hospital patients.

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Experimental

Materials and Methods

Identification and susceptibility testing of clinical *Proteus* spp. strains. *Proteus* spp. strains were isolated from 2009 to 2017 from patients of the Antoni Jurasz University Hospital No 1 in Bydgoszcz, Poland. Basic information, i.e., age, sex of patients, and the samples collected were determined using laboratory data. Details, i.e.: short-term (14 days) mortality after bacteremia, length of stay, period from admission to the hospital to the onset of *Proteus* spp. bacteremia, presence of urinal or central venal catheters, the previous surgery, and additional diseases were determined by using electronic administrative data. We did not find any detailed information about 15 patients hospitalized in 2009–2010; they were excluded from epidemiological analyzes.

The strains were identified at the Department of Microbiology: a) between 2009 and 2011 the identification was based on colony's morphology and the results of biochemical reactions (API 32E, BioMériuex), b) between 2011 and 2014 – on biochemical reaction results included in the VITEK GN cards (BioMériuex), and c) since 2014 – by using MALDI-TOF MS technique (Microflex, Bruker).

Susceptibility of *Proteus* spp. strains was examined: in 2009 and 2010 by using the disk diffusion method and interpreted in accordance with the Clinical and Laboratory Standard Institute (CLSI 2009) recommendation; since 2011 – using VITEK AST cards (BioMériuex) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2016). The meropenem susceptibility was evaluated since 2016.

Extended-spectrum beta-lactamases (ESBLs) were examined according to the CLSI recommendation by using the double disk synergic test (DDST).

Primary bacteremia was defined as the bacteremia for which no source of infection was documented. Bacteremia was defined as secondary when laboratory examination showed infection by the same microorganism at a distant site in the same time or up to three days earlier.

Until the investigation, the selected strains were stored in BHI with 20.0% glycerol at 70°C.

Genetic similarity and phenotypic characterization of *P. mirabilis* strains. A detailed analysis was performed for 26 strains isolated from 11 (A-K) randomly chosen patients with *P. mirabilis* bacteremia. Strains were isolated from three different samples from four patients, and from two different samples – from seven patients. Two patients had urethral catheters, and one – peripheral venous catheter.

Susceptibility patterns. The susceptibility assays of *P. mirabilis* strains were done using the diffusion method according to CLSI recommendation, including the following antibiotics in discs (Emapol): trimethoprim TRM (5 µg), norfloxacin NOR (10 µg), netilmicin NET (30 µg), tobramicin TOB (10 µg), ticarcilin TIC (75 µg), amikacin AMK (30 µg), imipenem IMP (10 µg), ciprofloxacin CIP (5 µg), cephoperazone CEP (75 µg), piperacillin PIP (100 µg), ampicillin AM (10 µg), ticarcillin with clavulanic acid TIM (85 µg), cefuroxime CXM (30 µg), ampicillin with sulbactam UNA (20 µg), gentamicin GEN (10 µg). All results were interpreted according to EUCAST (2016).

Biofilm formation. Biofilm formation by 26 *P. mirabilis* strains was evaluated based on the method described previously by Kwiecińska-Piróg et al. (2013a). The method is based on the measurement of absorbance of formazan (dissolution product of 2,3,5-triphenyl-tetrazolium chloride, TTC; Avantor; $\lambda = 470$ nm) (BioTek; Synergy HT Multi-detection; KC4 program). The assay was done in three independent repetitions. Two reference strains from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 6538 (strong biofilm former) and *Escherichia coli* ATCC 35218 (weak biofilm former) were used as the positive and negative control, respectively.

The A value was calculated based on the average (x) and threefold standard deviation (SD) of blank absorbance. The strains which had an absorbance above the A value were classified as being able to biofilm formation. All strains with an absorbance in the range between A and $2 \times A$ were classified as weak biofilm formers (W), in the range between $2 \times A$ and $4 \times A$ – as moderate biofilm formers (M), and above $4 \times A$ – as strong biofilm formers (S).

Dienes phenomenon testing. To examine the Dienes phenomenon of *P. mirabilis* strains, single colonies, previously cultured on MacConkey Agar, were inoculated as points on blood agar plates (Oxoid). The strains isolated from the same patient were inoculated on one plate. Plates were incubated at 37°C. After 24 hours, demarcation lines between strains were observed. When strains were non-identical, colonies were separated by the demarcation line (0.5–2 mm wide) (Fig. 1A). When strains were identical – there was any demarcation line (Sabbuda et al. 2003) (Fig. 1B).

