The Prevailing O Serogroups Among the Serologically Differentiated Clinical *Proteus* Spp. Strains in Poland

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Abstract

In the years 2006-2011, 617 *Proteus* spp. strains isolated mostly from urine and wounds or other clinical sources from infected individuals were collected in Łódź, Poland. *P. mirabilis* species was dominating (86.9%), followed by *P. genomospecies*, *P. vulgaris*, and *P. penneri*. Ninety four per cent strains were recognized as S (smooth) forms. *P. mirabilis* exhibited the most intensive swarming growth. Serological studies (involving ELISA – enzyme-linked immunosorbent assay and Western blotting using native and adsorbed rabbit antisera) enabled classification of 80% S isolates into respective *Proteus* O serogroups among the 83 ones, described so far. The remaining strains seemed to be serologically unique. Detailed serological analysis confirmed that the recently described in Poland O78 serogroup is the most widespread. Also, 14 other serogroups have been found to predominate, being represented by ten or more strains. No unique structural feature of the prevalent O serotypes has been indicated. The observed big serological variety may suggest a low rate of *Proteus* spp. strains' transmission between patients or rapid changes in their lipopolysaccharides. However, the prevalence of some O serogroups indicates that particular serotypes may be in some ways beneficial to the strains producing these kinds of O antigen.

Introduction

Proteus spp. bacteria are peritrichously flagellated members of *Morganellaceae* family distinguished from the other representatives of the order *Enterobacterales* by intensive and spectacular swarming growth on solid media as a result of the multicellular differentiation phenomenon [1, 2]. These bacilli are detected in natural environments (water or soil) and in many species of wild and domestic animals. They have also been found to be a component of natural faecal microflora in a part of human population. On the other hand, *Proteus* spp. are opportunistic pathogens, which may affect mainly immunocompromised individuals and cause infections of the urinary tract and wounds, bacteraemia, abscesses in many organs or other infections [3]. Among the pathogenic species, *P. mirabilis* is most common followed by less frequently isolated *P. vulgaris*, *P. penneri*, *P. hauseri*, and three genomospecies without names (*P. genomospecies* 4, 5, and 6). Lack of biochemical reactions which would reliably distinguish between different *P. genomospecies* resulted in limited knowledge on their characteristics and isolation frequency [4, 5], when compared to well-described *P. mirabilis*. Urinary tract infections (UTIs) of *P. mirabilis* aetiology are frequently catheter-associated – CAUTIs, recurrent and dangerous due to the frequent and severe complications like *pyelonephritis* and *urolithiasis* [2].

Among numerous virulence factors of *Proteus* bacteria, the lipopolysaccharide (LPS) should be emphasized as an endotoxin and an important antigen determining the serological specificity of *Proteus* spp. strains. The LPS core region in the genus *Proteus* is structurally and serologically diverse, which has led to the formation of the R typing *Proteus* scheme containing 11 R serotypes [6]. However, mainly the highly immunogenic O-polysaccharide region determines the serological specificity of LPS S forms, which has become a basis for the classification of *Proteus* strains into 83 O serogroups [7, 8]. The first O type scheme for *P. mirabilis* and *P. vulgaris* strains including 49 O serogroups was founded by Kauffmann [9]. Later, Larsson [10] indicated among them O3, O6, O10, O11, O13, O23, O24, O26, O27, O28, O29, and

O30 as the most prevalent serogroups in many countries. The Kauffmann's scheme was further extended with other O serogroups containing all *Proteus* species [7] but only a few *P. penneri* and *P. mirabilis* strains come from Poland [11, 12]. Gathering a new wide collection of more than 600 *Proteus* spp. clinical isolates from the Łódź region (central Poland) enabled us to study their serological differentiation. Up to date, serologically unique strains from the collection have formed six new serogroups O77-O82 [13-18] and two new subgroups in O8 and O11 serogroups [19, 20]. In this work the serological variety of the whole collection of *Proteus* spp. strains is shown focusing on *Proteus* O serogroups most widespread in central Poland.

Methods

Bacterial strains, LPSs, physiological tests

The studied clinical *Proteus* spp. strains from different sources (Fig. 1) were kindly provided in 2006-2011 by four big medical laboratories in Łódź (Poland): in Barlicki Hospital, in Biegański Hospital, „Synevo”, „Diagnostyka”. Some strains were isolated from faeces of 189 volunteers using McConkey and Nogrady media plates and recognized as forming the urease-positive and lactose-negative colonies, as described [20]. All isolates were identified by the cultivation on the media proposed by Senior [21], modified as described [13], additional tests were applied for the strains recognized as *P.* genomospecies, as described [4, 15] (Table 1). The strains were retained in stocks (Luria Broth cultures with 25% glycerol) at -80°C.

S-R tests included both the thermal stability in the boiled broth medium test and the pseudoagglutination test [22]. Additionally, the swarming growth ability on Luria Broth medium with 1.5% agar was studied, according to Wilkerson and Niederhoffer [23].

