The Incidence and Distribution of Colicinogenic and Colicin-sensitive *Escherichia coli* in the Gastro-intestinal Tract of the Pig

By M. C. L. DE ALWIS AND J. R. THOMLINSON

Department of Veterinary Pathology, University of Liverpool, Liverpool, L69 3BX

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SUMMARY

Eleven *Escherichia coli* serotypes recognized as being pathogenic for the pig and 231 commensal strains of *E. coli* from pigs were examined for colicinogeny and colicin sensitivity. Of the commensal strains, 43.7% were colicinogenic, and produced 11 known colicins and others that could not be identified. The only pathogenic strains shown to be colicinogenic were those of the O141 serogroup which produced colicin B.

All the pathogenic strains were sensitive to colicins produced by the commensal strains. The implications of these findings are discussed in relation to the dominance of pathogenic serotypes in colibacillosis.

INTRODUCTION

Relatively few investigations have been made into the colicinogenic properties of *Escherichia coli* from the gastro-intestinal tract of the pig. Levine & Tanimoto (1960) observed that colicinogenic strains were present in 83.7% of the pigs they investigated. More recently, Vasenius (1967) found a higher incidence of colicinogenic *E. coli* in pigs suffering from colibacillosis than in normal animals and concluded that the ability to produce colicin may be at least a contributory factor in the preservation of potentially pathogenic serotypes among the intestinal flora of healthy pigs. On the other hand, Craven, Miniats & Barnum (1971) concluded from their experiments with dual-infected gnotobiotic pigs that colicin production did not appear to influence bacterial antagonism in the alimentary tract and that other factors such as the differential growth rates of individual strains were probably responsible. A strain of *E. coli* producing an unidentified colicin was capable of dominating colicin-resistant mutants and a non-colicinogenic strain was capable of dominating certain colicin-sensitive strains.

The present work formed part of an investigation into factors that may lead to the dominance of pathogenic *Escherichia coli* strains in the gastro-intestinal tract of the pig. Colicins were identified and sensitivity patterns were investigated in commensal and pathogenic strains with a view to determining whether colicins may play any part in the stabilization of the coliform enteric flora.

MATERIALS

Sources of commensal Escherichia coli

A total of 231 strains from healthy pigs derived from two sources were isolated on 5% sheep blood agar. All these strains possessed the characteristic biochemical features of *Escherichia coli* and, since they failed to type with antisera to any of the recognized porcine

Table 1. *Colicin sensitivity of the standard indicator strains of Escherichia coli*

Indicator strain	Colicins									
	A	B	E ₁	E ₂	E ₃	G	Ia	Ib	K	V
CL142	S	S	S	S	S	S	S	S	S	S
CL136	R	S	R	S	S	S	S	S	S	S
CL135	S	R	S	S	S	S	S	S	S	R
CL137	S	S	S	R	S	S	S	S	S	S
CL145	s	S	R	R	R	S	S	S	S	S
CL144	S	R	S	S	S	s	S	S	S	S
CL223	S	S	S	S	S	S	R	S	S	S
CL232	S	S	S	S	S	S	S	R	S	S
CL146	R	S	S	S	S	S	S	S	R	S
CL205	S	S	S	S	S	S	R	R	S	R
CL184	S	S	R	S	S	S	R	R	S	S
CL201	R	S	R	R	R	S	S	S	S	S

S = Sensitive; s = partly sensitive; R = resistant.

pathogenic serotypes, they were regarded as commensals.

Source A. One hundred non-haemolytic and 27 haemolytic strains were isolated from swabs taken from various regions of the gastro-intestinal tracts of 20 freshly slaughtered pigs at an abattoir in Liverpool.

Source B. Eighty-two non-haemolytic and 22 haemolytic strains were isolated from rectal swabs obtained from 70 weaned pigs selected at random from a large herd in Scotland.

Sources of pathogenic Escherichia coli

Eleven haemolytic *Escherichia coli* strains, kindly supplied by Mr W. J. Sojka and recognized as pathogenic for the pig, were examined for colicin production and sensitivity. These strains represented the following 'OK' groups - 0141:K85a, b(B), 88a, b(L); 0141:K85a, b(B); 0141:K85a, c(B); 0141:K85a, c(b), 88a, b(L); 0138:K81(B); 0138:K81(B), 88a, c(L); 08:K87(B?), 88a, b(L); 08:K87(B?), 88a, c(L); 0139:K82(B); 0147:K79(B), 88a, c(L); and 0149:K91, 88a, c(L).

