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Swarming Growth and Resistance of *Proteus penneri* and *Proteus vulgaris* Strains to Normal Human Serum*

Wzrost rozpełzły i oporność szczepów *Proteus penneri* i *Proteus vulgaris* na działanie normalnej surowicy ludzkiej

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. *Proteus* sp. strains isolated from patients with urinary tract infection (UTI) are often insensitive to the bactericidal action of normal human serum (NHS) which poses a clinical problem. The swarming phenomenon is an especially important factor in cases of UTIs gained through the ascending route. Both these virulence factors are connected with the cell surface components of bacteria, including lipopolysaccharide (LPS).
Objectives. The resistance of *Proteus* bacilli to the bactericidal activity of NHS and the swarming phenomenon were investigated as well as the possible relationships between these virulence factors and the chemical structure of LPS.
Material and Methods. The research was carried out on *P. penneri* and *P. vulgaris* species. Two preparations of sera were tested with respect to the bactericidal action of NHS. The ability of bacteria to swarm was checked on broth agar plates. The length of the O-specific part of LPS was estimated after poliacrylamide gel electrophoresis (PAGE) and staining with silver nitrate.
Results. Among the 62 tested *Proteus* strains, over 62% of *Proteus vulgaris* and 50% of *Proteus penneri* strains were sensitive to the bactericidal action of NHS. However, the number of resistant strains grew dramatically when serum with blocked complement activation via the alternative pathway was used. From 102 of the *Proteus* sp. strains, only few were unable to swarm over the solid surface of the media. The remaining showed diverse ability to translocate.
Conclusions. There was no definite correlation between the chemical structure of the O-specific chains of lipopolysaccharides and sensitivity or resistance of the *Proteus* sp. strains to NHS. Also, no significant relationships were found between the length or the chemical structure of the O-specific chains of the bacterial LPSs and the swarming phenomenon (**Adv Clin Exp Med 2013, 22, 2, 165–175**).

Key words: *Proteus*, normal human serum, swarming growth, lipopolysaccharide.

Streszczenie

Wprowadzenie. Szczepy *Proteus* sp. izolowane od pacjentów z zakażeniem układu moczowego (z.u.m.) często są niewrażliwe na bakteriobójcze działanie normalnej surowicy ludzkiej (n.s.l.), co stanowi problem kliniczny. Wzrost rozpełzły bakterii jest niezwykle ważnym czynnikiem zwłaszcza w przypadkach z.u.m. rozwijających się drogą wstępującą. Obydwa czynniki chorobotwórczości mają związek ze strukturami powierzchni pałeczek, w tym także z lipopolisacharydem (LPS).
Cel pracy. Badaniom poddano oporność pałeczek *Proteus* na bakteriobójczą aktywność n.s.l. i wzrost rozpełzły, jak również przeprowadzono analizę ewentualnych korelacji między tymi czynnikami oraz strukturą chemiczną LPS.
Materiał i metody. Badaniami objęto szczepy *P. penneri* i *P. vulgaris*. Zastosowano dwa rodzaje surowic do badania bakteriobójczego działania n.s.l. Zdolność bakterii do wzrostu rozpełzłego sprawdzano na bulionowych płytkach

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zestalonych agarem. Długość części O-swoistej LPS oceniano po elektroforezie w żelu poliakrylamidowym i wysrebrzeniu żelu.
Wyniki. Wśród 62 badanych szczepów *Proteus* ponad 62% szczepów *P. vulgaris* i 50% szczepów *P. penneri* wykazało wrażliwość na działanie n.s.l. Zastosowanie surowicy z zablokowaną alternatywną drogą aktywacji dopełniacza spowodowało wzrost liczby szczepów opornych. Spośród 102 szczepów *Proteus* sp. tylko kilka nie miało zdolności pełzania po powierzchni podłoża stałego. Pozostałe wykazały zróżnicowaną zdolność przemieszczania się.
Wnioski. Nie zaobserwowano jednoznacznych korelacji między strukturą chemiczną łańcuchów części O-swoistej LPS i wrażliwością czy opornością szczepów *Proteus* sp. na działanie n.s.l. Nie znaleziono także wyraźnego związku między długością czy strukturą łańcuchów części O-swoistej LPS szczepów *Proteus* i wzrostem rozpełzłym (**Adv Clin Exp Med 2013, 22, 2, 165–175**).

Słowa kluczowe: *Proteus*, normalna surowica ludzka, wzrost rozpełzły, lipopolisacharyd.

