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The Susceptibility of α -haemolytic *Escherichia coli* Strains and Their Non-haemolytic Forms to the Bactericidal Action of Normal Cord Serum

Podatność szczepów *Escherichia coli* wytwarzających α -hemolizynę i ich niehemolitycznych form na bakteriobójcze działanie normalnej surowicy pępowinowej

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Abstract

Background. Haemolytic *E. coli* strains are significantly more resistant to the bactericidal activity of human serum and killing activity of human polymorphonuclear leukocytes. Some authors suggested that HlyA could be a factor increasing the serum resistance of *E. coli* rods.

Objectives. Comparison the susceptibility of α -haemolytic *E. coli* strains and their non-haemolytic mutant forms to the bactericidal action of normal cord serum (NCS).

Material and Methods. Fifty α -haemolytic *E. coli* strains freshly isolated from urine of children with urinary tract infections were collected and *hly*- mutant forms were derived from them by treatment with rifampicin or they were isolated as the spontaneous mutant forms. Normal cord serum was used for determination the susceptibility of tested *E. coli* strains to the bactericidal activity of serum. Plasmid DNA was isolated by the alkaline lysis method. Detection of plasmid DNA was carried out by agarose 0.8% w/v gel electrophoresis with TBE buffer and *E. coli* V517 strain were used as molecular mass standard.

Results. In this paper the susceptibility of 9 α -haemolytic *E. coli* strains and their non-haemolytic mutant forms to the bactericidal action of normal cord serum was compared. The differences in sensitivity between *E. coli hly*+ and their *hly*- mutant forms were observed only in 4 cases. Compared with *hly*- forms these *hly*+ *E. coli* strains were significantly more resistant to the action of serum. The authors compared also plasmid patterns of *E. coli hly*+ strains and their non-haemolytic mutant forms. Plasmid DNA was found in 8 of 9 pairs of strains tested. There was difference in plasmid DNA patterns only between one *E. coli* wild-type strain and its some non-haemolytic mutant forms.

Conclusions. The results have shown that only in case of some *E. coli* strains there were significant differences in susceptibility to the bactericidal action of NCS between α -haemolytic *E. coli* strains and their non-haemolytic mutant forms (Adv Clin Exp Med 2005, 14, 6, 1161–1167).

Key words: *E. coli*, complement, α -haemolysin, normal cord serum.

Streszczenie

Wprowadzenie. Hemolityczne szczepy *Escherichia coli* są bardziej odporne na bakteriobójcze działanie surowicy ludzkiej i aktywność bójczą ludzkich wielojądrzastych leukocytów. Niektórzy autorzy sugerują, że HlyA może być czynnikiem zwiększającym oporność na surowicę pałeczek *E. coli*.

Cel pracy. Porównanie podatności α -hemolitycznych szczepów *E. coli* i ich niehemolitycznych mutantów na bakteriobójcze działanie normalnej surowicy pępowinowej (NCS).

Materiał i metody. Z moczu dzieci z zakażeniem dróg moczowych wyizolowano pięćdziesiąt α -hemolitycznych szczepów *E. coli*. Formy *hly*-, niewytwarzające α -hemolizyny, uzyskano za pomocą ryfampicyny, bądź były to spontaniczne mutanty. Do określenia podatności testowanych szczepów *E. coli* na bakteriobójczą aktywność surowicy wykorzystano normalną surowicę pępowinową. Plazmidowe DNA izolowano metodą alkaliczną, a następnie przeprowadzono elektroforezę w 0,8% żelu agarozowym z buforem TBE. Szczep *E. coli* V517 wykorzystano jako standard masy molekularnej plazmidów.

Wyniki. Porównano podatność na bakteriobójczą aktywność NCS dziewięciu α -hemolitycznych szczepów *E. coli* i ich niehemolitycznych mutantów, które udało się wyizolować. Różnice we wrażliwości na surowicę między szczepami *E. coli hly+* i ich formami *hly-* zaobserwowano tylko w czterech przypadkach, gdzie szczepy *E. coli hly+* były bardziej odporne na działanie NCS w porównaniu do mutantów *hly-*. Porównano także profile plazmidowe szczepów *E. coli hly+* i ich niehemolitycznych mutantów. Plazmidowe DNA znaleziono w 8 na 9 testowanych par szczepów. Tylko w przypadku jednego szczepu zaobserwowano różnice w profilu plazmidowym między szczepem dzikim i niektórymi jego niehemolitycznymi mutantami.