Biomasses of the studied strains were obtained by 18-h cultivation on a nutrient broth medium supplemented with 0.2% glucose, with aeration, at 37°C. The strains were killed by 1% phenol, centrifuged, washed with distilled water and stored in +4°C (wet biomass) or lyophilized (dry biomass).

LPSs were extracted from strains' dry biomass using the classic phenol-water method, published by Westphal and Jann [24].

Serological investigation

We applied ELISA (enzyme-linked immunosorbent assay) and Western-blotting methods using bacterial biomasses and LPS preparations (extracted from selected studied strains or coming from our collection) as a source of O antigens as well as polyclonal rabbit sera specific to all the O serotypes reported in the genus *Proteus* (from our collection), as described earlier [15, 25]. In ELISA 50 ng of LPSs or 5 µg of the bacterial biomasses per well were applied for coating the F96 Maxisorp Nunc-Immuno plates. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was used as a substrate for peroxidase conjugated with the rabbit-IgG specific goat antibodies (Jackson ImmunoResearch). Antibody

titers were determined by measuring the absorbance (A_{405}) using Multiskan Go microplate reader (Thermo Scientific). Additionally, we used some antisera adsorbed by wet bacterial masses, according to the method by Drzewiecka *et al.* [13]. In Western blotting, antigens (bacterial biomass or LPS, 5-8 µg per lane) were separated during sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). Then, the samples were transferred to nitrocellulose (Whatman Schleicher & Schuel). The sera were diluted 1:500 in dot-blot-10% skimmed milk buffer. AP Conjugate Substrate Kit (Bio-Rad) was applied as a substrate of alkaline phosphatase conjugated with the rabbit-IgG specific goat antibodies (Jackson ImmunoResearch).

Results

Physiological features

Six hundred and seventeen strains were isolated in the Łódź city, central Poland (Fig. 1), in that number 606 ones from infected individuals. Urine isolates and wound isolates (including bacteria from pus, bedsores, fistulas, skin and wound swabs, and abscesses) were dominating. Other frequent sources of isolation were connected with the respiratory tract (bronchoaspirates, sputum, and the pharyngonasal cavity), faeces, and vagina, while strains from other sources were isolated rarely. Moreover, the bacteria (five *P. mirabilis* strains) were also detected in faecal samples taken from only five out of 181 healthy volunteers (2.8%) and in faecal samples from as many as six out of eight infected individuals (four *P. mirabilis*, one *P. vulgaris*, and one *P. genomospecies* 5/6 including four strains reported earlier [13, 14, 20]). Thus, it was shown that the 11 *Proteus* spp. strains were inhabiting the intestines of 5.8% studied volunteers.

The strains species were identified on the basis of the metabolic features of the isolates (Table 1). *P. mirabilis* species was definitely predominant, while the contribution of other detected *Proteus* species was much lower (Table 1). Totally, 41 isolates (6.6%) were found to belong to *P. genomospecies* 4 or 5/6, the two last are still impossible to distinguish on the grounds of their metabolic properties [4, 5], while no isolate was classified to *P. hauseri* species. Thus, *P. genomospecies* are isolated from clinical sources with a frequency comparable to that observed for *P. vulgaris* and all the isolates belonging to the so called *P. vulgaris* group together account for 11.7% of the whole collection, where *P. genomospecies* 4 is the rarest. Contrary to *P. mirabilis* and *P. penneri* (Fig. 1 a,b), among *P. vulgaris* and *P. genomospecies* the isolates from wounds were found to dominate, while the isolates from urine were less common (Fig. 1 c,d).

Table 1
Biochemical features and frequency of *Proteus* species occurrence (including the isolates reported earlier [13-20])

	<i>Proteus</i> species					
	<i>P. m.</i>	<i>P. v.</i>	<i>P. p.</i>	<i>P. h.</i>	<i>P. gsp. 4</i>	<i>P. gsp. 5/6</i>
Metabolic feature						
phenylalanine deaminase	+	+	+	+	+	+
mannose fermentation	-	-	-	-	-	-
urease production	+	+	+	+	+	+
indole production	-	+	-	+	+	+
ornithine decarboxylation	+	-	-	-	-	-
salicin fermentation	-	+	-	-	-	-
esculin hydrolysis	ns	+	ns	-	-	-
rhamnose fermentation	ns	ns	ns	-	+	-
lipase production	ns	ns	ns	-	+	+
DNase production	ns	ns	ns	-	+	+
Frequency						
strains no	536	31	9	0	9	32
percentage	86.9 %	5 %	1.5 %	0 %	1.5 %	5.2 %
ns, not studied <i>P.m.</i> , <i>Proteus mirabilis</i> <i>P.v.</i> , <i>Proteus vulgaris</i> <i>P.p.</i> , <i>Proteus penneri</i> <i>P.h.</i> , <i>Proteus hauseri</i> <i>P.gsp.</i> , <i>Proteus</i> genomospecies						