Gram-negative *Proteus* short rods are widely distributed in the natural environment. They can especially be found in polluted water and soil, where they play a significant role in decomposing organic matter of animal origin. They are also present in human and animal intestines. *Proteus* bacilli are opportunistic pathogens, nevertheless, under favorable conditions they can cause urinary tract infections, which may lead to severe complications, such as pyelonephritis or stone formation. These bacteria can also be etiological agents of nosocomial infections, infections of the respiratory tract, wound infections and rheumatoid arthritis [1–3].

Bacilli from the genus *Proteus* have developed several virulence factors such as fimbriae, flagella, urease, amino acid deaminases, proteases, hemolysins, swarming growth and LPS which enable them to colonize, survive and grow in the host organism [4, 5].

In a liquid medium, *Proteus* cells are motile, peritrichously flagellated (6–10 flagella per cell), 1.0–2.0 μm in length and they are called swimmer cells. When transferred to a solid medium, these bacteria undergo morphogenesis to swarmer cells, which are longer – 20–80 μm, multinucleated, nonseptated and characterized by a 50–500 fold increase in the number of flagella. The population of swarmer cells can swarm over the surface of a solidified media creating characteristic swarming zones which is known as a swarming phenomenon [6]. It is believed that the main stimulators of swarming growth are extracellular signals. A very important role could also be played by intracellular physiological parameters and contact with the surface. Among the inductors of swarming growth, there are media with great viscosity and the presence of glutamine (which initiate the process of differentiation of the cells and direct the process of migration) and agents which stop the rotation of the flagella [6]. There are several factors involved in the regulation of the swarming growth process. The most important are: *flh*DC flagellar operon, genes (*umoA*, *umoB*, *umoC*, *umoD*, *flaA*) and proteins (leucine-responsive regulatory protein, membrane proteins CcmA, regulatory RsbA protein) [7–12]. On the other hand, there is also research aimed at finding effective inhibitors of the swarming growth process. Very good future prospects have revealed a newly discovered active compound of ethanolic extract from *Lithrea molleoides* – (Z,Z)-5-(trideca-4′7′-dienyl)-resorcinol which expresses a strong inhibitory effect on swarming behavior [13].

One of the mechanisms which facilitates the survival of Gram-negative bacteria in the host macroorganism is resistance to the bactericidal action of normal human serum. The high percentage of the resistant strains isolated from ill people pose a serious clinical problem. Bacterial strains originating from cerebrospinal liquid, blood or UTI are very often resistant to the bactericidal action of NHS [14–18].

Resistance to the bactericidal activity of NHS and swarming growth phenomenon are most probably connected with the cell surface components of bacteria, including LPS. Translocation of the population of swarmer cells is facilitated by a capsular polysaccharide (CPS) and by long O-antigenic side chains of LPS. It is assumed that acidic O-antigenic polysaccharides diminish the surface friction during the migration of the population of swarmer cells over the solid surface of the media. This could be significant especially during ascending UTI. Lipopolysaccharide can also play an important role in the resistance of bacteria to NHS. In this activity, the crucial role seems to be that of the O-specific polysaccharide chain of LPS. The hydrophilic long chain of the O-specific part of LPS can protect the bacterial cell from the action of the hydrophobic membrane attack-complex (MAC) [19].

The susceptibility of *P. penneri* and *P. vulgaris* strains to the bactericidal action of NHS as well as the swarming phenomenon were examined. The research work undertaken was also aimed at showing the possible correlation between these two pathogenic factors and the chemical structure of the O-specific chains of *Proteus* LPSs.

Material and Methods

Bacterial Strains

Seventy-two *P. penneri* and thirty *P. vulgaris* strains were used in the study (Table 1). Clinical isolates were identified by the API 20E test (bioMèrieux).

Sera

The blood from four healthy donors was obtained from the Regional Centre of Blood Donation and Blood Health Service in Lodz, Franciszkanska 17/25. After the separation from the clot, the sera were mixed and stored in 0.5 ml portions at –74˚C.

Lipopolysaccharides

All analyzed LPSs from *Proteus* sp. strains were isolated using the phenol-water procedure [20].

LPSs derived from *P. penneri* strains were kindly obtained from the Department of General Microbiology of the University of Lodz in Poland.

Poliacrylamide Gel Electrophoresis and Silver Staining of LPSs

Samples containing 5 μg of *P. penneri* LPSs were dissolved in the sample buffer (0.167 M Tris; 1.67% DOC; glycerine; mercaptoethanol; bromophenol blue) and boiled for 10 minutes. Sodium deoxycholate poliacrylamide gel electrophoresis (DOC-PAGE) was carried out using 12.5% separating acrylamide gel and 3% stacking gel. For the first half hour, the electrophoresis was performed at 16 mA constant current per gel and for the rest of the time at 22 mA in the Tris-glycine buffer (25 mM Tris; 190 mM glycine pH 8.3 with the addition of 0.25% DOC) [21].