Wnioski. Tylko w przypadku niektórych testowanych szczepów *E. coli* stwierdzono istotne różnice w podatności na bakteriobójcze działanie NCS między α -hemolitycznymi szczepami *E. coli* i ich niehemolitycznymi mutantami (*Adv Clin Exp Med* 2005, 14, 6, 1161–1167).

Słowa kluczowe: *E. coli*, komplement, α -hemolizyna, normalna surowica pępowinowa.

Escherichia coli is a normal inhabitant of the digestive tract of humans and various animals [1–3]. However, pathogenic *E. coli* strains are able to cause intestinal and the extraintestinal infections such as neonatal meningitis, bacteraemia, pyelonephritis, cystitis and prostatitis. In contrast to non-pathogenic isolates, pathogenic *E. coli* strains produce pathogenicity factors which contribute to the capacity of strains to cause infectious diseases. These virulence factors facilitate colonization and invasion of the host, injury to host tissues and avoidance or disruption of host defence mechanisms.

α -Haemolysin (HlyA) is a protein toxin (~107 kDa) secreted by *E. coli* rods commonly associated with the extraintestinal infections such as urinary tract infections and septicemia [4–6]. HlyA is a member of a wider family of exotoxins, named RTX (repeats in toxin) [6, 7]. The formation of toxin-mediated pores in the membrane of attacked cells has been implicated as the common mechanism of its action. *E. coli* HlyA has a wide spectrum of cytotoxic activity. It targets erythrocytes, leukocytes, lymphocytes, endothelial cells and renal epithelial cells of many mammals' species. HlyA does not require a specific receptor and it could be a reason why this haemolysin can attack a wide range of target cells and can even bind to synthetic lipid membranes [7].

The bactericidal activity of serum caused by the complement system (C) is an important defence mechanism protecting the host organism against infections of Gram-negative bacteria [8, 9]. Many of these bacteria, especially those freshly isolated from patients, are sensitive to the lytic action of C, whereas some are resistant and therefore more virulent. The mechanism of the bacterial resistance to the bactericidal effect of serum is not fully understood but it is known that the structure of bacterial O-specific side chains of lipopolysaccharides, bacterial capsules and outer membrane proteins are the factors determining the resistance or sensitivity of bacteria to the bactericidal action of C [10, 11].

Haemolytic *E. coli* strains are significantly more resistant to the bactericidal activity of human serum and killing activity of human polymorphonuclear leukocytes [12, 13]. These authors suggested that HlyA could be a factor increasing the serum resistance of *E. coli* rods. To establish if this is true, the authors of the present paper have compared the susceptibility of α -haemolytic *E. coli* strains and their non-haemolytic mutant forms to the bactericidal action of normal cord serum.

Material and Methods

Bacterial Strains

Fifty *E. coli* strains freshly isolated from urine of children with urinary tract infections were collected. The children were hospitalised in the Lower Silesian J. Korczak Centre of Paediatrics in Wrocław. All strains were identified by API 20 E test.

Sera

Normal cord serum (NCS) was obtained from ten healthy newborns. The mothers of the newborns were not previously treated with antibiotics. The samples of NCS were collected and kept frozen in 0.25 ml portions at -70°C and pooled before use. A suitable volume of the sera was thawed immediately before experiments. Each portion was used only once.

Haemolytic Activity Assay

The α -haemolysin production was detected by the presence of characteristic zone of lysis on nutrient agar containing 5% sheep erythrocytes, with observation at 3 h and again after overnight incubation, as described by Beutin [14]. Strains having a clear halo on sheep blood agar plates were defined as haemolytic (*hly+*). Non-

haemolytic mutant forms (*hly*[−]) were obtained from 9 of 50 studied *E. coli hly*⁺ strains. In case of *E. coli* 5786 *hly*⁺ strain we isolated the spontaneous mutant forms (6 clones). The remaining eight *hly*[−] mutant forms were derived from 50 studied *E. coli hly*⁺ strains by treatment with rifampicin (300–500 mg/ml) [15].

