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COLICIN PRODUCTION IN RELATION TO PATHOGENICITY FACTORS IN STRAINS OF ESCHERICHIA COLI ISOLATED FROM THE INTESTINAL TRACT OF PIGLETS

By

Berit K. Djønne

DJØNNE, BERIT K.: *Colicin production in relation to pathogenicity factors in strains of Escherichia coli isolated from the intestinal tract of piglets.* Acta vet. scand. 1985, *26*, 145—152. — Three hundred and fifteen E. coli strains isolated from piglets dead due to neonatal E. coli diarrhea or traumatic lesions, were examined for presence of enterotoxin production, K-antigens 88 and 99, antibiotic resistance and colicin-production.

Almost 100 % of the E. coli strains positive on enterotoxin production produced colicin, while approx. 17 % of the non-enterotoxigenic strains did so.

Ninety-nine % of the E. coli strains harbouring K-antigens were found to produce colicin, while approx. 23 % of the strains without detectable K-antigens did so.

In strains where neither K-antigens nor enterotoxin production was found, resistance against tetracycline, neomycin and ampicillin, and sensitivity against streptomycin, was more commonly found among colicin-producing strains, than among strains without colicin production.

enteropathogenicity factors; K-antigens; enterotoxin production; virulens factors.

Colicins are proteins produced by Escherichia coli (E. coli) and related enterobacteria. Colicins have bacteriocidal effect primarily on strains of E. coli but also on some closely related organisms (*Bull & Meadow* 1978).

About 20 different colicins have been demonstrated (*Fredericq* 1964). One strain of E. coli can produce several colicins, which separately might act on other strains suceptible to these colicins.

In E. coli strains isolated from pigs, colicin production has not been found to correlate with pathogenicity (*Alwis & Thomlinson* 1973). In other animals, however, invasive E. coli strains with the colicin V plasmid are more pathogenic than strains without this plasmid (*Smith* 1974, *Smith & Huggins* 1976).

The aim of the present study was to examine E. coli strains isolated from the intestines of piglets for production of colicins, and to correlate this characteristic to the presence of pathogenicity factors in the same strains.

In E. coli, resistance to antibiotics has been found to be correlated to enterotoxin production and the presence of K-antigens. (*Gyles et al.* 1977, *Liven* 1979). In order to study the connection between colicin production and drug resistance without influence from other pathogenicity factors, E. coli strains without detectable enterotoxin production or K-antigens were tested for resistance to several antibiotics.

MATERIALS AND METHODS

Piglets

The investigation included 43 piglets between 1 and 14 days of age. The piglets originated from 29 herds located in Southern Norway. They were necropsied at the National Veterinary Institute, during 1982 and January 1983. Thirty piglets had gross lesions and bacteriological findings corresponding to those seen in neonatal E. coli diarrhea. In 13 piglets traumatic lesions were the main findings at necropsy.

E. coli strains

Bacteriological examination of the jejunal content was carried out according to standard cultural techniques on blood-agar and bromthymolblue-lactose agar. The plates were incubated at 37°C for approx. 20 h. The E. coli strains were identified by the IMVIC-test (Indole, Methylred, Voges-Proskauer, Citrate) and by their ability to produce gas from glucose. Six to 10 strains were isolated from each piglet.

Demonstration of O-antigens, K-antigens and enterotoxins

E. coli strains were examined for 0-antigen 2, 6, 8, 9, 32, 45, 64, 98, 101, 115, 124, 125 ab, 138, 139, 141, 145, 147, 149 and 157 by means of the tube agglutination test, and for the K-antigens 88 and 99 by means of the slide-agglutination test (*Søderlind* 1971, *Guinèe et al.* 1977). The strains were examined for production of heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) by the enzyme-linked immunosorbent assay (*Olsvik et al.* 1982)* and the suckling mouse test, respectively (*Dean et al.* 1972).

* Kindly performed at the Norwegian Defence Microbiological Laboratory, National Institute of Public Health, Oslo.