Thirty seven studied strains (6% of the collection) which were totally or partly non-thermal stable and agglutinated in 0.85% NaCl were recovered as R or S/R forms possessing LPS deficient in O polysaccharide or LPS with only one O-repeating unit, respectively. R forms constituted only 4.9% of *P. mirabilis* strains (26 out of all collected *P. mirabilis* isolates), while in *P. vulgaris* it was 9.7% (three out of

31 *P. vulgaris* isolates), in *P. genomospecies* 4 and 5/6 – 14.6% (six out of 41 isolates), and in *P. penneri* – 22.2% (two out of nine isolates). As expected, the majority of R strains were not able to swarm on the surface of solid Luria Broth medium. However, among the smooth strains, the non-swarming ones were also detected. Most of *P. mirabilis* isolates (more than 90%) were found to be able to swarm and 70% exhibited intensive swarming growth (on the distance >30 mm within 24 h). In the case of *P. penneri*, non-swarming strains constituted 22% (R forms only) while the other isolates were able to swarm effectively. The weakest swarming growth ability was observed among *P. vulgaris* and *P. genomospecies* isolates as 50 % of strains have not showed this ability or could swarm very weakly (<10 mm within 24 h).

Serological features

A total of the 580 S strains (possessing an O antigen in the LPS molecules) were serologically investigated to classify them into proper O serogroups in the genus *Proteus*. The results for 24 of these strains classified to O2, O8, O11, O77 – O82 serogroups, respectively, had been reported previously [13-20].

Biomasses of the S strains were studied in ELISA with rabbit polyclonal antisera specific to all the *Proteus* O serogroups described so far. Strong cross reactions comparable to the ones in the homologous systems (mostly to the titre 1:128000 or more) were the first indication on the serological type of the studied strains. But the obtained results were not always so clear.

Several biomasses were not recognized by any antisera (the O antigens present on the cells were possible to be masked by other cell wall components) and some cross reacted with more than one antiserum achieving various titres (other surface antigens may have also been recognized by the antibodies present in the used rabbit antisera). In these cases, LPS preparations were extracted from the biomasses by the phenol-water procedure [24]. Purified LPS antigens coated on the ELISA plates might be better exposed and better accessible to specific antibodies. Indeed, in consequence the O serotype of several such strains was identified.

These results allowed to recognize the similarity of most of the isolates (447/556 S forms studied in this work) to particular O serotypes among 83 *Proteus* O serogroups recognized so far, while the remaining strains seemed to be serologically unique and were considered to represent new O serogroups in the genus *Proteus*. One hundred and fifty six isolates have been preliminarily classified into 47 O serogroups (data not shown) formed by fewer than 10 studied strains. More than a half (291) of the studied here 556 S *Proteus* spp. clinical strains were initially considered as belonging to 14 predominating O serogroups consisting of 10 or more strains: O3, O6, O10, O11, O16, O18, O20, O27, O28, O30, O50, O60, O77, and O78.

To confirm that consideration, the sera specific to the 14 most prevalent O serotypes were adsorbed by the cross-reacting biomasses and their reactions with the homologous LPSs were analyzed in ELISA. In most cases, the reactions of the antisera in the homologous systems were totally abolished due to the

removal of all LPS-specific antibodies by the cross-reacting biomasses, indicating their antigenic identity and confirming the initial serological classification (Table 2). However, in some cases the adsorption was not complete and some remaining fractions of antibodies still reacted with homologous LPSs, although the reactions were obviously weaker than the ones of the native antisera (Table 2). In these 34 cases, some differences in the strains serospecificity were responsible for not full adsorption of the antisera.

Table 2

Cross reactions of studied strains with the respective O antisera (native and adsorbed by the studied strains' biomass) in ELISA.

O antiserum	Reciprocal titre of antiserum reacting with		
	homologous LPS	studied strains biomass	homologous LPS after adsorption (number of adsorbing strains)
03	256,000	32,000-512,000	< 1,000 (17) 2,000-8,000 (4)
06	64/128,000	32,000-128,000	<1,000 (19)
010	512,000	64,000-512,000	<1,000 (9)
011	256,000	64,000-512,000	4,000-32,000 (12)
016	256,000	32,000-512,000	<1,000 (31) 2,000-4,000 (2)
018	128,000	64,000-512,000	<1,000 (22)
020	512,000	256,000-2,048,000	16,000-64,000 (3)
027	1,024,000	64,000-2,048,000	<1,000 (11)
028	64/128,000	32,000-512,000	<1,000 (24)
030	512,000	128,000-1,024,000	<1,000 (18)
050	64/128,000	32,000-512,000	<1,000 (13) 4,000 (2)
060	512,000	64,000-512,000	<1,000 (12)
077	256,000	64,000-512,000	<1,000 (11)
078	512/1,024,000	64,000-512,000	4,000 (1)
		32,000-512,000	<1,000-1,000 (10)
			<1,000 (16)
			<1,000 (44) 2,000-32,000 (10)