Genetic similarity of *P. mirabilis* strains. Genetic similarity of *Proteus* spp. strains was evaluated by pulsed field gel electrophoresis (PFGE) using *Sfi*I (Fermentas) restriction enzyme cleavage, as previously described by Sabbuba et al. (2003) with own modifications, described by Kwiecińska-Piróg et al. (2013b). Comparative analysis was performed using the Quantitative One software according to Dice.

Statistical analysis. Statistical analysis was performed using Statistica 12.5 PL (StatSoft) software. The confidence interval for all tests was $\alpha = 0.05$. To compare variables, the chi^2 test with Yates' correction was used, if necessary.
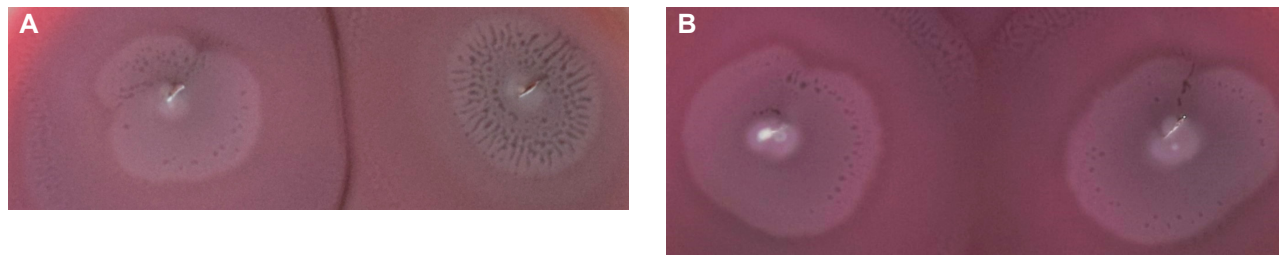
Fig. 1. The Dienes phenomenon: A) non-identical strains, B) identical strains.

Results

***Proteus* spp. blood stream infections.** In the examined time period (2009–2017), BSIs were found in 3956 cases in 2829 patients. BSIs caused by *Proteus* spp. were confirmed in 98 cases (2.5%) in 97 patients (3.4%). In one female patient, secondary urinary tract bacteremia was recognized two times with a six-months interval between symptoms (Table II).

Proteus spp. bacteremia was caused by *P. vulgaris* in five (5.2%) patients and by *P. mirabilis* in other patients (94.8%). In *P. vulgaris* bacteremia cases, four strains were isolated only from blood samples. One *P. vulgaris* bacteremia was related with a previous isolation of the same species strain from a chronic wound.

Median age for all patients with *Proteus* spp. bacteremia was 71 years. We found that 14-day mortality ratio of *Proteus* spp. bacteremia was 28.9%. None of

P. vulgaris strain was related with short-term mortality. We observed that elderly patients have a higher risk of mortality (Table I).

During the period analyzed, the most commonly used antibiotic for empiric therapy of BSIs was imipenem. Amongst 16 patients treated with imipenem, three (20.0%) of them died within 14 days. All *P. mirabilis* strains isolated from blood samples of these patients were susceptible to imipenem. Piperacillin with tazobactam was used in 15 cases of BSIs, and ceftriaxone – in 13 cases. The 14-days mortality rate for piperacillin with tazobactam was 26.7%, and for ceftriaxone – 44.0%.

Primary bacteremia was reported in 40 (40.8%) cases. The source of *P. mirabilis* secondary bacteremia was confirmed in 58 (59.2%) cases. In patients with secondary bacteremia, the main source of infection was the urinary tract (27; 46.6%), wounds of skin or soft tissues (14; 24.1%). In nine (9.2%) cases, *Proteus* spp.

Table I
Demographic and clinical parameters of patients with Proteus spp. BSIs (n = 83).

Variable		Death in 14 days (n = 24)	Survival (n = 59)	*p*-value*
Age (years), median (min-max)		77.5 (55–94)	67.0 (12–88)	0.0025**
Sex	Men, No. (%)	13 (54.2)	33 (55.9)	0.9229
	Women, No. (%)	11 (45.8)	26 (44.1)	
Length of stay in hospital (days), median (min-max)		19.5 (1–139)	38 (5–406)	0.0142**
Length of stay in hospital before *Proteus* spp. BSI (days), median (min-max)		9.5 (0–138)	7.5 (0–380)	1.0000**
Primary bacteremia, No. (%)		11 (45.8)	23 (39.0)	0.7420
Secondary bacteremia, No. (%)		13 (54.2)	36 (61.0)	
Adequate empiric antibiotic therapy, No. (%)		16 (66.7)	43 (72.9)	0.7648
More than one strain in blood culture, No. (%)		9 (37.5)	20 (34.0)	0.9537
ESBL *Proteus* spp., No. (%)		4 (16.7)	11 (18.6)	0.9185
Predisposing factors	Urinal catheter, No. (%)	18 (75.0)	44 (67.8)	0.8117
	Central Venus Catheter, No. (%)	15 (62.5)	28 (47.5)	0.3168
	Diabetes, No. (%)	7 (29.2)	10 (16.9)	0.3419
	Hospitalization during past half year, No. (%)	13 (54.2)	25 (42.4)	0.4625
	Cancer, No. (%)	3 (12.5)	4 (6.8)	0.6784
	Surgery during past half year, No. (%)	9 (37.5)	33 (55.9)	0.2003
	Cardio-vascular diseases, No. (%)	15 (62.5)	23 (39.0)	0.0879
	Chronic wounds, No. (%)	7 (29.2)	13 (22.0)	0.6849