Demonstration of antibiotic resistance

The E. coli strains were examined for resistance to antibiotics on Mac-Conkey agar. One colony of each strain was diluted in 10 ml of saline, and poured on the plates. Excess material was removed, and the plates were left at room temperature for about 30 min before disks (Ab-biodisk*) containing the following antibiotics were applied: Tetracycline (Te) — 30 μg, Streptomycin (S) — 30 μg, Neomycin (N) — 30 μg, Ampicillin (A) — 10 μg and Chloramphenicol (C) — 30 μg. The plates were kept at room temperature for 1—2 h, then incubated at 37°C for 20 h. The inhibition zone between the antibiotic disc and the bacterial growth was measured as recommendedb by AB-Biodisk.

Demonstration of colicin production

Th ability of E. coli strains to produce colicins was examined by a modification of the method described by *de Alwis & Thomlinson* (1973). Spot cultures of the strain to be tested were made on blood-agar. The cultures were incubated at 37°C for about 20 h, killed by exposure for chloroform vapour and left at room temperature for 30 min to remove residual chloroform. The indicator strain, Row**, was cultured on blood agar. One colony of Row from this medium was diluted in 5 ml of saline and poured on the plates with the chloroform-killed strains. Excess material was removed, and the plates were kept at room temperature for 1—2 h, then incubated at 37°C for about 20 h. If the inhibition zone between the spot culture and the indicator strain extended 1 mm or more the strain tested was regarded colicin producing.

Statistical methods

The Chi-square test at $P < 0.005$ or $P < 0.001$ were used for statistical significance testing.

RESULTS

From the 43 piglets 315 E. coli strains were isolated.

Enterotoxin production was found in 203 E. coli strains. All but one of these strains originated from the piglets dead due to neonatal E. coli diarrhea.

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* AB Biodisk, Pyramidvägen 7, S-171 36 Solna, Sweden.

** Kindly supplied by Dr. P. Fredericq, Institut de Microbiologie et Hygiéne de l'Université, Liège, Belgien.

In 112 E. coli strains no enterotoxin production was found. One of these strains originated from a piglet dead due to neonatal E. coli diarrhea, while the others were isolated from piglets assumed dead due to traumatic lesions.

The distribution of colicin production among the E. coli strains isolated in relation to enterotoxin production and the presence of K88 and K99 antigens is presented in Table 1.

Table 1. Colicin production in 315 E. coli strains with various patterns regarding O-antigens, K-antigens and enterotoxin production.

O-antigen, K-antigen and enterotoxin prduction	Number of colicin positive strains	Number of colicin negative strains
O 149, K88, LT+[1], ST+[2]	126	1
O—[3], K88, LT+, ST+	2	0
O9, K99, LT—[4], ST+	1	0
O64, K99, LT—, ST+	71	0
O8, K—[5], LT+, ST+	1	0
O—, K—, LT+, ST+	1	0
O147, K88, LT—, ST—[6]	0	2
O-var[7], K—, LT—, ST—	19	91
Total	221	94

1 Heat-labile toxin demonstrated.
2 Heat-stable toxin demonstrated.
3 No O-antigens demonstrated.
4 Heat-labile toxin not demonstrated.
5 K88 antigen and K99 antigen not demonstrated.
6 Heat-stable toxin not demonstrated.
7 O-antigens 8, 45, 64, 141, 147 or 157 or no O-antigens demonstrated.

There is a statistically significant difference ($P < 0.001$) in colicin production between E. coli strains producing enterotoxin (99.5 %) and strains where no enterotoxin production was found (16.9 %).

Colicin production was statistically more often found in strains where no K-antigens was demonstrated (23.2 %) ($P < 0.001$).

Of the 315 E. coli strains tested, 110 were designated "non-enteropathogenic" due to lack of demonstration of enterotoxin production and K-antigens. In 19 of these strains colicin production was demonstrated, while 91 strains were negative on colicin production (Table 1).

The relation between colicin production and antibiotic resistance in the 110 non-enteropatogenic E. coli strains is presented in Table 2.

Table 2. Colicin production in relation to resistance (R) or sensitivity (S) to antibiotics (in %) in 110 non-enteropathogenic[1] E. coli strains.