The nature of the cross reactions, observed in ELISA, was visualized in the Western-blotting method using LPS preparations obtained by proteinase-K treated biomasses as antigens (Fig. 2-4). The reactions are similar and comparable to the ones observed for the LPS preparations obtained using phenol-water

extraction, which is demonstrated on the example of strains classified to O77 serogroup (Fig. 2a,b). By the degradation of proteins, we could remove possible non-specific cross reactions caused by protein antigens rather than by O antigens [26]. Indeed, the initially observed in ELISA reactions of several biomasses not fully adsorbing the respective antisera, could have been caused by protein antigens, as they were not confirmed in Western blots. That was the case of four strains (Fig. 3b, paths 2-5) preliminarily classified into O10 serogroup, two strains (Fig. 3c, paths 8, 9) classified into O28 serogroup, and one strain (Fig. 3d, path 8) classified into O50 serogroup, which displayed completely no reaction after proteinase-K treatment in Western blots.

The Western-blotting technique also allowed localizing LPS epitopes responsible for the strong cross reactions initially observed in ELISA. The epitopes may have been present in the O-specific part and/or in the core region of the LPSs exposed on the biomasses of the studied strains. Indeed, among the strains preliminarily classified into O10 serogroup on the basis of strong reactions of their biomasses in ELISA, one isolate showed in Western blot reactions only in the fast-migrating fractions corresponding to the lipid A-core region, while there were no reactions observed for the slowly migrating LPS bands additionally possessing O-polysaccharide chains (Fig. 3b, path 8). Similar results were obtained in the case of as many as four strains (Fig. 3a, paths 2, 6, 7, and 9) initially classified into O3 serogroup. The O3 and O10 antisera are visibly rich in antibodies recognizing core-localized common epitopes and responsible for strong cross reactions observed both in ELISA and in Western-blotting. Obviously, these five strains cannot be included in O3 or O10 serogroups, although they may form R serogroups.

Noteworthy, the 15 strains initially classified into the most numerous O78, O16, and O11 serogroups but not fully adsorbing the respective O78, O16, or O11 antisera (Table 2) displayed in Western blots visible strong reactions of the slowly-migrating LPS molecules possessing O polysaccharides (O-PS), which is showed on the example of the strains (2-7) classified to O78 serogroup (Fig. 4a, paths 2-7). The use of the O78, O16, or O11 antisera adsorbed by the respective biomasses in the Western-blotting technique proved that the observed serological differences refer mainly to the O-specific parts of these strains' LPS. As the antisera contain small amounts of anti-core antibodies, the possible differences in core antigens were hard to be detected. The adsorbed O78, O16, and O11 antisera did not react with the respective adsorbing biomasses, which confirmed the properly conducted adsorption processes (control), but still in these three antisera there were remaining some fractions of antibodies recognizing the epitopes located in the homologous O antigens (slowly-migrating LPS bands) but absent in the studied strains. The Western-blotting results for these 15 strains were similar and they are shown on the example of O78 antiserum adsorbed with strain 2 (Fig. 4b). Thus, the 15 strains were recognized as representing different subgroups within O78, O16, or O11 serogroups, respectively.

Discussion

In the years 2006-2011 a total of 617 *Proteus* spp. strains were collected in Łódź, Poland (Fig. 1) including the 24 clinical strains reported earlier [13-20]. What is worth noticing, no strains were collected from blood, although *Proteus* spp. bacteria may also cause severe bacteremia and sepsis [2, 27]. Urine

and wound isolates were prevailing especially among the ubiquitous *P. mirabilis*. Frequent isolation of *P. mirabilis* (Table 1) is not surprising, as the species is regarded as the most virulent and widespread species in the genus *Proteus*, especially in patients suffering from nosocomial urinary tract and wound infections and it is still the most frequently isolated species among *Proteus* spp. [2]. *P.* genomospecies 4, 5, 6, or *P. hauseri* are not usually distinguished from *P. vulgaris* in the routine laboratory work and so there are very few reports available on their occurrence in patients. In comparison, O'Hara *et al.* [4] and Janda *et al.* [5] studying several *P. hauseri*, *P.* genomospecies 4, 5, and 6 strains, mostly from human sources, found *P.* genomospecies 5 and 6 to be the most numerous. Our result confirm the low frequency of *P. penneri* isolation from clinical sources [28, 29]. R forms which are regarded as less virulent, were found among the isolates belonging to all the identified *Proteus* species. However, in the most pathogenic *P. mirabilis* species, the smallest number of R forms was detected and the best swarming ability was noticed. The feature of swarming is considered to be an important virulence factor of *P. mirabilis* bacteria and may be a form of their adaptation to the host environment (especially urinary tract). Moreover, *P. mirabilis* swarming cells facilitate an entry into the urinary tract of other non-motile species, which often leads to following polymicrobial UTIs [30]. *P. vulgaris* isolates demonstrated weaker swarming growth in comparison to *P. penneri* strains, similarly to the results achieved by Kwil *et al.* [30].

