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Serotyping of *Proteus mirabilis* Clinical Strains Based on Lipopolysaccharide O-Polysaccharide and Core Oligosaccharide Structures*

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Abstract—The aim of this work was to serotype *Proteus mirabilis* urinary tract infection (UTI) strains based on chemically defined O-antigens with the use of two clinical collections from Sweden and Poland consisting of 99 and 24 UTI strains, respectively. A simple two-step serotyping scheme was proposed using enzyme immunoassay with heat-stable surface antigens of *Proteus* cells and immunoblotting with isolated lipopolysaccharides (LPSs). Using polyclonal anti-*P. mirabilis* rabbit antisera, 50 Swedish and 8 Polish strains were classified into serogroups O10, O38, O36, O30, O17, O23, O9, O40, O49, O27, O5, O13, O24, O14, and O33. From the Swedish strains, 10 belonged to serogroup O10 and five to each of serogroups O38, O36, and O9. Therefore, none of the O-serogroups was predominant. The majority of the serotyped clinical strains possess acidic O-antigens containing uronic acids and various acidic non-carbohydrate substituents. In immunoblotting, antisera cross-reacted with both O-antigen and core of LPSs. The core region of 19 LPSs bound a single serum, and that of 12 LPSs bound more than two sera. Following bioinformatic analysis of the available sequences, a molecular approach to the prediction of *Proteus* core oligosaccharide structures was proposed. The identification of the core type of *P. mirabilis* R110, derived from a serogroup O3 wild strain, using restriction fragments length polymorphism analysis of galacturonic acid transferase is shown as an example. In summary, the most frequent O-serogroups among *P. mirabilis* UTI stains were identified. The diversity of serological reactions of LPSs is useful for serotyping of *P. mirabilis* clinical isolates. A possible role of the acidic components of O-antigens in UTI is discussed.

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Key words: lipopolysaccharide, O-antigen, *Proteus mirabilis*, serology, serotyping, glycosyl transferase

Proteus mirabilis belongs to the *Enterobacteriaceae* family. It is a Gram-negative, facultatively anaerobic bacterium occurring in soil, polluted water, and in the normal microflora of human and animal intestines. *Proteus mirabilis* bacteria are opportunistic pathogens responsible for infections of skin wounds and the human upper and lower urinary tract (UTI). *Proteus mirabilis* antigens may also play a role in rheumatoid arthritis [1].

Proteus cell wall contains an outer membrane with a lipopolysaccharide (LPS, endotoxin), an important virulence factor of *P. mirabilis* [2, 3]. LPS is also crucial in the identification of *Proteus* strains. The serological specificity of the bacteria is determined by structural differences in the O-polysaccharide chain (OPS, O-antigen) of LPS. Based on this, *Proteus* strains are classified into about 80 O-serogroups [4, 5]. Their serological heterogeneity is due to a high diversity in composition and structure of OPS [6]. *Proteus* OPS are branched or linear polysaccharides built up of oligosaccharide repeating units. All OPS contain amino sugars, such as D-glucosamine (GlcN) or D-galactosamine (GalN), and other common monosaccharide components including D-glucose, D-galactose, L-rhamnose (L-Rha), D-glucuronic acid (GlcA), and D-galacturonic acid (GalA), as well as some rarely occurring

Abbreviations: EIA, enzyme immunoassay; LPS, lipopolysaccharide; OPS, O-polysaccharide; RFLP, restriction fragment length polymorphism; UTI, urinary tract infections.

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sugars. It is typical that various non-carbohydrate components occur in *Proteus* OPSs [6].

At present, the prevalence of particular *P. mirabilis* O-serogroups among clinical isolates is unknown. Previously, *P. mirabilis* strains isolated in clinical settings have been found to belong to serogroups O3, O10, O11, O13, O23, O27, and O30. Now, knowing the defined structures of *P. mirabilis* O-antigens and having rabbit sera against heat-stable antigens enabled us to perform new serotyping studies. *Proteus mirabilis* laboratory strains representing each O-serogroup with chemically defined OPSs were used as serological testing standards, and a variety of serological methods were applied, mainly enzyme immunoassay (EIA) and immunoblotting. Rabbit sera against heat-killed bacteria were used to reveal the major components of OPSs responsible for binding specific antibodies. In addition, the O-antigens were modified by chemical methods, and changes in the serological reactivity were evaluated. Synthetic antigens, such as amides of uronic acids with amino acids, were used as well. In most cases cross-reactions of antisera were observed with heterologous antigens that were similar or identical to the tested antigens. The most informative was immunoblotting as it showed which part of LPS (OPS or core oligosaccharide) is recognized by antibodies. In some instances, EIA data were not confirmed by immunoblotting. In most cases the decision about the nature of epitopes was made based on several positive reactions demonstrated by at least two methods [4].

In the present study, 20 polyclonal rabbit sera specific for different O-polysaccharides were used for the serotyping of 123 clinical isolates of *P. mirabilis* from Swedish and Polish hospitals. The serological reactivity of the clinical strains and standard laboratory strains with defined O-antigen structures were compared. A molecular approach to *P. mirabilis* strain identification based on core structures is proposed.

MATERIALS AND METHODS

Bacterial strains, growth, and isolation of lipopolysaccharides. *Proteus mirabilis* laboratory strains were obtained from the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague), 99 and 24 clinical strains were from the Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden and from the Urology Department, Swietokrzyskie Oncology Center, Kielce, Poland, respectively.