Standard colicin-sensitive and colicinogenic strains

Standard strains of *Escherichia coli* and *Shigella* having specific patterns of colicin sensitivity or known colicinogenic properties were kindly provided by Professor P. Fredericq and Dr M. J. Lewis. Table 1 shows the colicin sensitivity patterns of these various strains and their colicinogenic properties are shown in Table 2. In addition, five indicator strains with specific patterns of colicin sensitivity were selected from the commensal *E. coli* isolated from pigs. Of these, strain F3 was specifically sensitive to colicins D and F, F7 to colicins D and J, F10 to colicin D only, F57 to colicins D and H, and C18 to colicins D and S₃.

Colicin tests

The method was similar to that of Fredericq (1948). Spot cultures of up to eight strains to be tested for colicin production were made on nutrient agar plates (pH 7.4) in glass Petri dishes of 10 cm diameter. The cultures were incubated at 37 °C for 24 h, killed by exposure to chloroform vapour, incubated at 37 °C for 30 min to remove any residual chloroform and flooded with a 6 to 8 h broth culture of an indicator strain. Excess broth was removed and, after incubation at 37 °C for a further 24 h, the plates were examined for zones of inhibition.

Table 2. *Standard colicinogenic strains of Escherichia and Shigella*

Source	Strain	Colicin produced
Dr M. J. Lewis	<i>Escherichia coli</i>	
	CL123	A
	CL121	B
	CL122	D
	CL136	E ₁
	CL137	E ₂
	CL124	E ₃
	CL126	G
	CL223	Ia
	CL232	Ib
	G 144, G 206	K
	G 275	V
Professor P. Fredericq	<i>Escherichia coli</i>	
	CA57	C
	CA42	F
	CA58	H
	CA62	J+I
	<i>Shigella boydii</i> P1	S ₁
	<i>S. sonnei</i> P9	S ₃ +I
	<i>S. dispar</i> P14	S ₄
	<i>S. dispar</i> P15	S ₅

Colicins A, B, E (E₁, E₂ and E₃), G, Ia, Ib, K and V were identified on the basis of the specific pattern of resistance of the indicator strains. Colicins D, F, H, J and S₃ were identified on the basis of specific sensitivity of indicator strains to these colicins.

Colicin sensitivity was determined by the use of similar tests with the standard colicinogenic strains, in which the *Escherichia coli* strains under investigation were used as indicators.

RESULTS

Incidence of colicinogenic Escherichia coli

Commensal strains. Of the 231 commensal strains tested, 101 (43.7%) were colicinogenic. The colicins identified were A, B, E (E₁, E₂ and E₃), F, G, H, I, J, K, S₃ and V (Table 3), and were produced by 68 of the strains. Of these, colicin E was the most common. The colicins produced by the remaining 33 colicinogenic strains could not be identified with the indicator strains available.

The percentage of colicinogenic strains isolated from the caecum, colon and rectum of source A was similar to that from the rectal swabs of source B (Table 3) but the small intestine of source A yielded a lower proportion of colicinogenic strains.

Pathogenic strains. Of the 11 pathogenic *Escherichia coli* strains, only four, 0141:K85a, b(B), 88a, b(L), 0141:K85a, b(B), 0141:K85a, c(B) and 0141:K85a, c(B), 88a, b(L), were colicinogenic. Colicin B was produced by all four strains.

Colicin sensitivity

Commensal strains. Of the 231 commensal strains examined, 221 (95.6%) showed colicin sensitivity. Of these, 67 were sensitive to only one colicin. Of the remainder, 70 were sensitive to five or more colicins and two were sensitive to 14. No regularity was observed in the sensitivity pattern and the strains could not be classified into a reasonable number of groups on the basis of their sensitivity patterns.

Table 3. *Colicins produced by commensal strains of Escherichia coli from pigs*

Source	Site of isolation	Haemolysis	No. of strains examined	No. of strains colicinogenic	Colicinogenic (%)	Colicin															
						A	B	D	E	F	G	H	I	J	K	S ₃	V	U*			
A	Small intestine	—	54	11	20·3	1	0	0	2	0	1	1	2	0	2	1	3	5			
		+	15	3	20·0	0	0	0	1	0	0	0	0	0	0	0	0	2			
	Caecum and colon	—	27	18	66·6	1	1	0	3	2	0	2	0	1	0	1	2	10			
		+	9	1	11·1	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
B	Rectum	—	19	9	47·3	0	0	0	6	0	1	0	1	0	0	1	0	2			
		+	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	Faeces	—	82	50	60·9	8	0	0	17	2	6	3	9	2	4	7	1	12			
		+	22	9	40·9	1	0	0	1	1	0	1	2	1	0	1	3	2			
Total			231	101	43·7	11	1	0	30	5	8	7	14	4	6	11	10	33			

* No. of strains producing unidentified colicines.