Serological studies confirmed that the studied strains possess various core types which is typical in the genus *Proteus*. Frequently, strains forming a common R serogroup do not belong to a common O serogroup and the strains classified into one O serogroup may differ in their core-region serospecificity [6, 11]. Thus, the use of antisera which are rich in core-specific antibodies is informative, but in the studies aimed at classifying *Proteus* spp. strains into proper O serogroups may be misleading. [19]. A good solution might be the use in the preliminary classification studies of the antisera deprived of core-specific antibodies removed by adsorption, which is hard to obtain, or the application of further detailed tests allowing a precise serological classification of strains on the basis of their O antigens serospecificity, as presented in this work. Among the studied strains, 13 isolates preliminarily classified into the respective predominating O serogroups were further excluded on the basis of subsequent detailed studies.

Our studies showed a big serological diversity among *Proteus* spp. strains isolated recently from patients in central Poland. However, half of the isolates (299) including 18 strains reported before [13-15, 19] belong to 15 most numerous O serogroups, comprising ten or more studied strains (Fig. 5). O78 serogroup is the biggest one, formed by 61 studied strains (10.5% of 580 collected S strains). This serogroup together with O77 and O79 serogroups were described as new ones not long ago by Drzewiecka *et al.* [13, 14] and Arbatsky *et al.* [15] and they currently include only the isolates from Poland. O50 serogroup, in turn, was reported in 2003 [32] and has so far been represented by *P. mirabilis* strain TG 332 described earlier as being serologically unique [33]. O60 serogroup was also formed in 2003 [34] for a non-clinical *P. myxofaciens* isolate from a gypsy larva. Lately, the species has been proposed to be excluded from the genus *Proteus* [35]. However, our results clearly indicate that O60 serotype exists among *P. mirabilis* and *P.* genomospecies clinical strains. The other 10 most prevalent serogroups have been included in the first classic serological scheme proposed for *P. mirabilis* and *P. vulgaris* bacteria [9]. What is interesting, the majority of these predominant serogroups had been previously reported as

frequently found among hundreds of clinical *P. mirabilis* and *P. vulgaris* isolates from urine, faeces, blood, or unknown sources. Summarizing these data, Larsson [10] indicated O3 serogroup as dominating in all reports and O10, O13, O26, O28, and O30 serogroups as the most prevalent ones. O6, O11, O23, O24, O27, and O29 serogroups were also widely distributed. Analyzing the serological properties of 99 Swedish and 24 Polish *P. mirabilis* strains from urinary tract infections, Kaca *et al.* [12] also found O10 and O30 to be the most numerous serogroups. However, the authors applied only 20 *Proteus* O-specific sera in their studies so the attachment of the strains to the other prevalent serogroups (*e.g.* O6, O26, O28, or O29) was not analysed. Research on *P. penneri* strains revealed that clinical strains and isolates from faeces of healthy people in the USA, Great Britain, Canada, France, Germany, and single strains from other countries belonged mostly to O17, O61, O64, and O65 serogroups [11, 36] including also *P. mirabilis* and/or *P. vulgaris* strains [7]. In the presented studies three *P. penneri* isolates were classified into O10 serogroup. Several *P. genomospecies* strains were classified into O11, O60, O78, and O79 serogroups and two *P. vulgaris* isolates into O27 and O78 serogroups. Thus, some of the prevalent serogroups turned out to be multi-species (Table 3), including not only the ubiquitous *P. mirabilis* but also the other species.

O78, O16, and O11 serogroups seem to be heterogenous. Further chemical analysis may show the differences in the structures of O antigens, responsible for their slight serological variety observed within these serogroups and will allow the formation of new subserogroups or new cross-reacting O serogroups.

It can be seen that for many years, *Proteus* spp. strains belonging to O3, O6, O10, O11, O27, O28, and O30 serogroups have been most frequently isolated in many different countries. What is worth noticing, the *Proteus* O60 antigen structure resembles that of the *Proteus* O30 antigen [7] so before the O60 serogroup was created in 2003 [34], O60 isolates could be classified to O30 serogroup due to their mutual similarity and strong cross reactivity revealed in our work (data not shown). It should be noted that at present, O78 serogroup obviously seems to be the most prevalent among Polish patients from the Łódź region accounting for 10.5% of all S isolates, although its predominance is not big. The prevalence of the serotypes dominating over others may be connected with some features of their O antigens. However, it is difficult to indicate common structural O-PS features (Table 3) distinguishing the biggest serogroups from the smaller ones, *e.g.* the compounds present exclusively in the frequent O antigens and, simultaneously, absent in the rarely found O serotypes [7]. Among the 15 most numerous serotypes, both branched and linear O-PS repeating units can be found, which are built of three-to-five sugars, including glucose, galactose, and their derivatives frequently occurring in *Proteus* O-PSs. The rest of 2-amino-2-deoxy-D-glucose (D-GlcN) is the most frequent constituent building all the dominating serotypes except O3, however, it is also the most widely distributed component of *Proteus* O serotypes [7]. Some of these frequent O antigens contain also rare sugars like altruronic acid or deoxytalose and other compounds like amino acids or ribitol phosphate and all of them are acetylated. None of these features is exceptional or atypical for *Proteus* O antigens. For example, the repeating unit of the most ubiquitous O78 antigen is composed of five different sugars and sugar derivatives. Three of them constitute the main chain and two form side branches. This antigen is not characterized by any unusual or unique structural feature. It shares disaccharide fragments with several *Proteus* O antigens, but no cross reactions have been noticed [14].