Proteus mirabilis laboratory strains were cultivated under controlled aerobic conditions in nutrient broth (BTL, Poland). Cells were harvested at the end of the logarithmic growth phase, centrifuged (5000*g*, 30 min), washed with distilled water, and lyophilized. LPS was obtained by extraction of the bacterial mass with a phenol–water mixture [7] and purified by treatment with cold aq 50% CCl_3CO_2H followed by dialysis of the supernatant [8]. The LPS preparations obtained were essentially free of nucleic acid and contained <2.5% proteins. LPS preparations from the isolated clinical strains were not purified and were designated below as crude LPSs.

Serological techniques. Anti-*P. mirabilis* polyclonal rabbit sera were obtained, and EIA with LPS as antigen were performed as described [8]. Prior to use, LPS stock solution (1 mg/ml) in phosphate-buffered saline was shaken thoroughly and sonicated. SDS-PAGE was performed according to the procedure of Laemmli using 4% stacking gel and 12.5% resolving gel [9]. The gels were stained with silver nitrate (Sigma, USA) [10] or, for immunoblotting with diluted (1 : 250) anti-*P. mirabilis* polyclonal sera, LPSs were electroblotted from the gels onto nitrocellulose sheets (Schleicher and Schuell, Germany) as described [8, 11]. Secondary antibodies used in immunoblotting were the same as in EIA, and the color development reagent was 4-chloro-1-naphtol (Sigma) with H_2O_2 as substrate.

Identification of the galacturonic acid (GalA) transferase gene. Bioinformatic analysis was performed using programs available on-line on the NCBI server. Genes of the *waa* cluster as well as the *arn* operon, coding for phosphoethanolamine and GalA transferases were identified using Blink and BLAST database. Analysis was performed on *P. mirabilis* HI4320 genomic sequence (Accession No. NC_010554) and verified on *P. mirabilis* ATCC 29906 sequence (Accession No. NZ_ACLE00000000). The gene for GalA transferase was found using its similarities to the WabO transferase gene from *Klebsiella pneumoniae* [12]. Primers were designed with Primer-BLAST.

For polymerase chain reaction (PCR), genomic DNA matrix was prepared by boiling, and the presence of the GalA transferase gene was determined by restriction fragment length polymorphism (RFLP)-PCR analysis with the following primers: sense 5′-TTA AGT ATT GTG GTG GCG TTT-3′, antisense 5′-AAC CCG GCT CAA AGT AAT AAC C-3′. Then the PCR product was digested for 18 h with 2 U of the restriction enzyme Sau3AI (New England Biolabs, USA). The original PCR product and the product of Sau3AI digestion were run on 2% agarose gel and stained with ethidium bromide.

RESULTS

Serological epitopes of *P. mirabilis* strains and the serotyping scheme. Twenty sera were used for the serotyping of *P. mirabilis* clinical isolates. Purified LPSs from laboratory strains were used as control for each serological reaction with clinical strains. The structures of the OPSs of the control LPSs are presented in Table 1. Examples of proposed serological epitopes of *P. mirabilis*

Table 1. Structures of the O-polysaccharides of *P. mirabilis*

Serogroup	Strains	Structure of the O-polysaccharide
O3a,b	S1959	α-D-Gal*p*A6(L-Lys)-(1→4)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→ α-D-Glc*p*-(1→2)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→
O5	PrK 12/57	→4)-α-D-Glc*p*NAc3,6Ac$_2$-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→3)-β-D-Glc*p*NAc-(1→
O7	PrK 15/57	β-D-Qui*p*4NMal-(1→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→
O9	PrK 18/57	→4)-α-D-Gal*p*A-(1→2)-β-D-Rib*f*3Ac-(1→4)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→
O10	PrK 19/57, PrK 20/57, HJ 4320	α-L-Alt*p*A-(1→3)-α-D-Gal*p*NAc-(1→3)-α-D-Gal*p*A-(1→3)-α-D-Glc*p*NAc-(1→
O11	PrK 24/57	β-D-Glc*p*NAc-(1→2)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*A6(L-Thr)-(1→3)-β-D-Glc*p*NAc-(1→ α-D-Glc*p*-(1→6)
O13	PrK 26/57	α-D-Gal*p*A6(R-Cet-L-Lys)-(1→4)-β-D-Glc*p*NAc-(1→ →3)-α-D-Gal*p*-(1→3)
O14a,b	PrK 28/57, PrK 29/57	R-Cet-Etn*P*→6)-α-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*6Ac-(1→3)-β-D-Gal*p*NAc-(1→
O17	PrK 32/57	Etn*P*→6)-β-D-Fuc*p*3N(*R*3HOBu)-(1→6)-α-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→3)-α-D-Glc*p*NAc-(1→
O18	PrK 34/57	Cho*P*→4)-α-D-Glc*p*NAc-1-*P*-(O→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1 → α-D-Glc*p*-(1→4)
O20	PrK 38/57	α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→ →3)-α-D-Glc*p*NAc-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→
O23a,b,c	PrK 41/57	→2)-β-D-Gal*p*A-(1→3)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*A-(1→3)-β-D-Glc*p*NAc-(1→
O24	PrK 47/57	β-D-Gal*p*3,4(S-Pyr)→3)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→
O27	PrK 50/57	β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A6(L-Lys)-(1→3)-α-D-Gal*p*A6(L-Ala)-(1→3)-β-D-Glc*p*NAc-(1→ Etn*P*→6)
O30	PrK 53/57	→4)-β-D-Glc*p*A-(1→6)-α-D-Gal*p*NAc-(1→6)-β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc4Ac-(1→
O33	PrK 59/57, D52	D-Rib-ol-5-*P*→3)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→ Etn*P*→6)
O36	PrK 62/57	→2)-β-D-Rib*f*-(1→4)-β-D-Gal*p*A-(1→4)-α-D-Glc*p*NAc6Ac-(1→4)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→
O38	PrK 64/57	AcEtn*P*→6)-β-D-Qui*p*4N(Ac-D-Asp)-(1→6)-α-D-Glc*p*-(1→3)-α-D-Gal*p*A-(1→4)-α-D-Glc*p*NAc-(1→
O40	PrK 66/57	→3)-β-D-Glc*p*NAc4(R-Lac)-(1→3)-α-D-Gal*p*-(1→3)-D-Gro-1-*P*-(O→3)-β-D-Glc*p*NAc-(1→
O49	PrK 75/57	α-D-Quip4NSuc-(1→4)-α-D-Gal*p*A-(1→3)-α-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

