

## Colicin V Production by Clinical Isolates of *Escherichia coli*

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Strains of *Escherichia coli* isolated from random fecal samples, urines of hospitalized and nonhospitalized patients who had urinary tract infections, and blood of patients with septicemia were examined for colicin V production. The percentage of ColV<sup>+</sup> strains isolated from blood (31.6%) or from urines of hospitalized patients with urinary tract infections (26.2%) was significantly greater ( $P < 0.01$ ) than the percentage isolated from feces (13.6%). The colicin V immunity determinant of ColV,I-K94 conferred immunity to 26% of the type V colicins produced by clinical isolates. Of the ColV<sup>+</sup> strains studied, 63.6% produced at least one other type of colicin.

Colicins are antibacterial proteins produced by some strains of *Escherichia coli* and related enterobacteria. They are classified into about 20 types (A, B, I, V, etc.) according to their effects on a set of strains which are resistant to particular colicins (8). Smith (21, 22) found that about 78% of *E. coli* strains responsible for generalized infections in livestock (calves, chicks, and lambs) produce colicin V; elimination of the plasmids coding for colicin V from these strains invariably reduced their virulence for experimental animals, whereas virulence was restored to its original level when ColV plasmids were reintroduced into the strains by conjugation.

Numerous ColV plasmids increase bacterial resistance to host defense mechanisms (21, 22). Two virulence determinants present on ColV plasmids have been identified. A determinant (*iss*) which increases resistance to complement-mediated antibacterial defense mechanisms in normal serum occurs on ColV,I-K94 (2). Other ColV plasmids code for an iron transport system (24, 27), in some cases in addition to possessing the *iss* determinant (24). The determinants for colicin V and for increased serum resistance are closely linked in ColV,I-K94. No effect on virulence can be ascribed to the plasmid genes coding for colicin V (2, 16) or for immunity to colicin V (2). But because virulence determinants are frequently present on ColV plasmids (2, 21, 22), colicin V production is an important (and readily detectable) indicator of the presence of virulence plasmids.

Minshew et al. (14) estimated the proportion of colicin V producers among strains isolated from urine, blood, and a variety of other extraintestinal sources. They found that colicin V producers were more common among isolates from these sources than from the feces of healthy

persons. The method they used to detect colicin V producers was to compare the effects of colicinogenic strains on a ColV<sup>+</sup> (and therefore colicin V-immune) strain and on a ColV<sup>-</sup> strain of *E. coli* K-12; ColV<sup>+</sup> strains inhibit the latter but not the former.

We have examined a larger sample of strains by using a different method for detecting colicin V producers so that we can also identify strains producing V colicins of different immunity groups. We found that the colicin V immunity determinant of ColV,I-K94 conferred immunity to only 26% of type V colicins. In addition, we detected ColV<sup>+</sup> strains which produced other colicins in addition to colicin V. In our sample, 63.6% of V<sup>+</sup> strains also produced one or more other types of colicin. Presumably because of these differences in the method for detecting colicin V producers, we found a higher proportion of ColV<sup>+</sup> among isolates responsible for extraintestinal infections than was found in previous surveys (14, 18). Our results indicate that ColV-associated virulence determinants are more significant in human infections than was previously thought.

### MATERIALS AND METHODS

**Bacteria and isolation methods.** Clinical strains of *E. coli* which were tested for colicin production were isolated in the Central Microbiology Laboratory of the Federated Dublin Voluntary Hospitals.

Only organisms isolated from blood cultures which were deemed to be clinically significant were tested for colicin production. Blood cultures were prepared by adding 5 ml of blood to 100 ml of nutrient broth no. 2 (CM67, Oxoid Ltd.) and 0.05% (wt/vol) of Liquoid (sodium polyanethanol sulfonate) and incubating the cultures aerobically at 37°C. Anaerobic blood cultures were made by adding 5 ml of blood to 100 ml of Brewer Thioglycollate Broth (CM23, Oxoid Ltd., London,

England) as recommended by Stokes (*Blood culture technique*, Association of Clinical Pathologists Broad-sheet, no. 81, April 1974) which was incubated at 37°C. Aerobic and anaerobic cultures were subcultured 1, 2, and 3 days and 1 week after inoculation.

The *E. coli* strains that we used were isolated from blood during the period from 1972 to 1978 and were stored on nutrient agar slopes. The years and numbers of isolates tested from each year were the following: 1972, 24; 1973, 19; 1974, 23; 1975, 18; 1976, 23; 1977, 36; 1978, 53. Only one isolate from each patient was tested for colicin production. There were no significant differences in the proportion of colicin-producers or of colicin V producers among isolates from different years, so the results from all years were pooled in the data analyzed below.

*E. coli* strains from the urines of hospitalized patients who had urinary tract infections were collected during 1978, and those from the urines of nonhospitalized patients were collected during 1977. Urinary tract infection was considered to be present when there was a bacterial count of  $>10^5$  organisms ml<sup>-1</sup> of urine. Urine was cultured on MacConkey agar (CM7B, Oxoid).