* *Chi^2* test, ** U Mann-Whitney test

Table II
The strains isolated from patients with *P. mirabilis* (n = 93) and *P. vulgaris* (n = 5) bacteremia.

| Year | *Protteus* spp. bacteremia | Secondary *Proteus* spp. bacteremia | | | | | | | Isolation of *Proteus* spp. not related directly to bacteremia | | | | | | | | | |
| | | Total | Source of secondary bacteremia (strains isolated at the time/up to three days before isolation from blood sample) | | | | | Before bacteremia (more than three days) | | | | | After bacteremia | | | | |
			Urinary tract	Respiratory tract	Wounds	Blood catheter	Peritoneum	Respiratory tract	Wounds	Urinary tract	Blood catheter	Peritoneum	Respiratory tract	Wounds	Urinary tract	Blood catheter	Peritoneum
2009	12	8 (66.7%)	7	1	0	0	0	1	0	2	0	0	0	1	1	0	0
2010	12	8 (66.7%)	4	0	2	2	0	0	2	0	0	0	0	4	1	2	0
2011	7	4 (57.1%)	1	0	2	0	1	0	2	0	1	1	0	0	1	0	0
2012	4	4 (100.0%)	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0
2013	14	8 (57.1%)	2	2	2	2	0	3	2	1	0	0	0	2	0	1	1
2014	14	9 (64.3%)	5	0	3	1	0	0	2	1	0	0	1	0	0	0	1
2015	9	4 (44.4%)	1	1	2	0	0	0	0	0	0	0	1	1	1	0	0
2016	15	8 (53.3%)	5	1	1	1	0	0	1	1	0	0	1	1	0	0	0
2017	11	5 (45.5%)	1	2	1	0	1	0	0	1	0	0	2	2	1	1	0
Total	98	58 (59.2%)	27	8	14	7	2	4	9	7	1	1	5	11	5	5	2

bacteremia occurred three days prior to isolation of the same bacterial species from wound swab, and in seven (7.1%) – from urine samples (Table II). One or more weeks after *P. mirabilis* bacteremia, strains of the same species were isolated from wound swabs (11; 11.2%), central venous catheter samples (5; 5.1%) and urine collected by a catheter (5; 5.1%).

All *Proteus* spp. strains isolated in 2016 and 2017 (n = 26) were susceptible to meropenem. Most strains were susceptible to piperacillin with tazobactam (95.9%), ceftazidime (87.8%) and amikacin (86.7%) (Table III). More than half of the strains examined (51.0%) were resistant to co-trimoxazole. Resistance to ciprofloxacin was reported in 30 (30.6%) strains. All 40 strains isolated from primary bacteremia cases were susceptible to piperacillin with tazobactam. No significant differences in the antimicrobial susceptibility of strains isolated from primary and secondary bacteremia were found (Table III).

ESBLs were found in 18 (18.3%) out of 98 *Proteus* spp. strains. Among all ESBL positive strains, 6 (33.3%) were isolated in 2016, and 5 (27.8%) in 2017. None of the *P. vulgaris* strains produced ESBLs.

We compared the antimicrobial drugs susceptibility of ESBLs-positive (ESBLs(+)) and ESBLs-negative (ESBLs(−)) strains using the *chi²* test (Fig. 2). Among the ESBLs(−) strains, all (n = 80) were susceptible to piperacillin with tazobactam. The ESBLs(+) strains were more resistant to all penicillins tested with beta-lactamases inhibitors and cephalosporins. Among the

ESBLs(+) strains, more strains were non-susceptible to ciprofloxacin and co-trimoxazole than among ESBLs(−) bacteria (Fig. 2).

Susceptibility patterns. The susceptibility patterns of isolates from different specimens of the same patient were similar, except of the isolate B3 that was genetically different from B1 and B2 and was more resistant to antibiotics than other two isolates collected from the same patient. Isolate J2, collected from urine four days before the bacteremia, were resistant to trimethoprim while J1 were susceptible to this antibiotic (Table IV).