OPSs recognized by the sera (marked in bold) are shown and observed cross-reactions are described below.

P. mirabilis O7 (PrK 15/57) [13]. Both in EIA and immunoblotting, anti-O7 serum cross-reacted with *P. mirabilis* O49 LPS. Their common OPS fragment is marked in the *P. mirabilis* O7 OPS structure above. The O49-antigen (its structure is shown below) contains 4-amino-4-deoxyquinovose (Qui4N) substituted by succinic acid (Suc) rather than malonic acid (Mal) in the O7-antigen. Most likely, a weak inhibitory potency of the O49 OPS is accounted for different N-substituents on Qui4N.

Qui*p*4NMal
1
↓
6
→3)-β-D-Glc*p*NAc-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→.

P. mirabilis O9 (PrK 18/57) [14]. The presence of ribose in the furanose form and its O-acetylation play the most important role in binding anti-O9 antibodies. O-Deacetylation abolished reactivity with anti-O9 serum. The β-D-Rib*f*3Ac-(1→4)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc trisaccharide fragment is shared by OPS of *P. vulgaris* O25 and is responsible for a strong cross-reaction with this strain.

→4)-α-D-Gal*p*A-(1→2)-**β-D-Rib*f*3Ac-(1→**4)-β-D--Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→.

P. mirabilis O20 (PrK 38/57) [15]. A cross-reactivity of anti-O20 serum with *P. mirabilis* O7 LPS in EIA, inhibition of the cross-reaction, and results of immunoblotting suggest that the β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc disaccharide fragment is important for anti-O20 antibodies binding. *Proteus mirabilis* O18 also cross-reacted with anti-O20 serum in immunoblotting due to the presence of a similar disaccharide fragment in its OPS.

α-D-Glc*p*-(1→2)-β-D-Gal*p*
1
↓
4
→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→.

P. mirabilis O18 (PrK 34/57) [16]. In the case of *P. mirabilis* O18, data of inhibition with dephosphorylated oligosaccharides showed that the main role in binding antibodies is played by the phosphate groups that interlink the glucosamine and glucose residues. Interestingly, phosphocholine and lateral glucose residues do not seem to be involved in binding. Seven out of 99 Swedish clinical strains reacted with anti-O18 serum. One of them, *P. mirabilis* 1784, reacted as strongly as the homologous strain *P. mirabilis* O18 (PrK 34/57). This showed that strain 1784 belongs to the O18 serogroup. Immunoblotting revealed also cross-reactivity with *P. mirabilis* O49 LPS.

α-D-Glc*p*
Cho*P* 1
↓ ↓
4 4
→3)-α-D-Glc*p*NAc-**1-*P*-(O→6)-β-D-Glc*p*-(1→3)-β-**D--Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→.

P. mirabilis O38 (PrK 64/57) [17]. The 2-acetamidoethyl phosphate (AcEtnP) residue contributed most significantly to the serospecificity of the *P. mirabilis* O38 LPS as shown by the lack of reaction of the dephosphorylated OPS of this strain in EIA inhibition in the homologous test system. The α-D-Glc*p*NAc-(1→3)-β-D-Qui*p*4N(Ac-D-Asp) disaccharide is shared by *Providencia stuartii* O33 LPS and is responsible for its strong cross-reaction with anti-O38 serum.

AcEtn*P*
↓
6
→4)-α-D-Glc*p*NAc-(1→3)-β-D-Qui*p*4N(Ac-D-Asp)--(1→6)- α-D-Glc*p*-(1→3)-α-D-Gal*p*A-(1→.

P. mirabilis O40 (PrK 66/57) [18]. A weak serological cross-reactivity of anti-O40 serum with *P. vulgaris* O15 and *P. penneri* 19 LPSs suggested a role of the GlcNAc disaccharide and a lactic acid (Lac) residue in the serological specificity of the *P. mirabilis* O40 LPS.

→3)-β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc4(*R*-Lac)--(1→3)-α-D-Gal*p*-(1→3)-Gro-1-*P*-(O→.

P. mirabilis O49 (PrK 75/57) [13]. Cross-reactivity of *P. mirabilis* O49 and O7 indicated that α-D-Qui*p*4N is a part of an epitope(s). That replacement of succinic acid by malonic acid in the *P. mirabilis* O7 LPS did not abolish the cross-reaction may be due to the presence of a similar disaccharide fragment in the main chain of both OPSs.

