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THE EFFECT OF *VIR* PLASMID AND *K31* ANTIGEN – ASSOCIATED RESISTANCE OF *ESCHERICHIA COLI* ON THE BACTERICIDAL ACTIVITY OF HUMAN AND ANIMAL SERA[1]

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Summary. Studies carried out on smooth strains of *E. coli H209* differing by the presence of *K31* antigen and *Vir* plasmid showed that both the *K* antigen and *Vir* plasmid protect bacteria against bactericidal activity of serum. The protective role of the *Vir* plasmid was also displayed in the studies on rough *E. coli*.

Human and animal sera show a bactericidal activity against various Gram-negative bacteria. However, a large number of strains remains insensitive to their action, which was also found by Grzybek-Hryncewicz et al. (1981); Jankowski et al. (1981). Some authors are of the opinion that there is a relation between pathogenicity and resistance of bacteria to the activity of sera (Munn et al. 1982, Pai, DeStaphano 1982, Nakamura, Katsuno 1974). These observations were confirmed by experimental infections in animals (Durac, Beeson 1977, Archer, Fekety 1976). In recent years intensive studies aimed at elucidation of the reasons conditioning resistance of bacteria to serum activity have been performed. It has been found that many components of the outer membrane are responsible for this phenomenon. This concerns first of all the structure of lipopolysaccharides (Tee, Scott 1980), lipides (Akiyama, Inoue 1977), proteins (Taylor, Parton 1977) and predominance of certain *O* and *K* antigens among strains resistant to the activity of sera (McCabe et al. 1978, Stevens et al. 1978, Taylor, Robinson 1980). Moreover, it was showed that the presence of the *Col V* and *R* plasmids in bacteria can also determine a reduced sensitivity to bactericidal activity of serum (Binns et al. 1979, Moll et al. 1980).

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The purpose of this paper was to determine the role of the *Vir* plasmid determining pathogenicity and that of *K31* antigen in protection against bactericidal activity of human and animal sera.

MATERIAL AND METHODS

Strains and plasmids. The characteristic and origin of *E.coli* strains and plasmids applied in the studies are given in Table 1.

Table 1. Characterization of *E. coli* strains used in the studies

Strain	Phenotype (plasmid)	Characterization	Origin
H209 09K31:H14	K^+Vir^+	Strain, which the *Vir* plasmid was transmitted to from *E. coli S5*	W. Smith
H209 09K31-H14	K^-Vir^+	A spontaneous derivative without antigen *K31*	M. Mulczyk J. A. Morris
H209 09K31:H14	K^+Vir^-	*H209* without plasmid *Vir*	
H209 09K31-H14	K^-Vir^-	Strain without antigen *K31* and plasmid *Vir*	
711 R (rough)	Vir^-	Strain *K12*	J. A. Morris
711 R	Vir^+	Plasmid *Vir* transmitted from *E. coli S5*	
J53	*RPI*	*Tc, Ap, Km	N. Datta
J53	*R27*	Tc	N. Datta
J53	*R64*	Tc, Sm	N. Datta
J53	*R100.1*	Tc, Cm, Su, Sm	K. N. Timmis
V517	*PYA517A*	Cr, Km, Ap	K. Taylor

* resistance to drugs determined by the presence of plasmid and concerning;
Tc – tetracycline
Sm – streptomycine
Cm – chloramphenikol
Cr – carbenicyline
Ap – ampicyline
Su – sulphanamide
Km – kanamycine
Vir – plasmid determining virulence of *E. coli* bacilla.

Sera. The following sera were used in the studies: a) human serum, b) guinea pig serum, c) calf serum and d) rabbit serum. The method of taking sera and their storage were described previously (Jankowski et al. 1981). In some experiments sera from neonates were used. Sera were taken from three neonates after a normal birth, mixed together and stored in the amounts of 0.1 ml at –20°C. In the experiments with smooth strains *H209* 50% serum concentrations were used. In the case of the strain *R 711* sera of neonates were used in the lowest concentrations – 2.5% and those of rabbits – 1%. The sera were diluted in physiological solution (0.15M).