Conclusion

In general, clinical *Proteus* spp. isolates from central Poland are characterized by high serological diversity and include the representatives of almost all described O serogroups. These results may suggest that either *Proteus* spp. strains do not intensively transmit from one patient to another or they are able to rapidly change their antigenic specificity by avoiding the immune response from host organisms, increasing their own virulence and adapting to environmental conditions. Such examples of antigenic variation are known in other Gram-negative genera and may be caused either by mutations in O antigen genes or by inter/intra-species lateral transfer of O antigen genes by homologous recombination, IS elements, plasmids, or bacteriophages [37].

The genus *Proteus* is currently divided into 83 O serogroups [8] and their number continually grows exceeding numbers of serogroups in many other Gram-negative bacteria like the genera: *Shigella* or *Salmonella* (each 46 O serogroups), *Yersinia* (~35 O serogroups), *Citrobacter* (43 O serogroups), *Pseudomonas* (~30 O serogroups), and others, except for *Escherichia coli* (~180 O serogroups) and *Vibrio cholerae* (~200 O serogroups) [37, 38]. Despite the high serological diversity among *Proteus* bacilli, the results of the presented research confirm the persisting for many years predominance of some serogroups reported earlier [10] and reveal new prevalent O serotypes (especially O78). The presence of the representatives of different *Proteus* species among these serogroups suggests that the bacteria acquire these special O phenotypes as a result of adaptation in convergence processes. Thus, these O antigens may be in some ways attractive and beneficial to the expressing bacteria. It will be reasonable to investigate a clonal homology of one-species isolates belonging to the respective predominating O serogroups. Their diversity would confirm the special role of a particular O-antigen type in their survival and virulence.

The appointment of the most virulent O serotypes among the *Proteus* spp. bacilli would allow developing methods, which might enable rapid detection and eradication of such strains.

Declarations

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Authors contribution:

Dominika Drzewiecka led the project and acquired the funding. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Dominika Drzewiecka and all authors commented on previous versions of the manuscript, read and approved the final manuscript.

Data availability statement:

The data and materials are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Ethical approval:

No approval of research ethics committees was required.

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Statement of Informed Consent:

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References

1. Adeolu M, Alnajar S, Naushad S, Gupta RS (2016) Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for *Enterobacterales* nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *Int J Syst Evol Microbiol* 66:5575–5599, <https://doi.org/10.1099/ijsem.0.001485>
2. Armbruster CE, Mobley HLT, Pearson MM (2018) Pathogenesis of *Proteus mirabilis* *EcoSal Plus* 8:1-123, <https://doi.org/10.1128/ecosalplus.ESP-0009-2017>
3. Drzewiecka D (2016) Significance and roles of *Proteus* bacteria in natural environments. *Microb Ecol* 72:741–758, <https://doi.org/10.1007/s00248-015-0720-6>
4. O'Hara CM, Brenner FW, Steigerwalt AG, Hill BC, Holmes B, Grimont PAD, Hawkey PM, Penner JL, Miller JM, Brenner D (2000) Classification of *Proteus vulgaris* biogroup 3 with recognition of *Proteus hauseri* nov., nom. rev. and unnamed *Proteus* genomospecies 4, 5 and 6. *Int J Syst Evol Microbiol* 50:1869-1875
5. Janda JM, Abbot SL, Khashe S, Probert W (2001) Biochemical identification and characterization of DNA groups within the *Proteus vulgaris* *J Clin Microbiol* 39(4):1231-1234
6. Palusiak A (2016) Classification of *Proteus penneri* lipopolysaccharides into core region serotypes. *Med Microbiol Immunol* 205:615–624, <https://doi.org/10.1007/s00430-016-0468-8>