α-D-Qui*p*4NSuc
1
↓
4
→3)-β-D-Glc*p*NAc-(1→2)-α-D-Gal*p*A-(1→3)-α-L--Rha*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→.

Based on chemically defined O-antigens, a scheme for the serotyping of clinical *P. mirabilis* strains was proposed (Fig. 1). The initial classification of clinical strains into particular O-serogroups was based on EIA data with 1 : 500 diluted rabbit antisera. Dry bacterial mass was

used as antigen. In the next step, the *P. mirabilis* antigens that showed the highest reactivity were examined with serially diluted sera (from 1 : 500 to 1 : 24,000). Finally, the preliminary serotyping by EIA was confirmed by immunoblotting with crude LPSs as antigens. At each step, *P. mirabilis* laboratory strains and their purified LPSs were used as control.

Serotyping of *P. mirabilis* clinical strains. Ninety-nine and 24 *P. mirabilis* clinical strains from Swedish and Polish collections were tested according to the scheme presented in Fig. 1. As an example, the serotyping of *P. mirabilis* strains from Swedish collection reacting with rabbit anti-*P. mirabilis* O38 serum is shown in Fig. 2. Ten Swedish clinical strains (Fig. 2, gray bars) reacted with anti-O38 serum as strong as control (*P. mirabilis* O38 strain PrK 64/57; Fig. 2, black bars). Then, these 10 selected strains were examined with serially diluted anti-O38 serum. The strongest reaction (titre 50% – 1 : 3500) was observed for the homologous strain PrK 64/57. Only two strains, 67 and 111, bind antibodies in serum diluted 1 : 2000 and 1 : 800, respectively. Titre 50% for eight remaining eight strains was observed with serum diluted below 1 : 500.

For final serotyping of clinical isolates, immunoblotting with crude LPSs as antigens was performed (Fig. 3). The reactivity of anti-O38 serum with heterologous LPSs and the purified homologous LPS of *P. mirabilis* PrK 64/57 was different. Strong reactions with both core oligosaccharide and OPS parts of LPS were observed for seven of the examined strains. Only slow migrating bands of LPS with high molecular mass OPS from three more strains were recognized by rabbit antibodies. These findings enabled a preliminarily assignment of the ten strains to serogroup O38. However, strain 98 was not classified owing to its weak cross-reactivity with anti-O38 serum. Strains 57, 62, 65, and 67 were excluded from serogroup O38 as they cross-reacted with other sera. LPSs from strain 57 and 62 showed stronger and specific reactions with anti-O49 and anti-O17 sera, respectively, and strains 65 and 67 reacted with anti-O9 serum (data not shown). Therefore, clinical isolates 36, 38, 42, 111, and 112 were classified as those of the O38 serogroup, and isolates 57, 62, 65, and 67 as putative "O38" strains with a minor O38 antigen(s). The summary of the serotyping of strains from the Swedish collection is presented in Table 2.

In general, only few strains are classified to each serogroup. In several cases, the precise classification was possible. However, strong cross-reactivity with different sera made the final decision on some strains impossible. As a result, 50 from 99 strains of the Swedish collection were serotyped. Most often occurred strains of serogroup O10 (10 strains) as well as serogroups O30 (6 strains), and O38, O36, and O9 (five strains of each). No strains were found to belong to serogroups O3, O7, O11, O18, or O20. In the Polish collection, eight from 24 strains were classi-

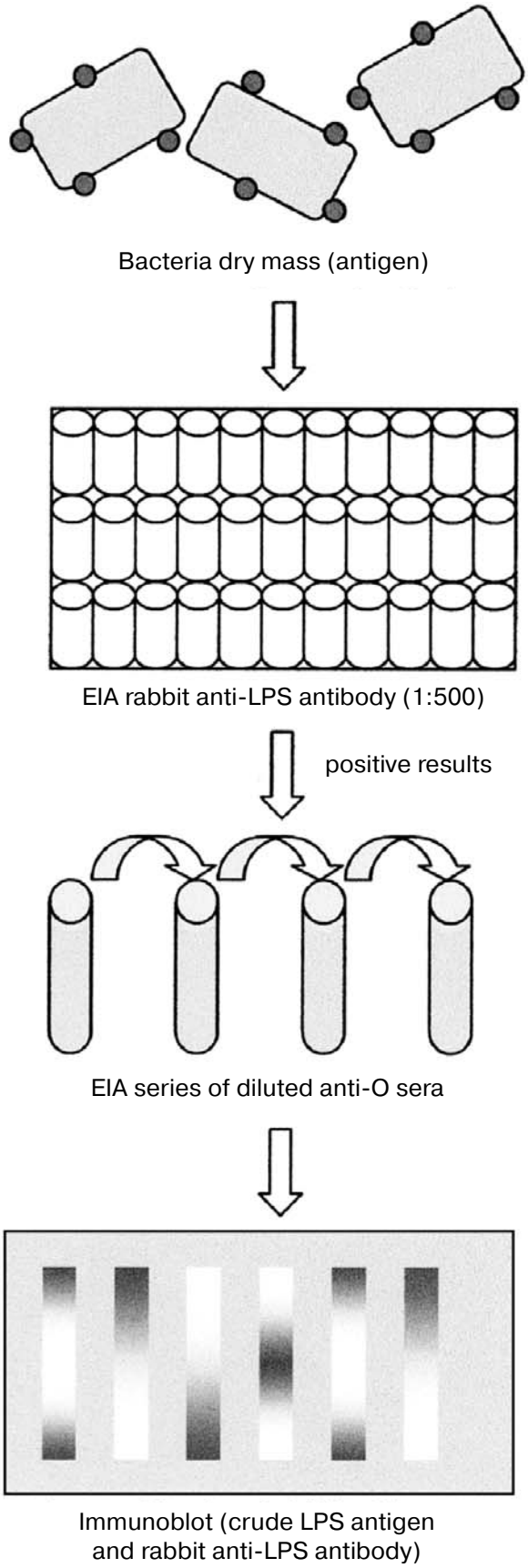

Fig. 1. Scheme of serotyping of clinical strains.