After DOC-PAGE of LPSs, the gels were washed in a mixture of ethanol, acetic acid and water at a ratio of 8:1:11. Then, the gels were stained with silver nitrate according to the method by Tsai and Frasch [22]. This technique contained three stages. The first included oxidation (with 0.007% periodate in the washing solution for 5 minutes), the second involved the process of staining with 0.14% silver nitrate with the addition of 0.34% ammonia and 0.019 N sodium hydroxide for 10 minutes. The last phase was designed to bring about coloration by using a mixture of 0.005% citric acid and 0.0185% formaldehyde.

Bactericidal Activity of NHS

Bacteria at approximately 10^7 CFU/ml were mixed with an equal volume of serum and incubated for up to 3 hours at 37°C. The bactericidal activity of NHS was determined immediately after mixing the bacteria with the serum (time 0), and after 1 and 3 h incubation. Then the samples were diluted and

Table 1. The list of *Proteus* sp. strains

Tabela 1. Lista szczepów *Proteus* sp.

Strain (Szczep)	Number of strains (Liczba szczepów)	Description (Opis)	Source (Pochodzenie)
Proteus penneri			
1–45	45	laboratory strains	CDC, Atlanta, USA
50–58	9	laboratory strains	Institute of Epidemiology, Wernigerode, Germany
61–74, 33515, 25463 26555, 33352	14 4	laboratory strains ATCC	Institute of Microbiology and Immunology, University of Lodz, Poland
Proteus vulgaris			
5/57, 9/57, 10/57, 17/57, 25/57, 30/57, 32/57, 37/57, 39/57, 40/57, 43/57, 44/57, 48/57, 55/57, 56/57, 57/57, 63/57, 65/57, 70/57, 71/57, 72/57, 73/57	22	CCTC	The Czechoslovak National Collection of Type Cultures
18984, 4635	2	CCUG	Collection of Cultures University of Goteborg
265/10, 309/11, 402, 599/10, 269/10, 10	6	clinical isolates	Institute of Microbiology and Immunology, University of Lodz, Poland

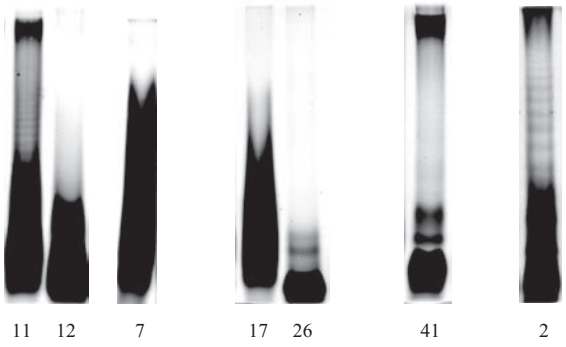

Fig. 1. Examples of silver stained DOC-PAGE electrophoregrams of *P. penneri* LPSs

7, 12, 17, 26 – strains with a short-length O-specific chain of LPS
2, 11, 41 – strains with a long O-specific chain of LPS

Ryc. 1. Przykłady rozdzielonych w żelu poliakrylamidowym i wysrebrzonych LPS szczepów *P. penneri*

7, 12, 17, 26 – szczepy z krótkołańcuchowymi częściami O-swoistymi LPS
2, 11, 41 – szczepy z długołańcuchowymi częściami O-swoistymi LPS

plated on agar medium. Subsequently, the number of colonies was scored taking as 100% their number grown from time 0. Those strains were regarded as resistant to bactericidal activity of NHS whose survival in the serum after 3 h of incubation was 50% of the initial number at time 0 [23]. A similar procedure was followed to investigate the bactericidal activity of the serum in which an alternative pathway of complement activation was blocked by 20 min. incubation of the serum at 50°C [23].

Swarming Growth

Bacterial strains were grown in an L broth medium (10 g of Bacto tryptone, 5 g of Bacto yeast extract, and 5 g of NaCl per liter). Single colonies, which were maintained on non-swarming LSW$^-$ agar (20 g of Bacto agar, 10 g of Bacto tryptone, 5 g of Bacto yeast extract, 0.4 g of NaCl, and 5 ml of glycerol per liter), were subsequently cultured in 5 ml of L broth at 37°C with rapid shaking (225 rpm) for 12–18 h. Then 10 µl of cultures was spotted on L broth agar (L broth containing 15 g of Bacto agar per liter) plates, previously dried to remove drops of water from the surface of the agar, and incubated at 37°C for 24 h [24]. After 24 h the concentric swarm zones were measured.