Bactericidal Activity of NCS

The bactericidal activity of NCS was determined as described previously [16]. Briefly, the strains were grown overnight, and then bacterial cells of early exponential growth phase were transferred to fresh nutrient broth and incubated at 37°C for 30 min. After incubation the bacterial cells were centrifuged (4000 rpm for 20 min). The bacteria were then added to 20, 30 or 50% NCS (serum was diluted with 0.1 M NaCl). Bacteria with serum were incubated in a water bath at 37°C. After 0, 60 and 180 min samples were collected, diluted and cultured on nutrient agar plates for 18 h at 37°C. The number of CFU at time zero was taken as 100%. Strains with a survival ratio not less than 50% after 180 min incubation in NCS were regarded as resistant. The results are means from three separate experiments.

Determination of the Level of C3 and C4

The level of C3 and C4 components of C in NCS was determined using specific antibodies. The nutrient agar plates were used with monospecific polyclonal antibodies (MEGA-TRADIN-Gliwice, Poland) anti-C3 and anti-C4 proteins. The assay was carried out according to the manufacturer's instructions.

Plasmid DNA Analysis

Plasmid DNA was isolated by the alkaline lysis method described by Maniatis et al. [17]. Detection of plasmid DNA was carried out by agarose 0.8% w/v gel electrophoresis with TBE buffer (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA, pH 8.0). The eight plasmids of *E. coli* V517 strain were used as molecular mass standards (pVA517A: 54.38 kb; pVA517B: 7.30 kb; pVA517C: 5.56 kb; pVA517D: 5.14 kb; pVA517E: 3.98 kb; pVA517F: 3.08 kb; pVA517G: 2.71 kb; pVA517H: 2.06 kb) [18]. Gel-Dok 2000 BioRad and Quantity One 4.0.3. computer program were used for analysing the gels.

Results

The first aim was to obtain non-haemolytic mutant forms from a total of 50 collected clinical isolates of *E. coli hly*⁺. Among all these 50 *E. coli hly*⁺ rods only in 9 cases *hly*[−] mutant forms were obtained. From *E. coli* 5786 *hly*⁺ strain we successfully isolated the spontaneous mutant forms (6 clones). The remaining *hly*[−] mutant forms were derived from eight *E. coli hly*⁺ strains by treatment 50 studied strains with rifampicin. In this way the authors isolated 5 clones from *E. coli* 79/D; 5 clones from *E. coli* 5703; 5 clones from *E. coli* 783; 2 clones from *E. coli* 5938; 2 clones from *E. coli* 49; 2 clones from *E. coli* 1092; 1 clone from *E. coli* 5697 and 1 clone from *E. coli* 5543.

The next experiments assessed the susceptibility of *E. coli hly*⁺ strains and their *hly*[−] mutant forms to the C-mediated bactericidal activity of NCS. The authors chose NCS for the experiments because they would like to test *E. coli* strains, which were isolated from urine of young children in serum, which approximately correspond with their age. NCS has a relatively low bactericidal activity because the level of proteins of the C system is lower than in normal adult serum [19]. In this case the level of C3 was 45.0 mg/dl (standard for a human serum is 55–120 mg/dl) and C4 was 14 mg/dl (standard for a human serum is 20–50 mg/dl).