Table 4. *The antibacterial spectrum of colicines against strains of Escherichia coli from healthy pigs*

Source	Site of isolation	Haemolysis	No. of strains examined	No. of strains sensitive to colicin																			
				A	B	C	D	E ₁	E ₂	E ₃	F	G	H	Ia	Ib	J	K	S ₁	S ₃	S ₄	S ₅	V	
A	Small intestine	—	54	6	8	0	54	11	16	12	19	4	1	0	0	18	4	0	19	0	4	6	
		+	15	0	4	0	13	8	7	1	8	1	0	0	0	8	1	0	7	0	1	2	
	Caecum and colon	—	27	1	1	0	25	1	3	2	2	2	1	0	0	3	1	0	3	0	0	0	
		+	9	0	0	0	9	4	6	1	8	2	1	0	0	4	1	0	5	0	1	1	
B	Rectum	—	19	0	0	0	19	3	6	0	6	1	0	0	0	2	1	0	4	0	1	1	
		+	3	0	0	0	2	2	1	2	0	1	0	0	0	2	1	0	2	0	1	1	
	Faeces	—	82	9	16	0	73	32	28	23	24	41	5	3	4	24	5	0	25	0	3	0	
		+	22	0	3	0	21	9	8	11	1	0	0	0	0	7	1	0	10	0	0	0	
Total				231	16	32	0	216	70	76	48	80	52	9	3	4	68	15	0	75	0	11	11

Colicin D showed the widest spectrum of activity and was active against 216 (93.5%) of the 231 commensal *Escherichia coli* strains. Other colicins showed varying degrees of activity (Table 4). For instance, colicins F, E₂, S₃, E₁ and J were active against 34.6, 32.9, 32.4, 30.3 and 29.4% of the commensal strains, respectively, while colicin B, which was the only one produced by any of the pathogenic serotypes, was active against only 13.8% of the commensal strains. Colicins C, S₁ and S₄ showed no activity.

Pathogenic strains. Each of the eight pathogenic strains tested (Table 5) was sensitive to the action of at least two colicins. The widest range of sensitivity was shown by the strains of 'O' group 141. Two of these were sensitive or partially sensitive to ten colicins and the other was sensitive to nine. *Escherichia coli* strain O8:K87(B), 88a, b(L) showed the narrowest range, and was sensitive to colicins D and F and partially sensitive to colicin E₂.

Colicin D had the widest spectrum of activity. Seven of the eight pathogenic strains were sensitive to this colicin and the other was partially sensitive. Of the other colicins, E₁, E₂ and F had the widest spectra of activity.

DISCUSSION

In these investigations, 43.7% of commensal *Escherichia coli* strains from pigs were shown to be colicinogenic, whereas the only pathogenic strains that showed colicinogenic activity were those of the O141 group. Vasenius (1967) found that 59.3% of *E. coli* strains isolated from pigs suffering from colibacillosis were colicinogenic, whereas only 17.2% from healthy pigs showed this property.

Earlier reports on the incidence of colicinogenic bacteria in the faeces of man and various animal species have shown some variation. Gardner (1950) examined 317 strains isolated mainly from human faeces and reported an incidence of 11.6% whereas Papavassiliou (1963) reported an incidence of 32.1% among *Escherichia coli* from human faeces, 28.6% from the dog, 26.3% from sheep and relatively few from rats and mice. In the present investigation, the incidence of colicinogeny was lower among strains isolated from the small intestine than from the rectum. This finding was not surprising since Vosti (1968) observed that, in human faeces, resident serotypes are more consistently colicinogenic than are transient ones and, according to Dixon (1960), the small numbers of organisms found in mammalian small intestines are transient, there being few resident organisms in this part of the intestine. However, it is clear that, at least in pigs, the site of isolation is important and results from the contents of the small intestine cannot be directly compared with those from the colon, rectum or faeces. The high incidence of colicinogeny observed in the commensal strains from pigs was consistent with the findings of Branche, Young, Robinet & Massey (1963), who observed a high incidence of colicinogeny when the multiplicity of serotypes was high and a low incidence when few serotypes were present. Serological analysis of the commensal strains (de Alwis, 1970) had revealed considerable heterogeneity.