Results

Studies of the bactericidal action of NHS were carried out on 33 *P. penneri* and 29 *P. vulgaris* strains. Detailed and summary results are shown in Tables 2 and 3, respectively.

It was observed that the survival of bacterial strains from both analyzed species after 3 h of incubation at 37°C with NHS was similar. However, a few more strains showed sensitivity to the serum (17 *P. penneri* and 18 *P. vulgaris* strains) than resistance (16 *P. penneri* and 11 *P. vulgaris* strains). Contrary to the results obtained for NHS, the percentage of the resistant strains grew dramatically when the serum with blocked complement activation via alternative pathway was used (76% of *P. penneri* and 97% of *P. vulgaris* strains, respectively).

Attempting to discover the relationships between the sensitivity of bacteria to NHS and the structure of the cell surface, the next stage of the investigations involved was LPS analysis. Sodium deoxycholate-PAGE of the lipopolysaccharides was applied. Poliacrylamide gel electrophoresis of 33 *P. penneri* LPSs revealed that 14 of them contained short-length O-specific chains of LPS (two were of R-type) and 9 of them appeared to be sensitive to NHS (Figure 1).

The second part of the research was focused on the very intriguing swarming phenomenon. When short *P. penneri* bacilli (swimmer cells) are transferred to a solid medium, they start to differentiate into long (20–80 µm), multinucleated, nonseptated cells containing even 500 fold more flagella (swarmer cells). The population of swarmer cells can migrate on the surface of solidified media creating characteristic swarming circles (Figure 2).

Detailed results of the swarming growth expression among 72 *P. penneri* and 30 *P. vulgaris* strains are shown in Tables 4 and 5. The largest number of investigated *P. vulgaris* strains expressed a strong ability to swarm (swarm zones 81–90 mm), however 6 strains appeared to be non-swarming. Most *P. penneri* strains were characterized by a strong and medium ability to swarm. Nevertheless, there was quite a large group of strains, consisting of 17 strains, which expressed very weak swarming growth. There were no strains unable to swarm within this species.

Discussion

The research on the bactericidal effect of normal human serum to bacterial cells was carried out on 33 *P. penneri* strains and 29 *P. vulgaris* strains. In the test, two kinds of sera preparations were applied. The normal serum and serum in which the alternative pathway of complement activation was blocked. When the normal serum was used, it appeared that 48% of *P. penneri* and 38% of *P. vulgaris* strains were resistant. The use of the

Table 2. Sensitivity of *P. penneri* and *P. vulgaris* strains to the action of different sera preparations

Tabela 2. Wrażliwość szczepów *P. penneri* i *P. vulgaris* na działanie różnych preparatów surowicy

Strain (Szczep)	NHS (n.s.l.)		Serum previously incubated for 20 min. at 50°C (Surowica inkubowana 20 min. w 50°C)		Strain (Szczep)	NHS (n.s.l.)		Serum previously incubated for 20 min. at 50°C (Surowica inkubowana 20 min. w 50°C)	
	survival of bacterial cells [%]	sensitivity of strain	survival of bacterial cells [%]	sensitivity of strain		survival of bacterial cells [%]	sensitivity of strain	survival of bacterial cells [%]	sensitivity of strain
P.p. 1	39	s	3858	r	*P.p.* 65	26	s	2667	r
P.p. 2	107	r	2080	r	*P.p.* 71	0	s	0	s
P.p. 3	676	r	1165	r	*P.v.* 4635	2	s	2455	r
P.p. 4	164	r	762	r	*P.v.* 18984	1	s	4770	r
P.p. 7	0	s	0	s	*P.v.* 5/57	14	s	117	r
P.p. 8	0	s	0	s	*P.v.* 9/57	190	r	253	r
P.p. 11	808	r	3516	r	*P.v.* 10/57	17	s	1268	r
P.p. 12	304	r	1337	r	*P.v.* 17/57	100	r	200	r
P.p. 14	137	r	1115	r	*P.v.* 25/57	1085	r	1028	r
P.p. 15	64	r	789	r	*P.v.* 30/57	0	s	104	r
P.p. 16	134	r	369	r	*P.v.* 32/57	1	s	2683	r
P.p. 17	9	s	2757	r	*P.v.* 37/57	12	s	524	r
P.p. 18	0	s	0	s	*P.v.* 39/57	12	s	78	r
P.p. 19	5	s	29	s	*P v.* 40/57	2	s	3027	r
P.p. 22	0	s	0	s	*P.v.* 43/57	0	s	1184	r
P.p. 25	3245	r	800	r	*P.v.* 44/57	281	r	1256	r
P.p. 26	0	s	0	s	*P.v.* 48/57	0	s	0	s
P.p. 34	348	r	4805	r	*P.v.* 55/57	0	s	1006	r
P.p. 35	1	s	2384	r	*P.v.* 56/57	0	s	633	r
P.p. 37	0	s	373	r	*P.v.* 57/57	765	r	1635	r
P.p. 40	0	s	113	r	*P.v.* 63/57	180	r	4370	r
P.p. 41	0	s	3906	r	*P.v.* 65/57	0	s	390	r
P.p. 42	0	s	0	s	*P.v.* 70/57	111	r	1170	r
P.p. 43	1417	r	1289	r	*P.v.* 71/57	72	r	566	r
P.p. 44	1	s	441	r	*P.v.* 72/57	700	r	1775	r
P.p. 51	721	r	1775	r	*P.v.* 73/57	0	s	2588	r
P.p. 52	4800	r	3665	r	*P.v.* 265/10	28	s	2336	r
P.p. 56	0	s	777	r	*P.v.* 309/11	1439	r	1364	r
P.p. 57	467	r	1916	r	*P.v.* 402	4243	r	1117	r
P.p. 58	73	r	1018	r	*P.v.* 599/10	3	s	1669	r
P.p. 62	594	r	928	r	*P.v.* 269/10	0	s	1876	r