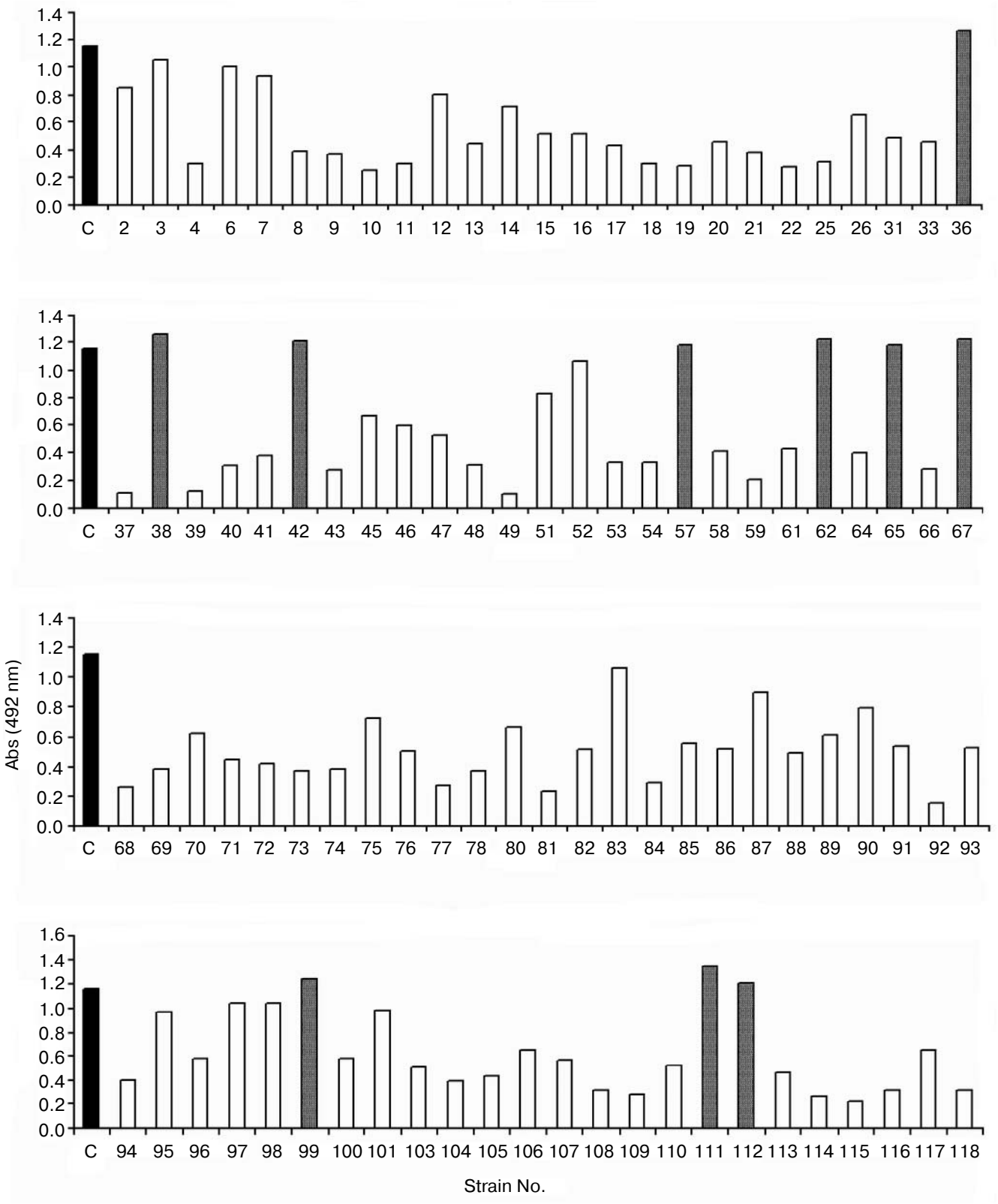

Fig. 2. EIA of Swedish collection strains with 1 : 500 diluted anti-*P. mirabilis* O38 (PrK 64/57) rabbit serum. Shown are reactions of dry bacterial mass of the homologous *P. mirabilis* O38 strain (PrK 64/57) (black bars) and 99 Swedish clinical strains (white or, for strains reacting as strongly as the homologous strain, gray bars).