Results of killing of *E. coli hly*⁺ strains and corresponding *hly*[−] mutant forms in NCS are shown in Tables 1–2. Differences in susceptibility of *E. coli hly*⁺ rods and their *hly*[−] mutant forms to the bactericidal action of NCS were estimated using the bacterial cell survival coefficient. Among the 9 *E. coli hly*⁺ strains and their *hly*[−] mutant forms, two groups could be discerned differing in their susceptibility to NCS what can be shown by comparison of their coefficients of survival in serum. In the first group (Table 1) essential differences in the sensitivity to the action of 20 and 30% NCS were found between α -haemolytic *E. coli* strains and their non-haemolytic mutant forms. All the α -haemolytic isolates were resistant to serum killing whereas *hly*[−] mutant forms were susceptible to both 20% and 30% NCS (in Table 1 are given the data only for 30% NCS). In this group of strains the survival coefficient after 180 min incubation in 30% NCS varied from 17.1 (*E. coli* 5786) to 481100.0 (*E. coli* 5703). The *hly*[−] mutant forms the most sensitive to the action of serum were *E. coli* 5703 (2) and 5703 (5). NCS at 30% concentration decreased the percent survival of these bacteria to 0.003 and 0.01 respectively after 180 min of incubation while *hly*⁺ strain proliferated in NCS. The authors did not test the suscepti-

Table 1. Bactericidal effect of 30% NCS on *E. coli hly+* strains and their *hly*– forms**Tabela 1.** Bakteriobójcze działanie 30% NCS na szczepy *E. coli hly+* i ich mutanty *hly*–

Strain No. (Szczep)	Time of incubation (Czas inkubacji) min			Percent of survival after 180 min (Procent przeżywalności po 180 min)	Survival coefficient (A)** (Współczynnik przeżywalności)
	0	60	180		
	CFU*	CFU	CFU		
5697 <i>hly+</i> 5697 (1) <i>hly</i> –	1.4×10^7 1.6×10^7	1.3×10^7 5.2×10^6	6.8×10^7 3.8×10^6	485.7 23.7	20.5
5543 <i>hly+</i> 5543 (1) <i>hly</i> –	3.1×10^7 1.7×10^7	5.7×10^7 4.0×10^5	1.3×10^8 2.2×10^5	419.3 1.3	322.5
5786 <i>hly+</i> 5786 (1) <i>hly</i> –	1.7×10^7 3.4×10^6	2.5×10^6 6.0×10^3	3.2×10^7 1.3×10^3	188.2 0.04	4705.0
5786 (2) <i>hly</i> –	1.4×10^7	6.3×10^4	2.9×10^5	2.07	90.9
5786 (3) <i>hly</i> –	1.0×10^7	3.4×10^5	1.1×10^6	11.0	17.1
5786 (4) <i>hly</i> –	7.1×10^6	3.4×10^4	6.0×10^3	0.08	2352.5
5786 (5) <i>hly</i> –	1.1×10^7	6.0×10^3	5.1×10^3	0.05	3764.0
5786 (6) <i>hly</i> –	8.1×10^6	4.3×10^3	5.8×10^3	0.07	2688.6
5703 <i>hly+</i> 5703 (1) <i>hly</i> –	9.7×10^6 9.2×10^6	1.4×10^7 7.7×10^6	1.4×10^8 4.5×10^5	1443.3 4.9	294.5
5703 (2) <i>hly</i> –	9.7×10^6	1.4×10^5	3.0×10^2	0.003	481100.0
5703 (3) <i>hly</i> –	1.0×10^7	6.1×10^5	5.5×10^5	5.5	262.4
5703 (4) <i>hly</i> –	1.2×10^7	9.7×10^5	3.8×10^3	0.03	48110.0
5703 (5) <i>hly</i> –	1.0×10^7	4.3×10^5	1.5×10^3	0.015	96220.0

* CFU – colony forming units (also relevant for Table 2).

** $A=b/c$, where: b is per cent of cells of *E. coli hly+* strain incubated in 30% or 50% NCS for 180 min, c is per cent of cells of *E. coli hly*– mutant form incubated in 30% or 50% NCS for 180 min (also relevant for Table 2).

* CFU – jednostka tworząca kolonie (dotyczy także tabeli 2).