s – sensitivity; r – resistance.
P.p. – P. penneri; P.v. – P. vulgaris.

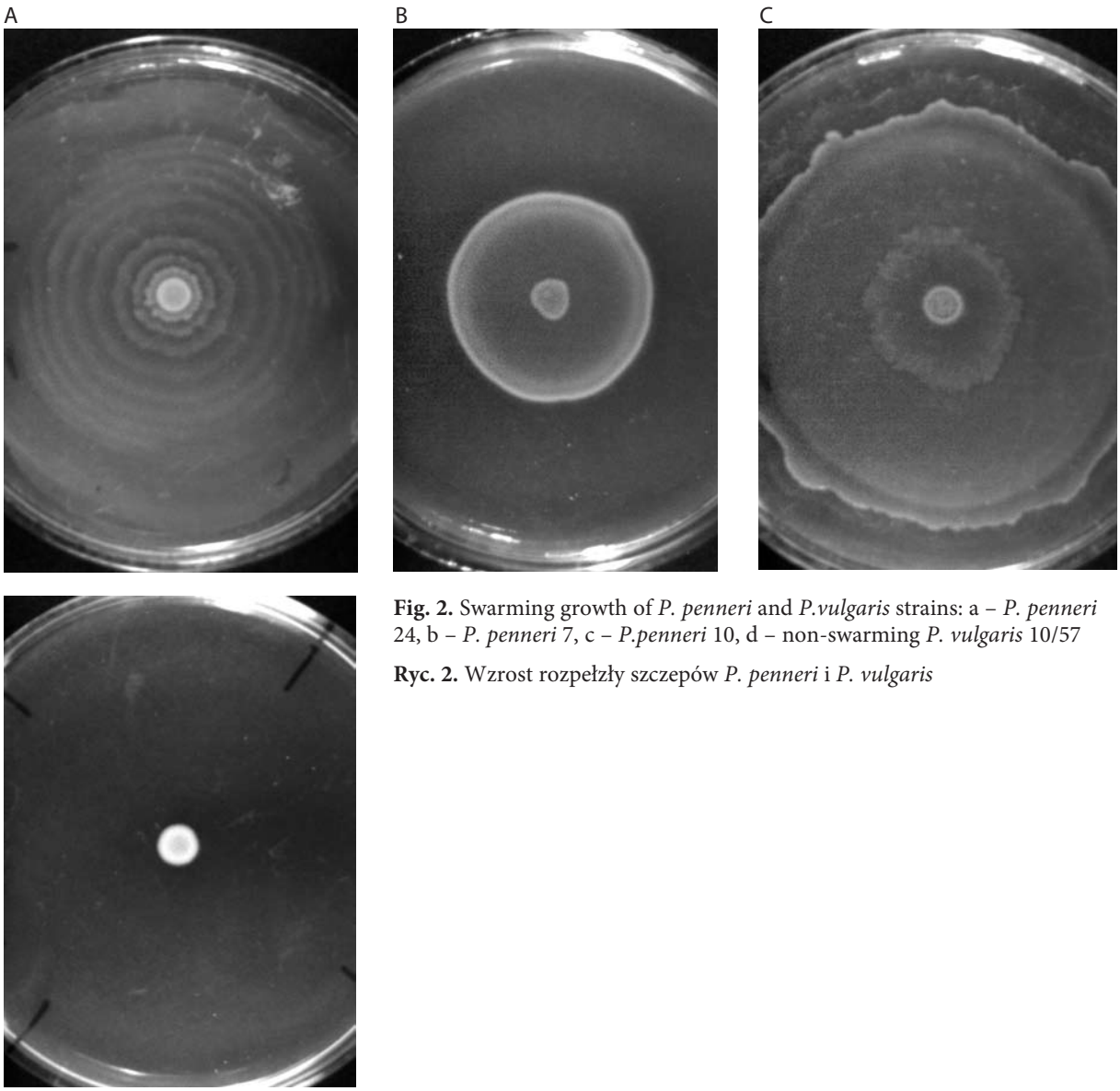

Fig. 2. Swarming growth of *P. penneri* and *P.vulgaris* strains: a – *P. penneri* 24, b – *P. penneri* 7, c – *P.penneri* 10, d – non-swarming *P. vulgaris* 10/57

Ryc. 2. Wzrost rozpełzły szczepów *P. penneri* i *P. vulgaris*

Table 3. Summary results of the bactericidal action of sera preparations

Tabela 3. Zbiorcze wyniki bakteriobójczego działania preparatów surowicy

Species (Gatunek)	Serum (Surowica)	The number of resistant strains (Liczba szczepów opornych)	% resistant strains (% szczepów opornych)	The number of sensitive strains (Liczba szczepów wrażliwych)	% sensitive strains (% szczepów wrażliwych)
P. penneri	NHS	16	48	17	52
	serum previously incubated for 20 min. at 50°C	25	76	8	24
P. vulgaris	NHS	11	38	18	62
	serum previously incubated for 20 min. at 50°C	28	97	1	3

Table 4. Swarming growth of *P. penneri* and *P. vulgaris* strains after 24 h of incubation

Tabela 4. Wzrost rozpełzły szczepów *P. penneri* i *P. vulgaris* po 24 h inkubacji

Strain (Szczep)	Swarming growth after 24 h [mm] (Wzrost rozpełzły po 24 h inkubacji [mm])	Strain (Szczep)	Swarming growth after 24 h [mm] (Wzrost rozpełzły po 24 h inkubacji [mm])	Strain (Szczep)	Swarming growth after 24 h [mm] (Wzrost rozpełzły po 24 h inkubacji [mm])
P. penneri 1	60	*P. penneri* 35	14	*P. penneri* 25463	5
P. penneri 2	35	*P. penneri* 36	63	*P. penneri* 26555	42
P. penneri 3	63	*P. penneri* 37	4	*P. penneri* 33352	39
P. penneri 4	85	*P. penneri* 38	38	*P. penneri* 33515	76
P. penneri 5	53	*P. penneri* 39	33	*P. vulgaris* 4635	85