Colicin production	Te[2]		S[3]		N[4]		A[5]		C[6]	
	R	S	R	S	R	S	R	S	R	S
Colicin positive strains (n = 19)	84.2	15.8	15.8	84.2	21.1	78.9	10.5	89.5	0	100
Colicin negative strains (n = 91)	11.0	89.0	63.7	36.3	1.1	98.9	0	100	0	100

1 Neither enterotoxin production nor K-antigens demonstrated.
2 Tetracycline
3 Streptomycin
4 Neomycin
5 Ampicillin
6 Chloramphenicol

Among the 110 non-enteropathogenic E. coli strains resistance to Te, N and A was more often seen in colicin-producing strains (84.2 %, 21.1 %, 10.5 %), than in strains without colicin production (11.0 %, 1.1 %, 0 %) ($P < 0.005$).

Resistance to S was more often demonstrated in E. coli strains without colicin production (63.7 %) than in colicin-producing strains (15.8 %) $P < 0.001$.

DISCUSSION

In the present investigation colicin production was demonstrated in almost 100 % of the E. coli strains where enterotoxin production or K-antigens 88 or 99 was found. About 18 % of the E. coli strains where neither enterotoxins nor K-antigens could be demonstrated was found to produce colicins.

Vasenius (1967) found that 52.4 % of the E. coli strains isolated from pigs affected with "colibacillosis" and 17 % of the E. coli strains isolated from healthy piglets produced colicin. *Larsen* (1976) reported from Denmark that 40.6 % of E. coli strains isolated from piglets with neonatal E. coli diarrhea produced colicin. *Craven & Barnum* (1971) found colicin production

in 42 % of the E. coli strains frem healthy piglets in Canada. The frequencies of colicin-producing strains found in the present examination are not directly comparable with those reported by *Vasenius, Larsen* and *Craven & Barnum*, due to the fact that colicin production is exclusively related to the presence of enteropathogenicity factors in E. coli strains.

The difference in the reported frequencies of colicin-producing strains may also be due to a difference in the characteristics of E. coli strains found in different countries at various periods. Sampling from different parts of the intestinal tract as well as the age of the piglets at the time of sampling may be of importance in this connection.

Genetic studies have demonstrated that determinants for enterotoxin production and K-antigens in enteropathogenic E. coli are transmissible (*Ørskov & Ørskov* 1966, *Smith & Linggood* 1971). In the present study colicin production was commonly found in E. coli carrying these enteropathogenicity factors. This justifies the suggestion that there may be a genetic basis for this correlation. Genes coding for colicin production may be transferred simultaneously with genetic determinants of some of the enteropathogenicity factors. *Franklin et al.* (1981) found that in E. coli 0149, genes coding for ST-production and genes coding for colicin production were located on the same plasmid.

In enteropathogenic E. coli, plasmids simultaneously coding for enterotoxin production and antibiotic resistance have been reported (*Tschäpe & Rische* 1974, *Gyles et al.* 1977). For this reason colicin production was studied in relation to the pattern of antibiotic resistance in the E. coli strains where no enteropathogenicity factors was demonstrated (Table 2). Among these strains resistance to Te, N and A, and sensitivity to S were more commonly found in colicin-producing strains than in strains without colicin production, indicating a correlation between antibiotic resistance and colicin production. This is in accordance with the results of *Swiderski & Lachowicz* (1974) who found that in E. coli the abilitis of colicin production and resistance to Te were transferred simultaneously.

The strong correlation between the presence of pathogenicity factors and production of colicins in intestinal E. coli strains demonstrated in this investigation justifies the suggestion that colicin production could be of some importance to the enteropathogenicity of E. coli strains. The present study, however,

gives no evidence to this suggestion, indicating the necessity of further studies in this context.

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REFERENCES

Bull, A. T. & P. M. Meadow: Companion to microbiology: Selected topics for further studies. Longman, London 1978, *VII*, p. 109—126.

Craven, J. A. & D. A. Barnum: Ecology of intestinal Escherichia coli in pigs. Canad. J. comp. Med. 1971, *35*, 324—331.

De Alwis, M. C. L. & J. R. Thomlinson: The incidence and distribution of colicinogenic and colicin-sensitive Escherichia coli in the gastro-intestinal tract of the pig. J. gen. Microbiol. 1973, *74*, 45—52.

Dean, A. G., Yi-Chuan Ching, R. G. Williams & L. B. Harden: Test for Escherichia coli enterotoxin using infant mice: Application in a study of diarrhea in children in Honolulu. J. inf. Dis. 1972, *125*, 407—411.