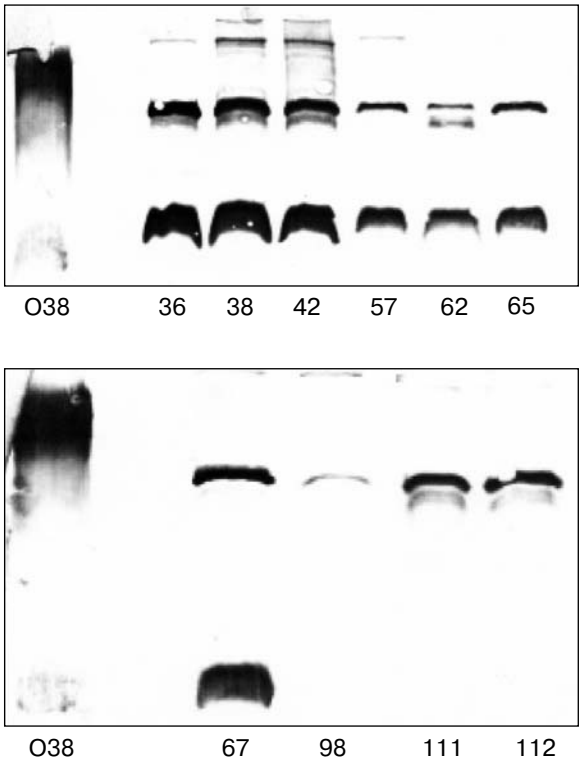

Fig. 3. Immunoblotting of the isolated LPSs with anti-*P. mirabilis* O38 (PrK 64/57) rabbit serum. Lane O38, the purified homologous LPS of *P. mirabilis* O38 (PrK 64/57); lanes 36, 38, 42, 57, 62, 65, 67, 111, and 112 are crude heterologous LPSs from the corresponding clinical strains of the Swedish collection.

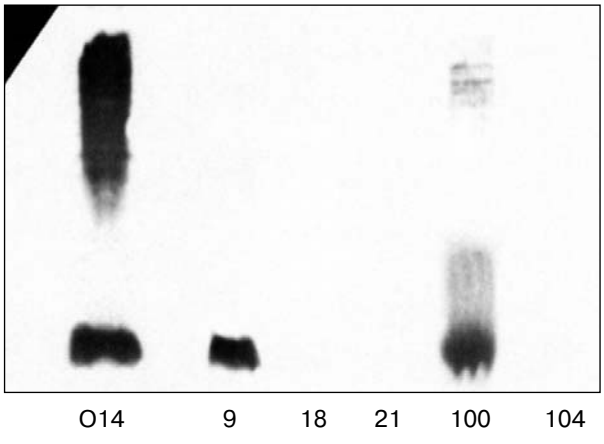

Fig. 4. Immunoblotting of the isolated LPSs with anti-*P. mirabilis* O14a,c (PrK 29/57); rabbit serum. Lane O14, the purified homologous LPS of *P. mirabilis* O14a,c (PrK 29/57); lanes 9, 18, 100, and 104 are heterologous LPSs from the corresponding clinical strains of the Swedish collection.

fied to various serogroups (Table 2). Several strains from the two collections tested showed strong reactivity with more than two sera and thus only a putative serotyping was possible.

Cross-reactivity of rabbit antisera with core oligosaccharides. In addition to the O-antigen, binding of the LPS core part by antibodies was observed in immunoblotting for several strains. As an example, reactions of anti-O14 serum were presented in Fig. 4. Strain 100 was classified to serogroup O14 based on a reaction with the OPS. However, in case of strain 9 only the core part was recognized, which corresponded to that of *P. mirabilis* O14 control laboratory strain. Therefore, strain 9 was not classified into serogroup O14. A summary of cross-reactivity of core oligosaccharides is presented in Table 3. The core part of 19 LPSs reacted with a single serum, five LPSs with two sera, and seven LPS with three or more sera.

Differentiation of *P. mirabilis* strains based on bioinformatic analysis and identification of glycosyl transferase. A growing number of polysaccharide structures and DNA sequence data allowed *in silico* analysis of the presence of

Table 2. Serotyping of Swedish and Polish *P. mirabilis* UTI strains

Serogroup	Number of classified strains	Number of putatively classified strains
Swedish collection		
O10	10	3
O30	6	4
O38	5	4
O36	5	1
O9	5	3
O17	4	
O23	3	2
O40	3	4
O27	2	1
O49	2	6
O5	1	1
O13	1	2
O14	1	
O24	1	1
O33	1	1
O20		4
Polish collection		
O3	2	
O18	2	
O38	2	
O7	1	
O40	1	
O14		2