P. penneri 6	60	*P. penneri* 40	3	*P. vulgaris* 18984	85
P. penneri 7	21	*P. penneri* 41	80	*P. vulgaris* 5/57	85
P. penneri 8	2	*P. penneri* 42	2	*P. vulgaris* 9/57	85
P. penneri 9	63	*P. penneri* 43	61	*P. vulgaris* 10/57	0
P. penneri 10	36	*P. penneri* 44	4	*P. vulgaris* 17/57	85
P. penneri 11	44	*P. penneri* 45	49	*P. vulgaris* 25/57	58
P. penneri 12	49	*P. penneri* 50	3	*P. vulgaris* 30/57	35
P. penneri 13	3	*P. penneri* 51	28	*P. vulgaris* 32/57	85
P. penneri 14	79	*P. penneri* 52	3	*P. vulgaris* 37/57	85
P. penneri 15	9	*P. penneri* 53	52	*P. vulgaris* 39/57	85
P. penneri 16	76	*P. penneri* 54	67	*P. vulgaris* 40/57	85
P. penneri 17	74	*P. penneri* 55	62	*P. vulgaris* 43/57	3
P. penneri 18	33	*P. penneri* 56	29	*P. vulgaris* 44/57	85
P. penneri 19	55	*P. penneri* 57	3	*P. vulgaris* 48/57	0
P. penneri 20	46	*P. penneri* 58	3	*P. vulgaris* 55/57	65
P. penneri 21	64	*P. penneri* 61	2	*P. vulgaris* 56/57	52
P. penneri 22	4	*P. penneri* 62	6	*P. vulgaris* 57/57	60
P. penneri 23	75	*P. penneri* 63	2	*P. vulgaris* 63/57	0
P. penneri 24	59	*P. penneri* 64	51	*P. vulgaris* 65/57	0
P. penneri 25	58	*P. penneri* 65	65	*P. vulgaris* 70/57	85
P. penneri 26	2	*P. penneri* 66	59	*P. vulgaris* 71/57	85
P. penneri 27	62	*P. penneri* 67	42	*P. vulgaris* 72/57	85
P. penneri 28	77	*P. penneri* 68	33	*P. vulgaris* 73/57	8
P. penneri 29	59	*P. penneri* 69	36	*P. vulgaris* 265/10	40
P. penneri 30	47	*P. penneri* 70	56	*P. vulgaris* 309/11	85
P. penneri 31	73	*P. penneri* 71	3	*P. vulgaris* 402	85
P. penneri 32	71	*P. penneri* 72	61	*P. vulgaris* 599/10	85
P. penneri 33	3	*P. penneri* 73	55	*P. vulgaris* 269/10	15
P. penneri 34	33	*P. penneri* 74	38	*P. vulgaris* 10	0