Isolates (213) of *E. coli* from consecutive specimens of feces were examined for colicin production. Of these, 57 were confirmed to be of enteropathogenic serotypes by tube agglutination tests. Of the 57 strains, 11 were definitely associated with diarrheal illness in infants; the rest were isolated in the course of screening patients on admission to a children's hospital. Also, 128 strains were isolated as commensals from the feces of adults which had been sent for routine laboratory examination, but these did not contain enteropatho-

gens. Specimens of feces were cultured directly on MacConkey agar and on Desoxycholate-Citrate Agar (CM35, Oxoid) and were also subcultured on these media after enrichment in Selenite Broth (CM395, Oxoid).

Bacteria were identified as *E. coli* if they produced indole, fermented lactose, and were urease negative. The identity of strains giving doubtful reactions was confirmed by using the API 20-E system (Ayerst Laboratories, N.Y.) for the identification of enterobacteria.

Bacteria that were used as standard colicinogenic strains or as indicator strains for typing colicins are listed in Tables 1 and 2.

**Testing for colicin production.** Bacteria were grown on plates of blood agar base no. 2 (CM271 Oxoid) (six cultures per plate) and then were replicated, by using a replicator with metal prongs, to a series of plates of diagnostic sensitivity test agar (CM261, Oxoid) which were incubated for 48 h at 37°C. The agar surfaces of the plates were exposed to chloroform vapor for 30 min and then exposed to air for 30 min. Each plate was overlaid with 5 ml of diagnostic sensitivity test agar containing 0.025 ml of an 18-h broth culture of an indicator strain.

**Statistical tests.** The significance of differences in the percentages of strains producing colicin V was determined by the method described by Bailey (1).

## RESULTS

**Isolation of colicin-resistant mutants.** Identifying the colicins produced by natural isolates of *E. coli* is complicated by the fact that

TABLE 1. *Colicinogenic strains*

Strain	Colicins produced	Source
CL123 <i>Citrobacter freundii</i> CA31	A-CA31	Laboratory stocks
KH573 <i>E. coli</i> K-12 W3110 (Co1E1-K30)	E1-K30	Laboratory stocks
KH293 <i>E. coli</i> K-12 AB1157 (Co1E2-P9)	E2-P9	Laboratory stocks
CL124 <i>E. coli</i> CA38 (Co1I <sup>-</sup> derivative)	E3-CA38	Laboratory stocks
SL711 <i>Salmonella typhimurium</i> LT2 SL497 (Co1K-K235)	K-K235	Laboratory stocks
M1248 <i>E. coli</i> 398	L-398	P. Reeves
M1250 <i>E. coli</i> 285	E3-285, N-285	P. Reeves
CL17 <i>Shigella dyspar</i> P15	S4-P15	Laboratory stocks
M1285 <i>E. coli</i> CA23	D-CA23, X-CA23	P. Reeves
KH175 <i>E. coli</i> K-12 AB1157 (Co1B,M-K98)	B-K98, M-K98	Laboratory stocks
KT357 <i>E. coli</i> K-12 W3110 (Co1D-CA23)	D-CA23	K. N. Timmis
CL7 <i>E. coli</i> CA46	G-CA46	Laboratory stocks
KH595 <i>E. coli</i> K-12 J5-3 (Co1Ia-CT2)	Ia-CT2	Laboratory stocks
KH596 <i>E. coli</i> K-12 J5-3 (Co1Ib-P9)	Ib-P9	Laboratory stocks
KH274 <i>E. coli</i> K-12 Co1M <sup>cys</sup> *trp <sup>+</sup>	M-K260	P. Fredericq
M1284 <i>E. coli</i> II	D,E1,I,Q	P. Reeves
KH500 <i>E. coli</i> K-12 AB1157 (Co1V,I-K94)	I-K94, V-K94	Laboratory stocks
KH576 <i>E. coli</i> K-12 W3110 (Co1V-K30)	V-K30	Laboratory stocks
KH932 <i>E. coli</i> B188	V-B188	H. W. Smith
KH862 <i>E. coli</i> K35	V-K35	P. Fredericq
KH863 <i>E. coli</i> K76	V-K76	P. Fredericq
KH864 <i>E. coli</i> K229	V-K229	P. Fredericq
KH865 <i>E. coli</i> K260	B,M,V-K260	P. Fredericq
KH866 <i>E. coli</i> K267	V-K267	P. Fredericq
KH868 <i>E. coli</i> K288	I-K288	P. Fredericq
KH872 <i>E. coli</i> K345	V-K345	P. Fredericq