7. Knirel YA, Perepelov AV, Kondakova AN, Senchenkova SN, Sidorczyk Z, Różalski A, Kaca W (2011) Structure and serology of O-antigens as the basis for classification of *Proteus* Innate Immun 17:70–96, <https://doi.org/10.1177/1753425909360668>
8. Siwinska M, Zablotni A, Levina EA, Shashkov AS, Ovchinnikova OG, Rozalski A, Knirel YA (2020) The unique structure of bacterial polysaccharides - immunochemical studies on the O antigen of *Proteus penneri* 4034-85 clinical strain classified into a new O83 *Proteus* Int J Biol Macromol 163:1168-1174, <https://doi.org/10.1016/j.ijbiomac.2020.07.012>
9. Kauffmann F (1966) *Proteus*. In: Kauffmann F (ed) The bacteriology of *Enterobacteriaceae*. Munksgaard, Copenhagen, pp 333–360
10. Larsson P (1984) Serology of *Proteus mirabilis* and *Proteus vulgaris*. Methods Microbiol 14:187–214
11. Drzewiecka D, Zych K, Sidorczyk Z (2004) Characterization and serological classification of a collection of *Proteus penneri* clinical strains. Arch Immunol Ther Exp 52:121-128
12. Kaca W, Glenska J, Lechowicz L, Grabowski S, Brauner A, Kwinkowski M (2011) Serotyping of *Proteus mirabilis* clinical strains based on lipopolysaccharide O polysaccharide and core oligosaccharide structures. Biochemistry (Moscow) 76(7):851-861, <https://doi.org/10.1134/S0006297911070169>
13. Drzewiecka D, Arbatsky NP, Shashkov AS, Stączek P, Knirel YA, Sidorczyk Z (2008) Structure and serological properties of the O-antigen of two clinical *Proteus mirabilis* strains classified into a new *Proteus* O77 serogroup. FEMS Immunol Med Microbiol 54:185-194, <https://doi.org/10.1111/j.1574-695X.2008.00462.x>
14. Drzewiecka D, Arbatsky NP, Stączek P, Shashkov AS, Knirel YA, Sidorczyk Z (2010) Structural and serological studies of the O-polysaccharide of strains from a newly created *Proteus* O78 serogroup prevalent in Polish patients. FEMS Immunol Med Microbiol 58:269-276, <https://doi.org/10.1111/j.1574-695X.2009.00632.x>
15. Arbatsky NP, Drzewiecka D, Palusiak A, Shashkov AS, Zablotni A, Siwińska M, Knirel YA (2013) Structure of a Kdo-containing O polysaccharide representing *Proteus* O79, a newly described serogroup for some clinical *Proteus* genomospecies isolates from Poland. Carbohydr Res 379:100-105, <https://doi.org/10.1016/j.carres.2013.07.001>
16. Siwińska M, Levina EA, Ovchinnikova OG, Drzewiecka D, Shashkov AS, Różalski A, Knirel YA (2015) Classification of a *Proteus penneri* clinical isolate with a unique O-antigen structure to a new *Proteus* serogroup, O80. Carbohydr Res 407:131-136, <https://doi.org/10.1016/j.carres.2015.02.003>
17. Zablotni A, Arbatsky NP, Drzewiecka D, Shashkov AS, Knirel YA (2018) **Chemical characterization and serological properties of a unique O-polysaccharide of the *Proteus mirabilis* Sm 99 clinical strain - identification of a new, O81, serotype.** Int J Biol Macromol 118:1131-1135, <https://doi.org/10.1016/j.ijbiomac.2018.06.174>
18. Siwinska M, Levina EA, Shashkov AS, Kalinchuk NA, Drzewiecka D, Knirel YA (2019) Structural and serological characterization of the O82 antigen of a *Proteus mirabilis* strain isolated from a patient in Poland. Carbohydr Res 486, <https://doi.org/10.1016/j.carres.2019.107831>