** $A=b/c$, gdzie: b – odsetek komórek szczepu *E. coli hly+* inkubowanego w 30% lub 50% NCS przez 180 min, c – odsetek komórek szczepu *E. coli hly*– inkubowanego w 30% lub 50% NCS przez 180 min (dotyczy także tabeli 2).

bility of these strains to the action of 50% NCS because *hly*– mutant forms were killed by NCS at even less concentration. In case of the other five strains tested (Table 2), there were no significant differences between the response of *E. coli hly+* strains and their *hly*– mutant forms to bactericidal action of NCS. Both the α -haemolytic isolates and *hly*– mutant forms were resistant to 20, 30 and 50% NCS although some *hly*– mutant forms proliferated less intensively (in Table 2 are given the data only for 50% NCS). The survival coefficient in this group of strains after 180 min incubation in 50% NCS was lower and varied from 0.3 (*E. coli* 1092) to 5.8 (*E. coli* 79/D).

The authors compared also plasmid patterns of *E. coli hly+* strains and their non-haemolytic mutant forms. Plasmid DNA was found in 8 of 9 pairs of strains tested. The majority of the strains carried two plasmids (strains: 5697 – 11.47, 1.6 kb; 5543 – 11.43, 1.35 kb; 5703 – 2.35, 1.5 kb; 79/D – 9.39, 1.5 kb and 783 – 5.5, 2.95 kb). Two strains had three plasmids (strains: 5786 – 12.51, 6.02, 2.56 kb and 1092 – 13.71, 6.43, 3.81 kb) and strain 49 one plasmid – 48.6 kb. There was differ-

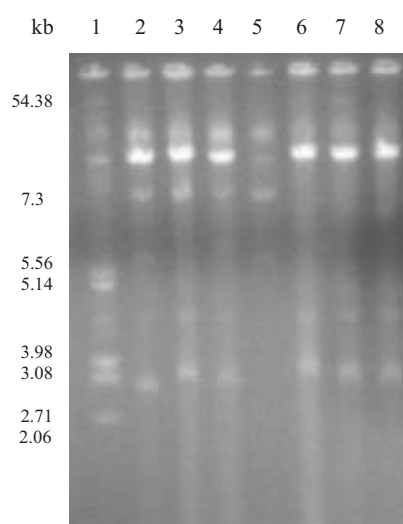
ence in plasmid DNA content only between *E. coli* 5786 *hly+* strain and some of its *hly*– mutant clones (Fig. 1). Clone number 3 carried only two plasmids (12.51 and 6.02 kb) and clones number 4, 5 and 6 had only one plasmid (2.56 kb). In case of the remaining strains there was no difference in plasmid patterns between the wild-type strain and its non-haemolytic mutant forms as showed on Fig. 2.

Discussion

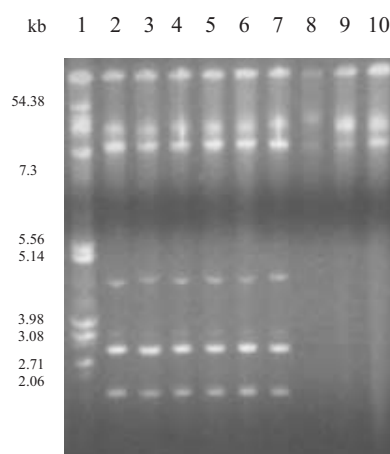
In this paper the susceptibility of α -haemolytic *E. coli* strains and their non-haemolytic forms to the bactericidal action of normal cord serum was compared. As previously described, HlyA may play certain role in the resistance of *E. coli* strains to the bactericidal activity of human serum [12]. Authors suggested that HlyA could be one of the virulence factors increasing the serum resistance of *E. coli* rods. This suggestion occurred not only from the significantly higher serum resistance found in α -haemolytic *E. coli* strains than in non-haemolytic ones, but mostly because authors

Table 2. Bactericidal activity of 50% NCS on *E. coli* *hly*⁺ strains and their *hly*[–] forms**Tabela 2.** Bakteriobójcze działanie 50% NCS na szczepy *E. coli* *hly*⁺ i ich mutanty *hly*[–]