On the other hand, the high incidence of colicinogeny observed by Vasenius (1967) in strains from diseased pigs may have been a reflexion of the dominance of one particular serogroup. Although, in the present investigation, O141 was the only serogroup to show colicinogeny, only one example of each pathogenic serotype was tested and Vasenius (1967) observed a high incidence of colicinogeny in serogroups other than O141, especially when they had been isolated from diseased pigs. A similar high incidence of colicinogeny has been observed in pathogenic *Escherichia coli* serotypes from children and from calves (Papavassiliou, 1961; Likhoded & Kudlai, 1963). Vosti (1968) has shown that individual strains within a serogroup may differ in their colicinogenic activity or in the colicin they produce,

Table 5. Colicin sensitivity of some pathogenic serotypes of *Escherichia coli* from pigs

<i>Escherichia coli</i>	Colicin														
	A	B	C	D	E ₁	E ₂	E ₃	F	G	H	Ia	Ib	J	K	V
'OK' groups															
o8:K87(B?), K88a, b(L)	R	R	R	S	R	s	R	S	R	R	R	R	R	R	R
o138:K81(B)	R	S	R	S	S	S	S	S	R	R	R	R	S	R	R
o138:K81(B), K88a, c(L)	R	S	R	S	S	S	s	S	R	R	R	R	S	R	R
o139:K82(B)	S	R	R	s	S	S	R	S	R	R	R	R	R	R	R
o141:K85a, b(B), K88a, b(L)	s	R	R	S	S	S	S	S	R	R	R	R	S	S	R
o141:K85a, b(B)	S	R	R	S	S	S	R	S	R	R	R	R	S	S	R
o141:K85a, c(B)	S	R	R	S	S	S	S	S	R	R	R	R	S	S	R
o149:K91, K88a, c(L)	R	S	R	S	s	R	R	R	R	R	R	R	S	R	R

R = Resistant; S = sensitive; s = partially sensitive.

and it is possible that conditions favouring the multiplication of certain colicinogenic strains may also favour the transfer of episomes controlling colicinogeny (Braude & Siemieniowski, 1968). In experimental shigella infections, Halbert (1948) observed an increase in the number of antagonistic coliforms after infection had occurred. Clearly, the results of investigations in which relatively small numbers of strains have been examined, or have been derived from a highly selected source such as a single outbreak of disease, must be interpreted with caution, and further clarification of these mechanisms will necessitate the examination of a large number of individual strains of each pathogenic serotype from a wide variety of sources.

The question of whether colicins play any part in the stabilization of the enteric flora is also a subject of controversy. Much of the evidence (Halbert & Swick, 1950; Friedman & Halbert, 1960; Braude & Siemieniowski, 1968; Ikari, Kenton & Young, 1969; Kelstrup & Gibbons, 1969; Craven *et al.* 1971) suggests that they do not contribute significantly to selective advantage in the intestinal tract where they are probably inactivated by free trypsin (Gratia & Betz-Bareau, 1946; Gardner, 1950), but that they may be active in infections of the peritoneal cavity or the urinary bladder. Since they occur at significant levels in serum and local exudates (Halbert & Swick, 1950), it is also possible that they may play a part in resistance to generalized infection.

A widely accepted feature in the pathogenesis of colibacillosis in pigs or other young animals is the dominance of one or other of the pathogenic *Escherichia coli* serotypes in the gastro-intestinal tract. The only colicin produced by any of the pathogenic serotypes tested in the present experiments was colicin B to which only a relatively small proportion of commensal strains was sensitive. However, all the pathogenic serotypes were sensitive to at least one of the colicins produced by commensal strains. These included 11 of the known colicins and others that were not identified. Thus it would seem unlikely that a pathogenic serotype would assert dominance in the gastro-intestinal tract of the pig through the action of its colicin but, under suitable environmental conditions, colicins produced by commensal strains may be capable of exerting control on the population of pathogenic serotypes. Individual colicins differ in their susceptibility to trypsin (Gardner, 1950) and it is noteworthy that colicin D, which has a wide spectrum of activity and is relatively resistant to trypsin, was not produced by any of the commensal strains from pigs. However, environmental conditions in the gastro-intestinal tract may differ in individual animal species and may also be influenced by diet. Whether conditions favourable to the action of colicins may occur in the gastro-intestinal tract of the pig is the subject of further investigation.

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