ESBLs were detected in five (19.2%) *P. mirabilis* isolates from two patients (D and G), treated in the Intensive Care Unit (G) and the Cardiology Department (D). Isolates D1, D2, G1, G2 and G3 had the same DNA profiles.

Biofilm formation. The A value was equal to 0.0654, based on the formazan absorbance of blank sample. The mean values (± SD) of absorbance recorded for biofilms formed by the references strains, were as follows: for *E. coli* ATCC 35218 – 0.1031 ± 0.01, and for *S. aureus* ATCC 6538 – 0.6931 ± 0.03. The results obtained for clinical *P. mirabilis* strains were shown in Table IV.

Weak biofilms were formed by 17 (65.4%) isolates, moderate biofilms by four (15.4%), and strong by five (19.2%) isolates. Most of the strains (8; 72.7%) isolated from blood samples formed weak biofilms. Two of three isolates collected from chronic wounds prior to bacteremia were able to form stronger biofilms than strains collected from blood samples. Two isolates

Table III
Antibacterial agents (Aas) susceptibility of *Proteus* spp. strains (n = 98) isolated from bacteremia.

Aas	Primary bacteremia Aas susceptibility						p^*	Secondary bacteremia Aas susceptibility						Total Aas susceptibility					
	S		I		R			S		I		R		S		I		R	
	n	%	n	%	n	%		n	%	n	%	n	%	n	%	n	%	n	%
AMC	9	22.5	24	60.0	7	17.5	0.4588	16	27.6	28	48.3	14	24.1	25	25.5	52	53.1	21	21.4
TZP	40	100.0	0	0.0	0	0.0	0.1316	54	93.1	0	0.0	4	6.9	94	95.9	0	0.0	4	4.1
CMX	28	70.0	0	0.0	12	30.0	0.6037	45	77.6	0	0.0	13	22.4	73	74.5	0	0.0	25	25.5
CTX	31	77.5	0	0.0	9	22.5	0.3393	46	79.3	0	0.0	12	20.7	77	78.6	0	0.0	21	21.4
CAZ	35	87.5	2	5.0	3	7.5	0.1697	51	87.9	0	0.0	7	12.1	86	87.8	2	2.0	10	10.2
FEP	32	80.0	2	5.0	6	15.0	0.4254	48	82.8	4	6.9	6	10.3	80	81.6	6	6.1	12	12.2
IMP	30	75.0	8	20.0	2	5.0	0.6062	49	84.5	8	13.8	1	1.7	79	80.6	16	16.3	3	3.1
GEN	29	72.5	1	2.5	10	25.0	0.3662	46	79.3	1	1.7	11	19.0	75	76.5	2	2.0	21	21.4
AMK	33	82.5	3	7.5	4	10.0	0.6311	52	89.7	4	6.9	2	3.4	85	86.7	7	7.1	6	6.1
CIP	24	60.0	1	2.5	15	37.5	0.4336	38	65.5	5	8.6	15	25.9	62	63.3	6	6.1	30	30.6
SXT	20	50.0	0	0.0	20	50.0	0.3165	28	48.3	0	0.0	30	51.7	48	49.0	0	0.0	50	51.0

* *chi^2* test – differences in the susceptibility of *Proteus* spp. strains isolated from primary and secondary bacteremia
S – susceptible, I – intermediate, R – resistant, AMC – amoxicillin + clavulanic acid, TZP – piperacillin with tazobactam,
CXM – cefuroxime, CTX – cefotaxime, CAZ – ceftazidime, FEP – cefepime, IMP – imipenem, GEN – gentamicin, AMK – amikacin,
CIP – ciprofloxacin, SXT – co-trimoxazole

from urine samples of catheterized patients formed moderate and strong biofilms, while isolates cultured from blood sample – weak biofilms. The isolates from venal catheters formed weak biofilms, similarly to isolates from blood samples.

Dienes phenomenon results. We found demarcation lines between strains J1 and J2, B1 and B3, and B2 and B3 (Table IV). Those strains were not genetically identical. Strain J1, from urine collected by a catheter, formed a stronger biofilm than J2 cultured from the blood sample. The B3 strain, cultured from the bedsore swab sample, formed a strong biofilm, in contrast to B1 and B2 isolates that formed a weak biofilm.

Genetic similarity of *P. mirabilis* isolates. Using PFGE we found that isolates collected from different clinical specimens were identical in 10 of 11 patients (Table IV). In five patients (K, G, H, E, and J), the same strains were found in other localizations before the bacteremia was confirmed.