Strain No. (Szczep)	Time of incubation (Czas inkubacji) min			Percent of survival after 180 min (Procent przeżywalności po 180 min)	Survival coefficient (A) (Współczynnik przeżywalności)
	0	60	180		
	CFU	CFU	CFU		
1092 <i>hly</i> ⁺	3.4×10^7	1.1×10^8	1.8×10^8	529.4	0.7 0.3
1092 (1) <i>hly</i> [–]	3.6×10^7	1.6×10^8	2.8×10^8	777.8	
1092 (2) <i>hly</i> [–]	1.1×10^7	4.9×10^7	1.7×10^8	1545.4	
5938 <i>hly</i> ⁺	1.5×10^7	7.4×10^7	2.5×10^8	1666.7	1.2 1.2
5938 (1) <i>hly</i> [–]	1.0×10^7	2.9×10^7	1.4×10^8	1400.0	
5938 (2) <i>hly</i> [–]	1.4×10^7	4.8×10^7	2.0×10^8	1428.6	
49 <i>hly</i> ⁺	2.5×10^7	7.5×10^7	3.9×10^8	1560.0	1.9 1.6
49 (1) <i>hly</i> [–]	2.2×10^7	8.8×10^7	1.8×10^8	818.2	
49 (2) <i>hly</i> [–]	2.6×10^7	1.0×10^8	2.5×10^8	961.5	
783 <i>hly</i> ⁺	8.5×10^6	1.6×10^7	8.6×10^7	1011.8	2.0 1.3 5.5 1.0 1.6
783 (1) <i>hly</i> [–]	1.3×10^7	3.9×10^7	6.6×10^7	507.7	
783 (2) <i>hly</i> [–]	1.6×10^7	4.3×10^7	1.2×10^8	750.0	
783 (3) <i>hly</i> [–]	4.5×10^5	2.8×10^5	8.3×10^5	184.4	
783 (4) <i>hly</i> [–]	1.5×10^7	9.3×10^6	1.5×10^8	1000.0	
783 (5) <i>hly</i> [–]	2.4×10^7	4.2×10^7	1.5×10^8	625.0	
79/D <i>hly</i> ⁺	1.5×10^7	3.6×10^7	1.0×10^8	666.7	4.1 4.0 2.5 5.8 3.0
79/D (1) <i>hly</i> [–]	2.5×10^7	1.4×10^7	4.1×10^7	164.0	
79/D (2) <i>hly</i> [–]	2.7×10^7	1.5×10^7	4.5×10^7	166.7	
79/D (3) <i>hly</i> [–]	3.1×10^7	3.1×10^7	8.1×10^7	261.3	
79/D (4) <i>hly</i> [–]	2.8×10^7	2.4×10^7	3.2×10^7	114.3	
79/D (5) <i>hly</i> [–]	2.9×10^7	1.3×10^7	6.5×10^7	224.1	

**Fig. 1.** Plasmid patterns of *E. coli* strains. Lane 1: *E. coli* V 517 used as a molecular mass marker; 2: *E. coli* 5786 *hly*⁺; 3: *E. coli* 5786 (1) *hly*[–]; 4: *E. coli* 5786 (2) *hly*[–]; 5: *E. coli* 5786 (3) *hly*[–]; 6: *E. coli* 5786 (4) *hly*[–]; 7: *E. coli* 5786 (5) *hly*[–]; 8: *E. coli* 5786 (6) *hly*[–]

Ryc. 1. Profil plazmidowy szczepów *E. coli*. Ścieżka 1: *E. coli* V 517 – marker mas molekularnych plazmidów; 2: *E. coli* 5786 *hly*⁺; 3: *E. coli* 5786 (1) *hly*[–]; 4: *E. coli* 5786 (2) *hly*[–]; 5: *E. coli* 5786 (3) *hly*[–]; 6: *E. coli* 5786 (4) *hly*[–]; 7: *E. coli* 5786 (5) *hly*[–]; 8: *E. coli* 5786 (6) *hly*[–]

**Fig. 2.** Plasmid patterns of *E. coli* strains. Lane 1: *E. coli* V517 used as a molecular mass marker; 2: *E. coli* 5703 *hly*⁺; 3: *E. coli* 5703 (1) *hly*[–]; 4: *E. coli* 5703 (2) *hly*[–]; 5: *E. coli* 5703 (3) *hly*[–]; 6: *E. coli* 5703 (4) *hly*[–]; 7: *E. coli* 5703 (5) *hly*[–]; 8: *E. coli* 5938 *hly*⁺; 9: *E. coli* 5938 (1) *hly*[–]; 10: *E. coli* 5938 (2) *hly*[–]