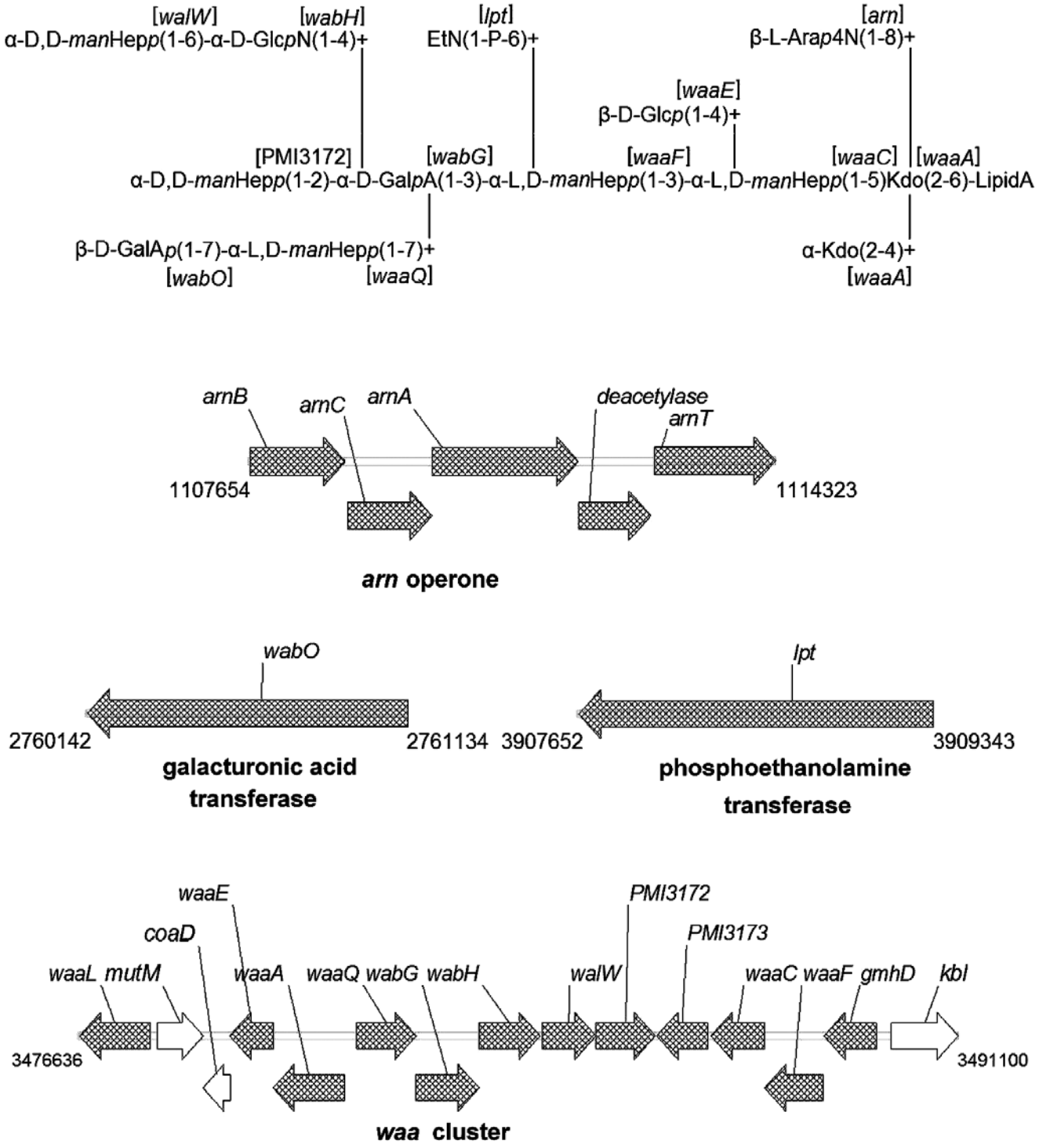

Fig. 5. Correlation between the structures of *P. mirabilis* core oligosaccharides and genes responsible for their biosynthesis. The functions of genes were assigned by comparison with sequences for enzymes whose function was defined earlier. Numbers indicate the gene positions on HI 2043 *P. mirabilis* genome sequence.

putative glycosyl transferases and strain differentiation on their basis. Bioinformatic analysis using sequences of several enzymes with defined function demonstrated a correlation between the complete structure of the LPS core of *P. mirabilis* O3a,b (OXK group) wild strain S1959, as well as rough mutant R110 derived thereof, and genes responsible for the core biosynthesis (Fig. 5). This core contains a GalA residue linked to a heptose residue at

Table 3. Cross-reactivity of core oligosaccharides of LPSs isolated from laboratory and clinical *P. mirabilis* strains with rabbit anti-O-sera

Serogroup	Clinical strain number	Core antigen reactivities with anti-O-serum
O9	64, 75, 99	O9
	65	O9, O38, O20, O7
	67	O9, O49, O38, O18
O10	9	O14
	26	O23, O3
	37	O5
	97	O9, O40, O18, O7
O14	100	O14
O17	19, 116	O35
	31	O9, O35
	62	O38, O35
O23	4, 18, 25	O23
O30	39	O20
	90	O40
O36	16	O13
	22	O23
O38	36	O49, O23, O38
	38	O9, O40, O49, O38, O20
	42	O40, O38
	111	O49, O18, O20
	112	O49, O18
O40	7, 52, 83	O40
O49	3	O49
	57	O40, O49, O38

position 7. In search for a gene for the corresponding glycosyltransferase, the gene of a *Klebsiella pneumoniae* strain having the same core fragment was used as its prototype [12]. It was found that this gene is localized outside the *waa* cluster. The RFLP-PCR analysis *in silico* confirmed identification of this gene in *P. mirabilis* O3a,b (S1959) strain. A PCR reaction with primers specific to this gene resulted in a product of an adequate size (about 540 bp), and, as expected, digestion with restrictase Sau3AI divided this product into two parts (about 400 and 140 bp) (Fig. 6).

DISCUSSION

The presence of certain *P. mirabilis* serogroups in patients' urine is not often documented. In this work, 123 clinical strains were classified by serotyping with 20 different sera. Strains belonging to serogroups O10, O38, O30, O36, O17, O23, and O9 were identified most often. The majority of serotyped *P. mirabilis* clinical strains possessed acidic O-antigens containing uronic acids and various acidic non-carbohydrate substituents. One may speculate that negative charged components of *Proteus* LPSs on the cell surface protect bacteria against killing by the immune response. The serogroup frequencies from previous studies are presented in Fig. 7. All serogroups but O3 and O11 were present in both our and former studies [19-21].