Strains J1 and J2, isolated from patient J, were genetically different and formed biofilms with various capacity, but they presented similar susceptibility patterns.
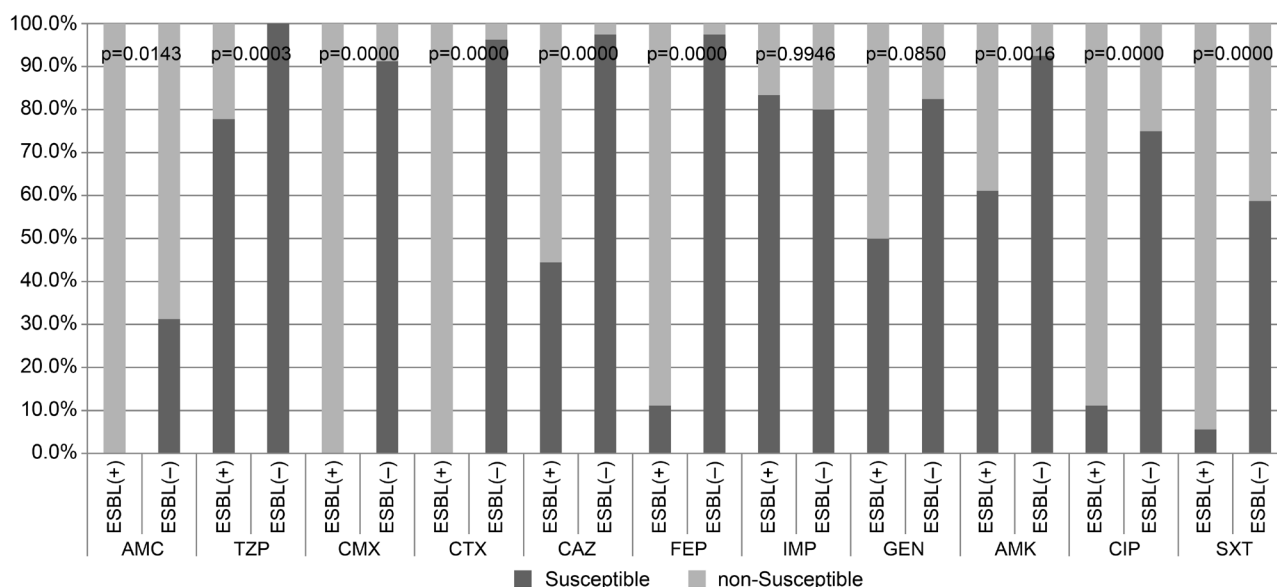

Fig. 2. Antibiotic susceptibility of ESBLs(+) and ESBLs(−) *Proteus* spp. strains (n = 98) isolated from bacteremia; *p*-value of *chi^2* testing between susceptible and non-susceptible (resistant and intermediate) strains; abbreviations of antibiotic names (see Table III).

Table IV
Genetic similarity of *P. mirabilis* isolates (n = 26).