Franklin, A., O. Søderlind & R. Møllby: Plasmids coding for enterotoxins, K88 antigen and colicins in porcine Escherichia coli strains of O-group 149. Med. Microbiol. Immunol. 1981, *170*, 63—72.

Fredericq, P.: A note on the classification of colicins. Institut de Microbiologie et Hygiène de l'Universitè, Liège, Belgien. Gustav Fisher Verlag, Stuttgart 1964.

Guinèe, P. A. M., J. Veldkamp & W. H. Jansen: Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of Escherichia coli. Infect. Immun. 1977, *15*, 676—678.

Gyles, C. L., S. Palchaudhuri & W. K. Maas: Naturally occurring plasmid carrying genes for enterotoxin production and drug resistance. Science 1977, *198*, 198—199.

Larsen, J. L.: Differences between enteropathogenic Escherichia coli strains isolated from neonatal E. coli diarrhoea (N.C.D.) and post weaning diarrhoea (P.W.D.) in pigs. Nord. Vet.-Med. 1976, *28*, 417—429.

Liven, E.: Relationship between production of enterotoxin and various drug resistance patterns in strains of Escherichia coli isolated from piglets suffering from colienterotoxemia. Acta vet. scand. 1979, *20*, 396—403.

Olsvik, Ø., O. T. Hushovd, B. P. Bredal, T. Bergan & M. Mathiesen: Production of enterotoxin by Escherichia coli at four, twenty-two and thirty-seven degrees centigrade. Eur. J. clin. Microbiol. 1982, *1*, 12—16.

Smith, H. W.: A search for transmissible pathogenic characters in invasive strains of Escherichia coli: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. J. gen. Microbiol. 1974, *83*, 95—111.

Smith, H. W. & M. B. Huggins: Further observations on the association of the colicine V plasmid of Escherichia coli with pathogenicity and with survival in the alimentary tract. J. gen. Microbiol. 1976, *92*, 335—350.

Smith, H. W. & M. A. Linggood: Observations on the pathogenic properties of the K88, Hly and Ent plasmids of Escherichia coli with particular reference to porcine diarrhoea. J. med. Microbiol. 1971, *4*, 467—485.

Swiderski, M. & T. H. Lachowicz: Occurrence and transfer of antibiotic resistance, colicinogeny and haemolysin production in Escherichia coli strains isolated from cases of colibacillosis. Acta microbiol. polonica 1974, *6*, 155—162.

Søderlind, O.: Studies on Escherichia coli in pigs II. Serological investigations. Zbl. Vet. Med. B. 1971, *18*, 569—590.

Tschäpe, H. & H. Rische: Die Virulenz-Plasmide der Enterobacteriaceae. (The virulens-plasmids of enterobacteriaceae). Z. Alg. Mikrobiol. 1974, *14*, 337—350.

Vasenius, H.: The colicinogenic properties of porcine Escherichia coli strains. Acta vet. scand. 1967, *8*, 195—200.

Ørskov, I. & F. Ørskov: Episom-carried surface antigen K88 of Escherichia coli. J. Bacteriol. 1966, *91*, 69—75.

SAMMENDRAG

Colicin produksjon relatert til enteropatogenitets faktorer hos stammer av Escherichia coli isolert fra tarmkanaler hos spedgris.

Forekomst av colicin produksjon, enterotoksin produksjon, K-antigen 88 og 99 og antibiotika resistens ble undersøkt hos 315 E. coli-stammer isolert fra tynntarmen hos spedgris døde pga. kolidiare eller traumatisering.

Colicin produksjon ble påvist hos nesten 100 % av alle enterotoksin-produserende E. coli-stammer, og hos 17 % av stammene uten påvisbar enterotoksin-produksjon.

Av alle E. coli-stammer med K-antigen produserte 99 % colicin. Av stammene hvor K-antigen ikke ble påvist produserte 23 % colicin.

Blant E. coli-stammer uten enterotoksin produksjon og uten K-antigen, ble resistens mot tetracyklin, neomycin og ampicillin samt sensitivitet mot streptomycin oftere påvist hos colicin-produserende stammer enn hos stammer som ikke produserte colicin.

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Reprints may be requested from: Berit K. Djønne, the National Veterinary Institute, P. O. Box 8156 Dep., 0033 Oslo 1, Norway.