Characteristic structural features of *Proteus* LPS core region may be used as markers for identification of strains. In the present study, cross-reactivity of rabbit anti-O sera with the core parts of the tested LPSs allowed us to propose a new serological designation and identification of *P. mirabilis* strains. For example, clinical strain 26 serotyped as belonging to O10 serogroup may be described as *P. mirabilis* O10;C23,3, where "C" refers to core epitopes characteristic of other serogroups (O23 and O3 in this case). Such an approach has been described earlier for designation *P. penneri* core epitopes by Z. Sidorczyk et al. [22]. The importance of terminal carbohydrate and non-carbohydrate groups for the serological reactivity has been discussed there too [22].

Serological identification of *P. mirabilis* strains based on defined OPS and core parts might be enhanced by DNA analysis. Bioinformatic analysis may help in the identification of the genes responsible for each stage of the LPS core synthesis. In our study, it enabled identification of specific glycosyl transferase genes by comparison with genes with defined functions found in other bacteria. Thus the GalA transferase gene was identified in *P. mirabilis* R110 and O3 by comparison with the transferase

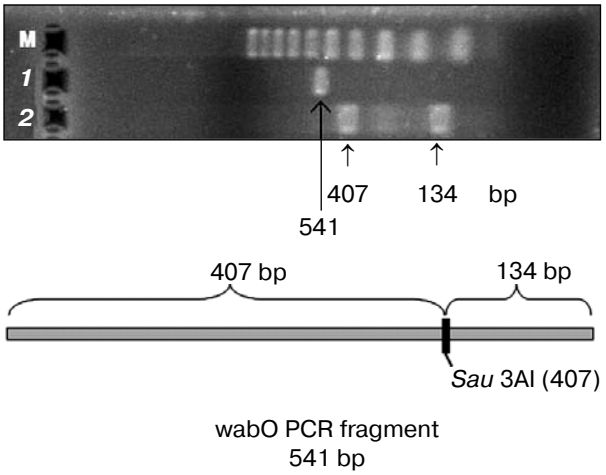

Fig. 6. RFLP analysis of PCR products with primers for GalA transferase gene of *P. mirabilis* O3a,b (S1959). Upper panel: electrophoresis or PCR and restriction analysis products. Lanes: M, size marker 100 bp ladder; *1*) PCR product; *2*) PCR product, digested with Sau3AI. Lower panel: *in silico* analysis of PCR-RFLP-amplified PCR product and localization of Sau3AI site.

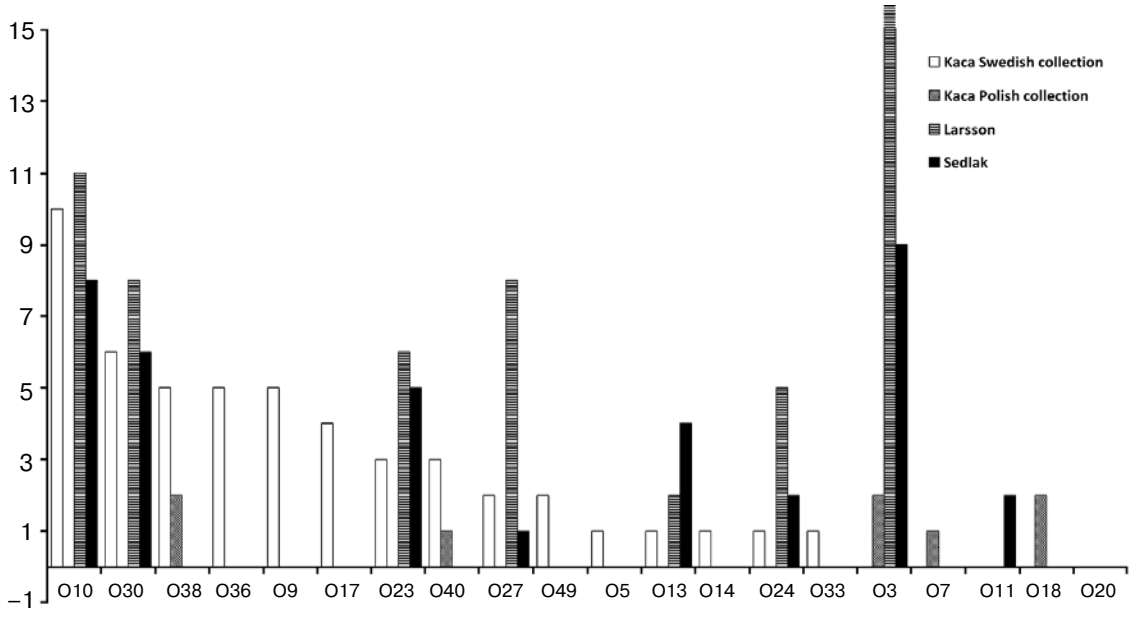

Fig. 7. Comparison of serogroup frequencies in *P. mirabilis* clinical strains.

WabO gene from *K. pneumoniae* [12]. The presence of the gene for this enzyme was revealed by the RFLP method. Recently, bioinformatic identification of genes involved in *P. mirabilis* LPS core biosynthesis was confirmed by complementation studies with *K. pneumoniae* having a similar LPS core structure [23].

In addition to the designation based on the serotyping, strain 26 might be described as *P. mirabilis* O10;C23,3;TGalA(1-7), where T indicates the presence of a specific transferase (in this example, transferase that 1,7-links GalA to heptose). Development of a system of multiplex PCR that is able to detect a series of different transferases in one experiment is now in progress in our lab. In conclusion, the combination of serological and molecular approaches provides a powerful tool for precise identification of *P. mirabilis* clinical strains.

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