Group	ID	Source		AM	PIP	TIC	CEP	CXM	IMP	UNA	TIM	AMK	GEN	NET	TOB	CIP	NOR	TRM		biofilm	
K	S26/K3	Bedsore swab	11/02	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	−	0.185 ± 0.01 (M)	
	S25/K2	Blood	26/02	S	S	S	S	S	I	S	S	S	S	S	S	S	I	R	−	0.1137 ± 0.01 (W)	Compatible
	S24/K1	Pus swab	27/01	S	S	S	S	S	I	S	S	S	S	S	S	S	I	R	−	0.1147 ± 0.02 (W)	
F	S12/F2	Blood	24/09	R	I	S	S	S	S	R	S	S	S	S	S	S	S	S	−	0.1206 ± 0.04 (W)	Compatible
	S13/F1	Urine by catheter	15/10	R	I	S	I	S	S	R	S	S	S	S	S	I	S	S	−	0.2090 ± 0.02 (M)	
G	S14/G3	Blood	29/12	R	R	S	R	S	S	R	S	S	S	S	S	S	I	R	+	0.1156 ± 0.02 (W)	Compatible
	S15/G2	Pus swab	30/11	R	R	S	I	S	S	R	I	S	S	S	S	I	I	S	+	0.2511 ± 0.02 (S)	
	S16/G1	Tissue	30/1	R	R	S	I	S	S	R	S	S	S	S	S	S	S	I	+	0.1007 ± 0.04 (W)	
H	S18/H2	Pus	17/11	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	−	0.0989 ± 0.01 (W)	Compatible
	S17/H1	Blood	22/11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	−	0.1112 ± 0.02 (W)	
E	S11/E2	Bedsore swab	30/06	R	I	R	S	S	S	S	I	S	R	S	S	R	R	R	−	0.8876 ± 0.05 (S)	Compatible
	S10/E1	Blood	06/07	R	R	R	I	S	S	S	R	S	R	S	I	R	R	R	−	0.7986 ± 0.04 (S)	
I	S19/I3	Blood	29/09	R	R	R	S	R	S	R	S	R	S	S	S	S	S	R	−	0.1212 ± 0.01 (W)	Compatible
	S20/I2	Venous catheter	28/09	R	R	R	S	R	S	R	S	R	S	S	S	S	S	R	−	0.1296 ± 0.02 (W)	
	S21/I1	Pus	15/10	R	R	R	S	R	S	R	S	R	S	S	S	S	S	R	−	0.1004 ± 0.02 (W)	
D	S9/D2	Bedsore swab	02/03	R	R	R	I	R	S	R	R	S	R	S	I	R	R	R	+	0.0956 ± 0.01 (W)	Compatible
	S8/D1	Blood	07/02	R	R	R	R	R	S	R	R	S	R	S	R	R	R	R	+	0.2197 ± 0.04 (M)	
C	S6/C2	Blood	30/04	R	R	R	R	R	S	S	R	S	R	S	I	I	S	R	−	0.0981 ± 0.01 (W)	Compatible
	S7/C1	Venous catheter	10/05	R	R	R	S	S	S	S	R	S	R	S	R	I	S	R	−	0.1126 ± 0.02 (W)	
J	S23/J2	Urine by catheter	14/11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	−	0.4262 ± 0.02 (S)	Incompatible
	S22/J1	Blood	18/11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	−	0.0887 ± 0.01 (W)	
A	S2/A2	Urine	13/03	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	−	0.1196 ± 0.03 (W)	Compatible
	S1/A1	Blood	14/03	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	−	0.1753 ± 0.04 (M)	
B	S5/B3	Bedsore swab	13/03	R	R	R	S	S	S	I	R	S	R	S	R	S	R	R	−	0.693 ± 0.02 (S)	Incompatible
	S4/B2	Blood	04/03	R	S	S	S	S	S	I	R	S	R	S	S	S	R	R	−	0.1032 ± 0.01 (W)	
	S3/B1	BAL	04/03	R	S	S	S	S	S	I	R	S	R	S	S	S	R	R	−	0.1125 ± 0.01 (W)	

S – Susceptible, I – Intermediate, R – Resistant, abbreviations of antibiotics – in the text; *(S) – Strong, (M) – Moderate, (W) – Weak biofilms

The J2 strain was isolated from urine sample four days before J1 was cultured from blood sample.

Two strains, B1 and B2, were genetically identical as it was shown by their macrorestriction profiles. They formed weak biofilms and had the same susceptibility toward antibiotics. Strain B3, isolated from chronic wound swab nine days after confirmation of *P. mirabilis* bacteremia, was similar to B1 and B2, but it formed a strong biofilm and was resistant to higher number of the antibiotics tested than B1 and B2 isolates.

Discussion

The *P. mirabilis* is isolated from 1% to 3% of all BSIs (Laupland et al. 2007; Sohn et al. 2011). Our study confirms these data. We also observed *Proteus* spp. BSIs in 2.5% of all BSIs investigated. Among *Enterobacterales*, *P. mirabilis* is the fourth Gram-negative bacteria species after *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. isolated from hospital-acquired BSIs (Sohn et al. 2011). BSIs are well-known cause of high mortality. We found that mortality rate of *P. mirabilis* BSIs was 28.9%. It is similar value to that obtained by Endimiani et al. (2005). They found that mortality rate attributable to *P. mirabilis* BSIs was 33.0%.

Laupland et al. (2014) showed that most cases of *P. mirabilis* bacteremia (18; 72.0%) have no documented sources and were recognized as primary bacteremia. In another seven (28.0%) cases, the urinary tract was confirmed to be the source of bacteremia. The opposite results were reported by Sohn et al. (2011). They detected primary bacteremia caused by *Proteus* spp. in 32.4% patients. The source of bacteria in secondary bacteremia was found mostly (35.1%) in the urinary tract and intra-abdominal cavity (21.6%) (Ahn et al. 2017). Infections of the urinary tract were admitted as the main source of BSIs also in the study by Tumbarello et al. (2012). They showed this phenomenon in BSIs caused by 19 (52.8%) multidrug resistant (MDR) and 28 (4.4%) non-MDR *P. mirabilis* strains.