Investigation of bactericidal activity of serum. Bactericidal activity of serum was determined by tracing the dynamics of that process during 60 min. incubation of bacteria in the respectively dilluted serum, added with renewed culture. The initial bacterial density in the sample at 0 time was about 10^5 bacteria ml. Samples were taken after corresponding time intervals, dilluted and placed on agar plates. After 18 h incubation the number of grown bacterial colonies was calculated taking the number of bacteria grown at 0 time as 100%. Those strains, the survival of

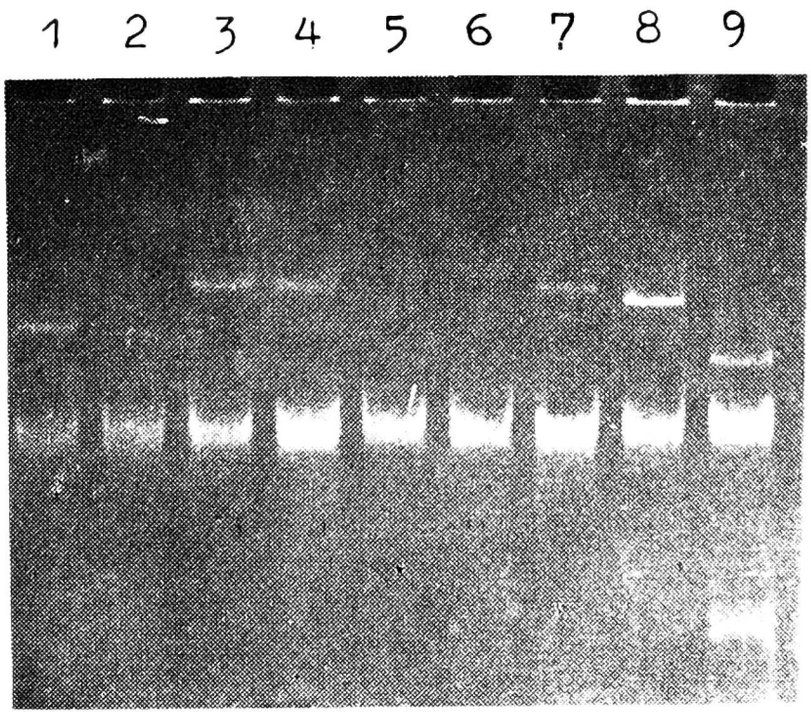

Fig. 3. Results of DNA electrophoresis of plasmids

1 – RPI, 2 – 711 Vir⁻, 3 – 711 Vir⁺, 4 – H209 Vir⁺, 5 – H209 Vir⁻, 6 – R27, 7 – R64, 8 – R100.1, 9 – PYA 517A.

which in a 50% serum after 60 min. was higher than 50% of the cells of the initial density, were recognized as resistant.

Isolation of plasmid DNA. The method of Mickel and Bauer (1976) was used: 0.5 ml of an overnight culture was mixed with an equal volume of a solution containing 75% ethanol, 2% phenol, 20 mM *Tris-HCl*, (pH 8.0), 10 mM *EDTA*. The cells were collected by centrifugation and resuspended in 50 µl of 20 mM *Tris-HCl* (pH 8.0), 1 mM *EDTA*. 5 µl of 25% sodium dodecyl sulphate was added and the solution incubated at 37°C for 10 min. The lysate was centrifuged at 12.800×g (eppendorf centrifuge) for 15 min., 40 µl of the supernatant was removed, mixed with 10 µl 0.025% bromophenol blue, and electrophoresed on a 0.7% agarose gel at 100-120 V. In order to observe DNA bands in UV (the short wavelength UV light), the agarose gel was placed in the solution of athydine bromide (0.4 µg/ml). The molecular weight of isolated DNA was calculated by the method of Mayers et al. (1976).

RESULTS

The bactericidal activity of 50% human and animal sera (calf serum, rabbit serum and guinea pig serum) against the strain of *E. coli H209 09 K31: H14 Vir*$^+$ and its variants devoid of *K31* antigen or *Vir* plasmid is given in Table 2. The importance

Table 2. The effect of the plasmid *Vir* and antigen *K31* on resistance of *E. coli H209* strain against bactericidal activity of normal sera

Variant of strain	Percent of bacteria survival in 50% sera				
	Human		Calf	Guinea pig	Rabbit
	adult	neonate			
	60'	60'	60'	60'	60'
K$^-$ *Vir*$^-$	0.03*	50.0	7.1	37.5	44.1
K$^-$ *Vir*$^+$	7.0	235.0	33.2	90.0	186.0
K$^+$ *Vir*$^-$	42.0	440.0	82.0	166.0	200.0
K$^+$ *Vir*$^+$	96.0	409.0	91.0	612.0	250.0