Ryc. 2. Profil plazmidowy szczepów *E. coli*. Ścieżka 1: *E. coli* V 517 – marker mas molekularnych plazmidów; 2: *E. coli* 5703 *hly*⁺; 3: *E. coli* 5703 (1) *hly*[–]; 4: *E. coli* 5703 (2) *hly*[–]; 5: *E. coli* 5703 (3) *hly*[–]; 6: *E. coli* 5703 (4) *hly*[–]; 7: *E. coli* 5703 (5) *hly*[–]; 8: *E. coli* 5938 *hly*⁺; 9: *E. coli* 5938 (1) *hly*[–]; 10: *E. coli* 5938 (2) *hly*[–]

ascertained that the wild-type α -haemolytic isolates were resistant to bactericidal activity of human serum while their non-haemolytic derivatives with reduced production of HlyA were killed by this serum. Presented results have shown that it is not always the truth. In case of some *E. coli* *hly*⁺ strains that were investigated by the authors no significant differences were found in susceptibility to the bactericidal action of NCS between α -haemolytic *E. coli* strains and their non-haemolytic mutant forms (Table 2). It is possible that the susceptibility to the bactericidal action of serum could depend on the amount of HlyA produced by *E. coli* rods. It is known that HlyA can have various and graded effects depending on its concentration [7]. At the low concentration (even far from the site of bacterial infection) HlyA can elicit pathological influence mainly on white cells. Such effects involve inhibition of mobility and chemotaxis of neutrophils and lymphocyte mitogenesis, apoptosis of T-lymphocytes, stimulation of the release of inflammatory lipid mediators (e.g. leukotrienes from granulocytes) and release of cytokines from monocytes and macrophages. At higher concentration (in the vicinity of the bacterium) the HlyA pore-formation can inhibit or even eliminate phagocytosis by polymorphonucleated cells and macrophages and reduce ability of neutrophils to kill bacteria [7]. The red blood cells undergo osmotic lysis, releasing their haemoglobin. Haemoglobin could thus become a source of iron for bacterial growth. The large amounts of HlyA may bind to the cell membrane in the contact region between bacteria and cells. Thus the toxin can destabilize the lipid bilayer and the cytoskeleton, causing cell disruption and extensive tissue damage, which may facilitate the spread of the bacteria through the epithelia and their penetration into the host tissues [7]. The mechanism of increased serum resistance by HlyA production is

likely connected with neutralisation of some serum components involved in bacterial killing by interaction with HlyA [20]. Serum resistance in *E. coli* strains due to HlyA production could be based on one of the mechanism: α -haemolysin releases terminal C components from the outer membrane and operates by interrupt the C cascade at the C3 level or by blocking the membrane-attacking complex to become integrated in the outer membrane [21, 22].

Genes for the production of haemolysin in most human *E. coli* isolates are located on the bacterial chromosome [23]. In majority of *E. coli* strains causing urosepsis haemolysin production is chromosomally encoded. Although the most number of haemolytic *E. coli* strains isolated from human sources carry the *hly* determinant on the chromosome, *hly* plasmids in human faecal or uropathogenic strains have also been described [23]. It might be a reason why *hly* determinant was removed by rifampicin or spontaneous lost at low frequency. Perhaps variable susceptibility of α -haemolytic *E. coli* strains and their non-haemolytic forms to the bactericidal action of NCS may depend on location of *hly* operon.

Sensitivity of bacteria to the bactericidal activity of complement depends on the structure and organisation of the bacterial outer membrane [24, 25]. Attention has been paid to lipopolysaccharide and outer membrane proteins, which are the outer membrane components as one of the factors determining the resistance of bacteria to the bactericidal action of C [25]. Initial research [26] showed that some *E. coli* *hly*⁺ strains and their non-haemolytic mutant forms differ in the outer membrane protein patterns. Further investigations are needed to elucidate if a loss of haemolytic phenotype is linked with changes at the bacterial cell-wall surface in cases of described remaining strains.

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