Our data confirms the findings of Sohn et al. (2011). In most cases (58; 59.2%), the bacteremia caused by *P. mirabilis* was secondary due to the presence of these bacteria in other body sites, particularly in the urinary tract (27; 46.6%). In 14 (24.1%) patients, we found *P. mirabilis* in wound swabs. Wound infected by *P. mirabilis* was confirmed as the source of BSIs in the patient of the Traumatic Care Unit in India (Endimiani et al. 2005).

Proteus spp. is inherently susceptible to co-trimoxazole (trimethoprim-sulfamethoxazole). In the present study we found that more than half (51.0%) of the *Proteus* spp. strains, isolated from patients with bacteremia, were resistant to co-trimoxazole. High resistance to co-trimoxazole was reported also in another paper (Wang et al. 2014). Among 158 examined strains isolated from blood samples during longitudinal nationwide study from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program, the resistance of *P. mirabilis* to co-trimoxazole was found in 65.2% strains. Similar percentage (69.2%) of co-trimoxazole resistance was reported also for ESBLs-producing *P. mirabilis* strains isolated from blood samples (Lebeaux et al. 2014). In this study, we found that almost all ESBLs(+) strains (17; 94.4%) were non-susceptible to co-trimoxazole. Lower percentages of resistant strains were found in the study by Tumbarello et al. (2012): 33.3% among non-MDR, and 30.6% among MDR *P. mirabilis* strains involved in BSIs development.

ESBLs were found by Sohn et al. (2010) in nine (24.3%) out of 37 *P. mirabilis* strains causing bacteremia from 2000 to 2009. In our study, we detected ESBLs in 18.4% *P. mirabilis* isolates causing bacteremia during the period evaluated. The highest number of ESBL-positive strains was found in 2016 (6; 33.3%). Tumbarello et al. (2012) noted that ESBLs were confirmed in 36 (36.4%) out of 99 *P. mirabilis* strains causing BSIs. MDRs were recorded for 36 (36.6%) strains tested.

Tumbarello et al. (2012) diagnosed *P. mirabilis* BSIs in 103 adult patients. The growth of the strains was inhibited by meropenem, amoxicillin with clavulanic acid, piperacillin with tazobactam, and amikacin. Ahn et al. (2017) confirmed that most *P. mirabilis* isolates from BSIs were susceptible to piperacillin with tazobactam (98.3%) and amikacin (93.5%). In the study by Wang et al. (2014), 58.9–87.3% of *P. mirabilis* strains isolated from blood samples were susceptible to gentamicin and amikacin, respectively. This is in agreement with the present results. *Proteus* spp. strains examined in this study were mostly susceptible to piperacillin with tazobactam (95.3%) and amikacin (86.7%).

Proteus spp. strains were generally susceptible to ciprofloxacin (67.1–73.4% of the strains) (Tumbarello et al. 2012; Wang et al. 2014). Tumbarello et al. (2012) found that susceptibility to ciprofloxacin is rare among MDR strains and was showed only by three (8.3%) out of 36 strains. Sohn et al. (2010) claim that a correlation between ciprofloxacin resistance and ESBLs production in *P. mirabilis* bacteremia may occur. This is in agreement with our results. We found significant differences in the susceptibility of ESBLs(+) and ESBLs(−) *Proteus* spp. strains to ciprofloxacin.

Sabbuba et al. (2003) investigated the genetic similarity of *P. mirabilis* strains isolated from hospitalized patients. They confirmed identical DNA profiles of the isolates from blood and urine of the same patient. In the present study, we report that strains isolated from blood samples of 10 out of 11 patients were identical with isolates cultured from other clinical specimens. The same strains were isolated from various specimens

also a few weeks after the bacteremia, that it is because of the survival of *Proteus* spp. strains despite the antibiotic treatment. The decrease in the effectiveness of antibiotic treatment may be related to the biofilm forming ability.

In most cases we observed that strains from clinical samples collected from infections related to biomaterials were able to form stronger biofilms than identical strains from other samples collected from these patients. The presence of biomaterials in body sites probably promotes biofilm formation, which is in agreement with the results obtained by Lebeaux et al. (2014) and Di Domenico et al. (2017). We also reported that isolates collected from chronic wounds prior to bacteremia were able to form stronger biofilms than isolates cultured from blood samples.

Conclusions

In most cases bacteremia caused by *Proteus* spp. was secondary to urinary tract infection.

Most of the strains isolated from *Proteus* spp. BSIs were susceptible to piperacillin with tazobactam, third generation of cephalosporins, and amikacin.

Elderly patients have a higher risk of mortality in consequence of *Proteus* spp. BSI.

Most strains isolated from blood samples were identical to isolates cultured from other clinical specimens of the same patient.