* means of three experiments

of the *K* antigen and pathogenicity traits (of the *Vir* plasmid) for sensitivity of these strains to serum were analysed. It was displayed that strains without *K* antigen are more susceptible to bactericidal serum activity than strains containing this antigen. The strain *K*$^-$*Vir*$^-$ is strongly killed by human serum. Calf, guinea pig and rabbit serum and that of human infants also induces bactericidal activity, though a little weaker. The strain *E. coli H209* with the *K* antigen (*K*$^+$*Vir*$^-$) survives in most sera and even multiplies (in serum of guinea pig, of rabbits and of human infants). Only in human serum it is killed 1000-fold less than the strain without the *K* antigen (*K*$^-$*Vir*$^-$).

Taking into account strains with the *Vir* plasmid, one can find a larger susceptibility of the strain not synthesizing the *K* antigen (*K*$^-$*Vir*$^+$) as the latter is killed in human and calf serum — in comparison with the strain containing the *K* antigen (*K*$^+$*Vir*$^+$) which is resistant to all the sera.

The importance of the *Vir* plasmid-associated pathogenicity in determination of bacterial resistance to bacteridical activity of sera was traced. The stability in sera of bacilli differing by the presence on absence of the *Vir* plasmid was compared in two series of experiments. *E. coli* bacilli devoid of the *K* antigen and containing this antigen were used. Bacilli without the *K* antigen and *Vir* plasmid (K^-Vir^-) are susceptible to all the sera. The strain K^-Vir^+ is more resistant. The bactericidal effect on it of human serum is 100-fold weaker in comparison with K^-Vir^- bacilli. It is also more weakly killed by calf and guinea pig serum, whereas in rabbit serum and in serum of human infants the strain K^-Vir^- multiplies. The strains K^+ with the Vir^+ plasmid or without it also display differentiation in the sensitivity to bactericidal serum activity. The first of them (K^+Vir^-) is susceptible to the action of human serum, while the strain K^+Vir^+ is resistant to that serum. In other sera the discussed strains are not killed and even multiply and it should be mentioned that multiplication of the strain K^+Vir^+ may be several-fold more intensive than that of the strain K^+Vir^-.

In another experiment the importance of the *Vir* plasmid as a protective agent against bactericidal serum activity was studied using rough strains (*R*) *E.coli 711v* (Vir^+) and *E. coli 711* (Vir^-). In view of the fact that *R* strains as a rule are very sensitive to sera the lowest concentrations of bactericidal sera were used in the studies. Figs. 1 and 2 present the bactericidal activity of the cord serum and rabbit serum against the mentioned strains. As follows from these data, the strains con-

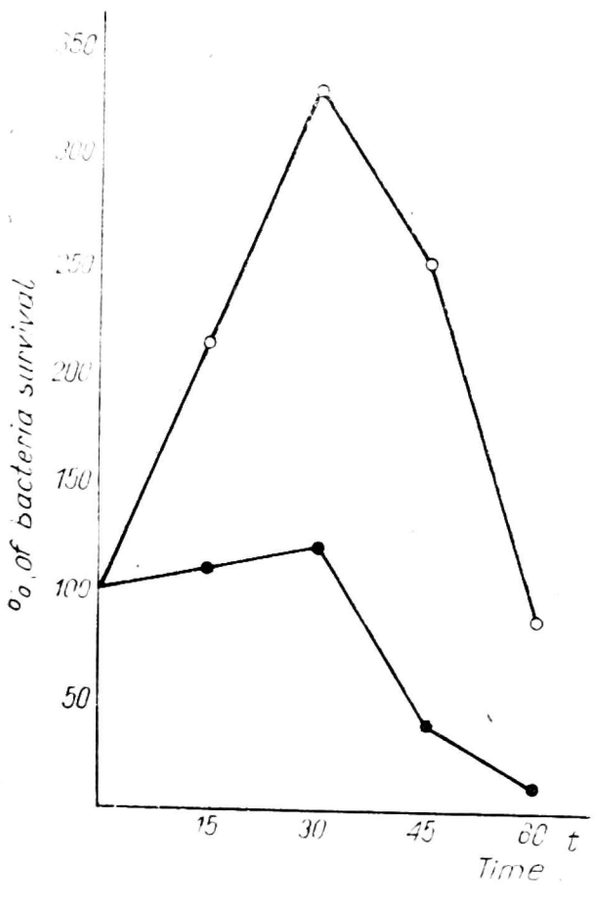

Fig. 1. Bactericidal activity of 2.5% neonate serum on the survival of *E. coli 711* Vir^+ (○) and Vir^- (●) strains