19. Drzewiecka D, Arbatsky NP, Kondakova AN, Shashkov AS, Knirel YA (2016) Structures and serospecificity of threonine-containing O polysaccharides of two clinical isolates belonging to the genus *Proteus* and their classification into O11 subserogroups. *J Med Microbiol* **65**(11):1260-1266, <https://doi.org/10.1099/jmm.0.000360>
20. Drzewiecka D, Shashkov AS, Arbatsky NP, Knirel YA (2016) Immunochemical characterization of the O antigens of two *Proteus* strains, O8-related antigen of *Proteus mirabilis* 12 B-r and O2-related antigen of *Proteus* genomospecies 5/6 12 B-k, infecting a hospitalized patient in Poland. *Microbiology* 162:789-797, <https://doi.org/10.1099/mic.0.000274>
21. Senior BW (1997) Media and tests to simplify the recognition and identification of members of the *Proteeae*. *J Med Microbiol* 46:39-44
22. Kotelko K, Gromska W, Papierz M, Sidorczyk Z, Krajewska D, Szer K (1977) Core region in *Proteus mirabilis* *J Hyg Epidemiol Microbiol Immunol* 21:271-284
23. Wilkerson ML, Niederhoffer EC (1995) Swarming characteristics of *Proteus mirabilis* under anaerobic and aerobic conditions. *Anaerobe* 1:345-350
24. Westphal O, Jann K (1965) Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. *Methods Carbohydr Chem* 5:83-89
25. Sidorczyk Z, Zych K, Toukach FV, Arbatsky NP, Zabłotni A, Shashkov AS, Knirel YA (2002) Structure of the O-polysaccharide and classification of *Proteus mirabilis* G1 in *Proteus* serogroup O3. *Eur J Biochem* 269:1406-1412, <https://doi.org/10.1046/j.1432-1033.2002.02782.x>
26. Palusiak A (2015) The antigens contributing to the serological cross-reactions of *Proteus* antisera with *Klebsiella* *Mol Immunol* 64:228-234, <https://doi.org/10.1016/j.molimm.2014.11.016>
27. Kim B-N, Kim NJ, Kim M-N, Kim YS, Woo J-H, Ryu J (2003) Bacteraemia due to tribe *Proteeae*: a review of 132 cases during a decade (1991–2000). *Scand J Infect Dis* 35:98-103
28. Drzewiecka D, Sidorczyk Z (2005) Characterization of *Proteus penneri* species – human opportunistic pathogens. *Post Mikrobiol* 44:113-126 (in Polish)
29. Palusiak A (2013) Immunochemical properties of *Proteus penneri* lipopolysaccharides – one of the major *Proteus* virulence factors. *Carbohydr Res* 380:16-22, <https://doi.org/10.1016/j.carres.2013.06.025>
30. Armbruster ChA, Mobley HLT (2012) Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nat Rev Microbiol* 10:743-754, <https://doi.org/10.1038/nrmicro2890>
31. Kwil I, Kaźmierczak D, Różalski A (2013) Swarming growth and resistance of *Proteus penneri* and *Proteus vulgaris* strains to normal human serum. *Adv Clin Exp Med* 22(2):165-175
32. Kołodziejska K, Kondakova AN, Zych K, Senchenkova SN, Shashkov AS, Knirel YA, Sidorczyk Z (2003) Structure of the O-polysaccharide of a serologically separate strain of *Proteus mirabilis*, TG 332, from a new proposed *Proteus* serogroup O50. *Carbohydr Res* 338(20):2105-2109
33. Penner JL, Hennessy JN (1980) Separate O-grouping schemes for serotyping clinical isolates of *Proteus mirabilis* and *Proteus vulgaris*. *J Clin Microbiol* 12:304–309

34. Sidorczyk Z, Kondakova AN, Zych K, Senchenkova SN, Shashkov AS, Drzewiecka D, Knirel YA (2003) Structure of the O-polysaccharide from *Proteus myxofaciens*. Classification of the bacterium into new *Proteus* O serogroup. Eur J Biochem 270:3182-3188

35. Giammanco GM, Grimont PA, Grimont F, Lefevre M, Giammanco G, Pignato S (2011) Phylogenetic analysis of the genera *Proteus*, *Morganella*, and *Providencia* by comparison of *rpoB* gene sequence of type and clinical strains suggests the reclassification of *Proteus myxofaciens* in a new genus, *Cosenzaea* nov., as *Cosenzaea myxofaciens* comb. nov. Int J Syst Evol Microbiol 61:1638-1644, <https://doi.org/10.1099/ijs.0.021964-0>

36. Sidorczyk Z, Toukach FV, Zych K, Arbatsky NP, Drzewiecka D, Ziółkowski A, Shashkov AS, Knirel YA (2002) Structural and serological characterization of the lipopolysaccharide from *Proteus penneri* 20 and classification of the cross-reacting *Proteus penneri* strains 10, 16, 18, 20, 32 and 45 in *Proteus* serogroup O17. Arch Immunol Ther Exp 50:345–350

37. Wang L, Wang Q, Reeves PR (2010) The variation of O-antigens in gram-negative bacteria. In: Wang X, Quinn PJ (eds) Endotoxins: structure, function and recognition, Springer, Dordrecht, Heidelberg, London, New York, pp 123-152

38. Knirel YA (2011) Structure of O-antigens. In: Knirel YA, Valvano MA (eds) Bacterial lipopolysaccharides. Structure, chemical synthesis, biogenesis and interaction with host cells, Springer, Wien, New York, pp 41-116

Table

Due to technical limitations, table 3 is only available as a download in the Supplemental Files section.

Figures

Figure 1

The sources of isolation of the whole collection of 617 *Proteus* spp. strains and separately: *P. mirabilis* isolates (a), *P. penneri* isolates (b), *P. genomospecies* isolates (c), and *P. vulgaris* isolates (d) [this work and 13-20].

a

b

Figure 2

Western blots of O77 LPS (1) and proteinase K-treated biomasses (a) or LPSs (b) of the strains (2-17) classified to O77 serogroup with O77-specific serum.

Figure 3

Western blots of homologous LPSs (1) and proteinase K-treated biomasses of selected strains (2-10) preliminarily classified to O3 serogroup (a), O10 serogroup (b), O28 serogroup (c), and O50 serogroup (d) with the respective antisera.

a

1 2 3 4 5 6 7

b

1 2

Figure 4

Western blots of O78 LPS (1) and proteinase K-treated biomasses of selected strains (2-6) classified into O78 serogroup with O78 antiserum (a) and O78 antiserum adsorbed by the strain 2 (b).

Figure 5

Classification of 580 *S. Proteus* spp. strains into O serogroups [this work and 13-20].

Supplementary Files

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