Table 5. Categories of migration of *P. penneri* and *P. vulgaris* strains

Tabela 5. Przedziały migracji szczepów *P. penneri* i *P. vulgaris*

Ability to swarm (Zdolność do migracji)	Range [mm] (Przedział [mm])	*P. penneri*		*P. vulgaris*	
		number of strains	[%]	number of strains	[%]
Unable to swarm (Niezdolny do pełzania)	0	0	0	5	17
Very weak (Bardzo słaba)	1–5	17	24	1	3
Weak (Słaba)	6–30	6	8	2	7
Medium (Średnia)	31–55	22	31	3	10
Strong (Silna)	56–80	26	36	3	10
Very strong (Bardzo silna)	81–90	1	1	16	53

serum with the inactivated alternative pathway of the complement caused a considerable increase in resistance among tested *P. penneri* and *P. vulgaris* strains to 76% and 97% respectively.

Taking into account the influence of the LPS structure on the bactericidal action of NHS, the authors extended their research on the LPS analysis. Lipopolysaccharides which are present on the surface of Gram-negative *Proteus* bacilli are antigenically highly heterogenic, mostly in the O-specific part of LPS. This is caused by structural differences of the O-specific polysaccharide chain of LPS (O-antigen). Depending on the number of the repeats of sugar constituents which are present in the O-PS, the authors can distinguish between long-chained and short-chained LPSs. The length of the side chain of the O-PS part could be visualized in an electrophoretic pattern in poliacrylamide gel after electrophoresis of LPS. All 33 preparations of LPS were separated in DOC-PAGE and silver stained. To estimate whether the O-antigenic part of LPS has long or short O-polysaccharide chains of LPS, in some cases the authors also used the data of western-blot analysis kindly provided by the Department of General Microbiology, University of Lodz (data not shown). In the group of analysed *P.penneri* strains, there were 14 which had a short-chained O-specific part of LPS, including two strains of the rough type (R-type), devoid of the O-antigenic part. Nine of them revealed sensitivity to NHS. This data is in agreement with the concept of the membrane attack complex action which is directly incorporated in the outer membrane of phenotypically rough cells or cells with LPS containing deep defects in LPS structure [19, 25]. The permanent hydrophobic bond of MAC with lipid A initiates the process of cell lysis.

It was observed that after a blockage of the alternative pathway of the complement among 14 strains with a short O-specific chain of LPS, only three remained sensitive and 6, which were previously sensitive to native serum, became resistant. This confirms the role of complement efficiency in eliminating microorganisms from the host organism.

Among 19 *P. penneri* strains with long-chain O-specific LPS, slightly more strains were resistant than sensitive to NHS. In the case of strains phenotypically smooth, synthesizing all three parts of LPS (O-specific, core and lipid A), the membrane attack complex undergoes deposition on long chains of the O-specific part of LPS, far away from the outer membrane of the cell. Such localization of MAC is due to the binding of C3b protein (the main constituent of convertases) with long chains of O-PS. MAC, which is in that case attached to the cell surface by weak hydrophilic bonds, cannot reach proper domains in OM and it is removed. This confirms the thesis that the outer membrane of bacterial cells surrounded by "the coat" of long chains of the O-specific LPS is not easily available for the membrane attack complex and this mechanism helps to protect microorganisms from lysis.