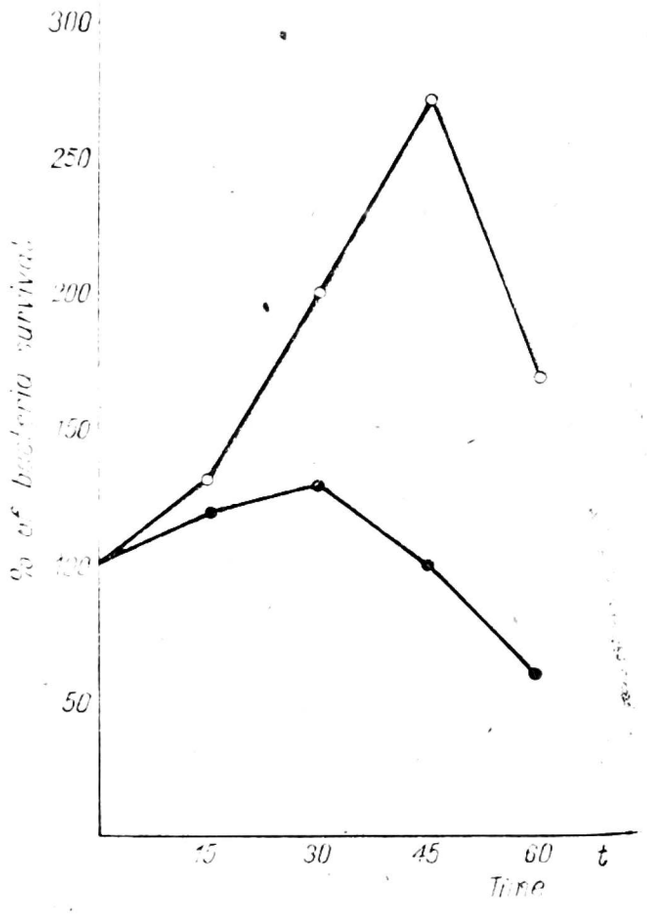

Fig. 2. Bactericidal activity of 1% rabbit serum on the survival of *E. coli 711* Vir^+ (○) and Vir^- (●) strains

taining the *Vir* plasmid show an increased resistance to bactericidal activity of sera.

In order to confirm the presence or absence of plasmids in the studied strains, DNA contained in the cells was isolated and electrophoresis was performed in agarose gel. In this way it was confirmed that the *Vir*$^-$ strains of both *E. coli H209* and *E. coli 711 Vir*$^-$ contain no plasmid DNA (Fig. 3).

Then, the molecular weight (m.w.) of the *Vir* plasmid was determined and compared with that of several other tested plasmids (Table 3, Fig. 3). It was found that

Table 3. A comparison of molecular weight of the *Vir* plasmid with that of several tested plasmids

Plasmid	Molecular weight (Mdal)
PYA VS 17A	35.8
RPI	40.0
R100.1	60.0
R64	76.0
Vir	76.0
R27	112.0

the m.w. of the *Vir* plasmid is 76 Mdaltons. In this respect it is identical to *R64* plasmid and is larger than *R100.1* plasmid, which also exerts a protective action against bactericidal activity of sera (Ogata et al. 1980).

DISCUSSION

The role of *K* antigens in the protective action against bactericidal activity of serum has not been completely explained so far. Some authors suggest their positive action (Glynn and Howard 1970, Howard and Glynn 1971, Gemski et al. 1980), others point out to a minimal or no importance of these polysaccharides in the discussed process (Mc Cabe et al. 1978, Pitt 1978, Taylor et al. 1979). The best studied in this respect were *K1* and *K27* antigens. Pathogenic strains of *E. coli* frequently contain *K1* antigen, which protects bacteria against serum activity (Robbins et al. 1974, Glode et al. 1977, Gemski et al. 1980, Opal et al. 1982). The presence of *K27* antigen, on the contrary, has no protective action (Taylor and Robinson 1980, Opal et al. 1982). Opal et al. (1982) found that *K27* antigen and probably other *K* antigens do not prevent bactericidal activity of human serum. In our studies in which we used the *E. coli H209* strain and its derivative form without ability to the synthesis of *K31* antigen, it was showed that the *K*$^-$ variant is definitely more sensitive to human and animals sera in comparison to the initial *K*$^+$ form. On the basis of these results it may be suggested that *K31* antigen, like *K1*, has a protective action. In the studies on the antigen structure of the discussed strains the authors Smith (1974) and Morris et al. (1982) detected no other differences except those concerning the presence of the *K* antigen.