Conflict of interest

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Ahn JY, Ann HW, Jeon Y, Ahn MY, Oh DH, Kim YC, Kim EJ, Song JE, Jung IY, Kim MH, et al. 2017. The impact of production of extended-spectrum β-lactamases on the 28-day mortality rate of patients with *Proteus mirabilis* bacteremia in Korea. BMC Infect Dis. 17:327.

Armbruster CE, Mobley HLY. 2012. Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. Nat Rev Microbiol. 10(11):743–754.

CLSI. 2009. Performance Standards for Antimicrobial Disk Susceptibility Tests. 11th ed. CLSI standard M02. Wayne (USA): Clinical and Laboratory Standards Institute.

Di Domenico EG, Farulla I, Prignano G, Gallo MT, Vespaziani M, Cavallo I, Sperduti I, Pontone M, Bordignon V, Cilli L, et al. 2017. Biofilm is a major virulence determinant in bacterial colonization of chronic skin ulcers independently from the multidrug resistant phenotype. Internat J Mol Sci, 18(5):1077.

Di Pilato V, Chiarelli A, Boinett CJ, Riccobono E, Harris SR, D'Andrea M, Thomson NR, Rossolini GM, Giani T. 2016. Complete genome sequence of the first KPC-type carbapenemase-positive *Proteus mirabilis* strain from a bloodstream infection. Genome Announc. 4(3):e00607–16.

Drzewiecka D. 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol. 72(4):741–758.

Endimiani A, Luzzaro F, Brigante G, Perilli M, Lombardi G, Amicosante G, Rossolini GM, Toniolo A. 2005. *Proteus mirabilis* bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 49:2598–2605.

EUCAST. 2016. Breakpoint tables for interpretation of MICs and zone diameters, version 8.0 [Internet]. London (UK): The European Committee on Antimicrobial Susceptibility Testing; [cited 2018 June 27]. Available from http://www.eucast.org/clinical_breakpoints/

Kim BN, Kim NJ, Kim MN, Kim YS, Woo JH, Ryu J. 2003. Bacteremia due to tribe *Proteeae*: a review of 132 cases during a decade (1991–2000). Scand J Infect Dis. 35(2):98–103.

Kwiecińska-Piróg J, Bogiel T, Gospodarek E. 2013a. Effects of ceftazidime and ciprofloxacin on biofilm formation in *Proteus mirabilis* rods. J Antibiot. 66(10):593–597.

Kwiecińska-Piróg J, Skowron K, Zniszczoł K, Gospodarek E. 2013b. The assessment of *Proteus mirabilis* susceptibility to ceftazidime and ciprofloxacin and the impact of these antibiotics at subinhibitory concentrations on *Proteus mirabilis* biofilms. Biomed Res Int. 2013:1–8.

Laupland KB, Church DL. 2014. Populations-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev. 27:647–664.

Laupland KB, Parkins MD, Ross T, Pitout JDD. 2007. Population-based laboratory surveillance for tribe *Proteeae* isolates in a large Canadian health region. Clin Microbiol Infect. 13(7):683–688.

Lebeaux D, Ghigo JM, Beloin C. 2014. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 78(3):510–543.

O'Hara CM, Brenner FW, Miller JM. 2000. Classification, identification and clinical significance of *Proteus, Providencia*, and *Morganella*. Clin Microbiol Rev. 13(4):534–546.

Różalski A, Kwil I, Torzewska A, Baranowska M, Stączek P. 2007. *Proteus* bacilli: features and virulence factors. Post Hig Med Dosw. 61:204–219.

Sabbuba NA, Mahenthiralingam E, Stickler DJ. 2003. Molecular epidemiology of *Proteus mirabilis* infections of the catheterized urinary tract. J Clin Microbiol. 41(11):4961–4965.

Sohn KM, Kang ChI, Joo EJ, Ha YE, Chung DR, Peck KR, Lee NY, Song JH. 2011. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Proteus mirabilis* bacteremia. Korean J Intern Med. 26:89–93.

Tumbarello M, Trecarichi EM, Fiori B, Losito AR, D'Inzeo T, Campana L, Ruggeri A, Di Meco E, Liberto E, Fadda G, et al. 2012. Multidrug-Resistant *Proteus mirabilis* bloodstream infections: risk factors and outcomes. Antimicrob Agents Chemother. 56(6):3224–3231.

Wang JT, Chen PC, Chang SC, Shiau YR, Wang HY, Lai JF, Huang IW, Tan MC, Lauderdale TL. 2014. TSAR Hospitals. Antimicrobial susceptibilities of *Proteus mirabilis*: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC Infec Dis. 14(1):486.

Watanakunakorn C, Perni SC. 1994. *Proteus mirabilis* bacteremia: a review of 176 cases during 1980–1992. Scand J Infect Dis. 26:361–367.