Taking into account that not all tested strains which had long side chains of LPS were resistant to NHS, the potential role of some other factors which make bacteria vulnerable to serum bactericidal activity should be highlighted. These agents most probably act in concert rather than individu-

ally. Lysozyme is probably one of them. By decomposing the mucopeptides which build the inner membrane of the cells, lysozyme facilitates MAC the access to the place of binding [23, 26]. Supposedly the activity of lysozyme depends on its muramidase activity which leads to degradation of the murein layer and the killing of the bacteria. However research on the heat-denaturated T4 lysozyme also revealed its non-enzymatic microbicidal activity, which is based on membrane disruption [27]. Also, the resistance of bacteria to the serum may be related to the production of proteases which inactivate the complement's proteins [28, 29].

Some papers report a relation between the chemical structure of the O-specific part of LPS and the sensitivity of bacterial cells to the action of NHS. Such research has been successfully done for the genus *Salmonella*. Analysis of the chemical structure of the O-specific part of LPS of strains *S. typhimurium* and *S. enteritidis* sensitive to the complement activity revealed that these cells did not possess rhamnose, mannose and abeqose [19].

As the authors found the idea of searching for the influence of the chemical structure of the O-specific part of LPS on the sensitivity of bacteria to NHS very interesting, the authors decided to do similar research for *Proteus* sp. strains. These structures were previously established [30]. Nevertheless, despite a detailed analysis, the authors did not manage to establish significant relationships between the chemical structure of the O-specific part of LPS and sensitivity to the action of NHS. It was observed that almost all *P. penneri* sensitive strains were characterized by the presence in their O-specific part of LPS N-acetylogalactosamine, and in some of them N-acetylogalactosamine and galacturonic acid. Nevertheless, these compounds were also found in LPSs of the resistant strains.

A very fascinating and thoroughly investigated feature of the genus *Proteus* is the swarming phenomenon [3, 5, 6]. There have been several reports on the possible relationships between the swarming growth of bacteria and the dynamic development of urinary tract infections particularly by the ascending route. It was documented that transferring *Proteus* bacilli from the liquid media to L-broth agar plates is the first signal for differentiating short swimmer cells into long swarmer cells. Owing to the coordinated movement of flagella, the population of hyperflagellated swarmer cells can translocate over the surface of the solid medium until they reach the consolidation phase. The process repeats and finally the surface of the solidified medium becomes covered with concentric circles of migration and consolidation stages. Analysis of this specific feature of *P. penneri* bacterial cells revealed that most of the strains after 24 h of incubation at 37°C were characterized by a strong (zone of swarming 56–80 mm) or medium (zone of swarming 31–55 mm) ability to swarm. Strong swarming was expressed by only one *P. penneri* strain – swarm zone 85 mm. There were also less numerous groups of strains which migrated weakly and very weakly. Among the tested strains, there were none unable to migrate. With respect to the 72 *P. penneri* strains, this special phenomenon was analyzed for 9 hours. Every hour the diameter of swarming zones was measured and an additional final measurement was done after 24 h of incubation. It appeared that the ability to swarm was visible at the earliest after 3 h of the plate incubation at 37°C (data not shown). Similar findings were described by Belas et al. [12]. In comparison to the data obtained for *P. vulgaris* cells, more than half of the tested strains expressed a very strong ability to swarm. On the other hand, there were 5 strains unable to migrate.

Translocation of swarmer cell populations is probably facilitated by capsular polysaccharide as well as long O-antigenic side chains of lipopolysaccharide. Analysis of the chemical structure of the O-antigenic part of LPS of *P. penneri* strains which presented a strong ability to swarm indicated the presence of galacturonic acid, rarely glucuronic acid and hexosamines – the most frequently glucosamine and galactosamine. Nevertheless, it has to be emphasized that such components were also found in the O-PS of strains which migrated very weakly. The authors did not notice a correlation between the total charge of the O-specific part of LPS and the swarming phenomenon. Most strains exhibited an acidic character of the O-PS and only few were neutral. The latter were found both among strains migrating very well and very weakly. So solely the chemical structure of LPS is not a sufficient factor to decide upon the strength of swarming growth.

Comparing the strength of the swarming ability and the sensitivity of the tested strains to normal human serum, the authors observed that among 17 *P. penneri* strains which were sensitive to NHS, most strains swarmed very weakly although four strains in this group revealed a strong swarming ability. Among *P. vulgaris* strains, there was an opposite situation where more sensitive strains swarmed very strongly over the surface of the solidified media.

To conclude, it is obvious that the virulence factors of *Proteus* bacilli are aimed at assuring the survival of the bacteria in the human organism. They act together although their direct relationships are not always so clearly visible *in vitro*. Both of the virulence factors of *Proteus* bacilli described in this paper are important and worthy of further study.

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