The *Vir* plasmid described by Smith (1974) increases pathogenicity of *E. coli* strains. This is related with the coding by this plasmid of the ability to produce toxin and to increase adhesion of bacteria to epithelial cells of the intestine (Morris et al. 1982). In our studies it was found that the *Vir* plasmid protects bacteria against bactericidal serum activity. It was shown not only on smooth (*H209*), but also on rough (*711*) strains of *E. coli*. This resistance is likely to be associated with the occurrence of an outer antigen in the cells, which depends on the *Vir* plasmid. This antigen was discovered by Smith (1974) in the strain *H209* and its presence was supported by Lopez-Alvarez and Gyles (1980) in several other strains of *E. coli*, which were found to have the *Vir* plasmid. The chemical structure of this antigen has not been described till now. In the studies on the importance of the *R* and *Col V* plasmids in the process of strain resistance to bactericidal serum activity the role of outer structures coded by these plasmids has been perceived (Moll et al. 1980, Taylor et al. 1979, Binns et al. 1979, Taylor and Robinson 1980). Moll et al. (1980) showed that in the case of the plasmid *R6*-5-determined resistance of bacteria to sera the factor protecting cells is a protein localized in the outer membrane with the n.w. of 25 000 daltons, whose copy number per cell is about 21 000. The synthesis of that protein is coded by the *traT* gene of the plasmid. It is responsible for outer exclusion. A detailed knowledge of the nature of antigen or antigens coded by the *Vir* plasmid genes permitted with certainty to determine thoroughly their role in raising bacterial resistance to bactericidal serum activity.

Noteworthy is the fact that *K31* antigen protects bacteria to a higher degree than does the *Vir* plasmid against bactericidal activity of all the studied sera. The degree of susceptibility of the variants of the strains *H209* to sera is as follows; $K^-Vir^- < K^-Vir^+ < K^+Vir^- < K^+Vir^+$. The highest resistance to sera of the variant K^+Vir^+ indicates that the both agents interact in the discussed process. Synergetic protective action of agents may be of large practical importance in case of bacterial infections.

CONCLUSIONS

1. *K31* antigen increases the resistance of *E. coli* bacilli to sera.
2. The *Vir* plasmid determines also *E. coli* resistance to sera, but to a lesser degree than does *K31* antigen.
3. *K31* antigen and *Vir* plasmid act synergetically increasing the resistance of *E. coli* bacilli to bactericidal activity of sera.

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OPORNOŚĆ PAŁECZEK *ESCHERICHIA COLI* NA BAKTERIOBÓJCZE DZIAŁANIE SUROWIC LUDZKICH I ZWIERZĘCYCH UWARUNKOWANA OBECNOŚCIĄ PLAZMIDU *VIR* I ANTYGENU *K31*

Streszczenie

W badaniach prowadzonych na gładkich szczepach *E. coli H209* różniących się obecnością antygenu *K31* i plazmidu *Vir* wykazano, że zarówno antygen *K* jak i plazmid *Vir* chroni bakterie przed bakteriobójczym działaniem surowicy. Ochronną rolę plazmidu *Vir* wykazano również w badaniach nad szorstkimi szczepami *E. coli*.

СОПРОТИВЛЯЕМОСТЬ ПАЛОЧЕК *ESCHERICHIA COLI* К БАКТЕРИЦИДНОМУ ДЕЙСТВИЮ СЫВОРОТКИ ЧЕЛОВЕКА И ЖИВОТНЫХ, ОБУСЛОВЛЕННАЯ НАЛИЧИЕМ ПЛАЗМИДА *Vir* И АНТИГЕНА *K31*

Резюме

Во время исследований, проведённых на гладких штаммах *E. coli H209*, отличающихся наличием в них антигена *K31* и плазмида *Vir*, обнаружено, что как антиген *K*, так и плазмид *Vir* защищают бактерии от бактерицидного действия сыворотки. Защитная функция плазмида *Vir* была также обнаружена в исследованиях шёрстких штаммов *E. coli*.