TABLE 2. Indicator strains

Strain	Relevant characteristics	Source and reference
KH215	<i>E. coli</i> $\phi$ Str <sup>r</sup> Nal <sup>r</sup>	Laboratory stocks
AB1133	<i>E. coli</i> K-12 <i>thi argE his proA thr leu ara mtl xyl galK lacY supE</i> Str <sup>r</sup> $\lambda^-$ F <sup>-</sup>	Laboratory stocks
P1205	AB1133 <i>tonA</i>	P. Reeves (7)
P585	AB1133 <i>tonB</i>	P. Reeves (6)
P295	AB1133 <i>cbt</i>	P. Reeves (6)
P535	AB1133 <i>exbC</i>	P. Reeves (6)
P625	AB1133 <i>cir</i>	P. Reeves (6)
P1235	AB1133 <i>cut</i>	P. Reeves (6)
KH1038	AB1157 made resistant to colicins B(D), G(H), I, L, M, A, E, K, N, S4, and X	This paper
KH1040	P1205 made resistant to colicins A, E, K, L, N, S4, and X	This paper
KH1041	P295 made resistant to colicins A, E, K, L, N, S4, and X	This paper
KH1043	P625 made resistant to colicins A, E, K, L, N, S4, and X	This paper
KH1044	P1235 made resistant to colicins A, E, K, L, N, S4, and X	This paper
KH1100	KH1038(pBH11); immune to colicin V-K94	This paper

many strains produce more than one type of colicin. In addition, many colicin-resistant mutants are insensitive to several types of colicin. Colicin-resistant mutants of existing indicator strains were therefore isolated so that colicin V-producing colonies could be readily identified. A strain (KH1038) was isolated which was resistant to all colicins listed in Table 1 except colicin V. KH1038 was derived from *E. coli* K-12 AB1157 by isolating three successive mutants resistant to colicins B, G, and I and by overlaying colicinogenic colonies with agar containing the indicator strain at a concentration of  $10^9$  cells ml<sup>-1</sup>. The mutants were screened for those which retained sensitivity to colicin V. From a mutant which was already resistant to colicins B, G and I, a derivative was isolated which was also resistant to all types of colicin belonging to the A group of Davies and Reeves (7). This was done by isolating a mutant from the region where inhibition zones produced by ColA<sup>+</sup>, ColE<sup>+</sup>, and ColK<sup>+</sup> bacteria overlapped. This strain was then tested for its resistance to all the colicins produced by strains listed in Table 1. The other colicin-resistant mutants listed in Table 2 (KH1040, KH1041, KH1043, and KH1044) were isolated and tested in the same way. The colicin-sensitivity patterns of P1205, P585, P295, P535, P625, and P1235 were as described by Davies and Reeves (6, 7).

To check the resistance spectra of indicators, standard Col<sup>+</sup> strains were always included in tests of the colicinogeny of clinical isolates because some indicator strains were outgrown by colicin-sensitive revertants in stock cultures. Plasmid pBH11 (2), a recombinant plasmid derived from ColV, I-K94, confers immunity to colicin V-K94 but not to colicin I-K94 and was added to KH1038 by transformation (3).

**Criteria for colicin V production.** The activity of colicins produced by clinical isolates was tested against the following indicator strains: KH215, AB1133, P585, P1235, KH1038, KH1040, KH1041, KH1043, KH1044, and KH1100. The resistance spectra of these strains are listed in Table 3. A colicin was designated colicin V if it inhibited KH1038 but was inactive against strain KH1044. The effect of colicins on other indicators was used to determine whether a strain produced other types of colicin in addition to colicin V. All strains judged to be ColV<sup>+</sup> from their effects on indicators formed large inhibition zones (>9 mm from the edge of the Col<sup>+</sup> colony to the edge of the inhibition zone), which is consistent with previous reports on the diffusibility of colicin V (19). When determining the types of colicin produced, no attempt was made to distinguish between colicins B and D, G and H, or Q and V, which have identical activity spectra against indicators (6, 7). All strains were tested on diagnostic sensitivity test agar because preliminary experiments showed that many ColV<sup>+</sup> bacteria did not produce inhibition zones on blood agar base or on nutrient agar.

Several strains which had been previously classified as colicin V producers were tested for their effect on the set of indicator strains used for analyzing clinical isolates. *E. coli* strains KH500, KH576, B188, K35, K76, K229, K260, K267, and K345 all gave the expected reactions when tested against the set of indicators (i.e., all were active against KH1038, but none were active against KH1044) except strains K260 and KH500; these two ColV<sup>+</sup> strains also produce colicins B and M (9) and colicin I (15), respectively. The isolate of strain K288 that we used was found to produce colicin I but not colicin V.

**Colicin V production by clinical isolates.** The percentages of ColV<sup>+</sup> strains among isolates from different sources are listed in Table 4. In comparison with fecal strains, a significantly greater percentage of strains from blood cultures or from the urine of hospitalized patients with urinary tract infections produced colicin V ( $P < 0.01$ ). There was a significantly greater proportion of ColV<sup>+</sup> isolates from blood than from feces; this was so whether comparisons were made between ColV<sup>+</sup> strains as a percentage of

TABLE 3. Resistance spectra of indicator strains

Indicator	Sensitivity (+) or insensitivity (-) to the following colicin:													
	A	B(D)	E1	E2	E3	G(H)	I	K	L	M	N	S4	V(Q)	X
KH215	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AB1133	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P585	+	-	+	+	+	-	-	+	+	-	+	+	-	+
P1235	+	+	+	+	+	+	+	+	+	+	+	+	-	+
KH1038	-	-	-	-	-	-	-	-	-	-	-	-	+	-
KH1040	-	+	-	-	-	+	+	-	-	-	-	-	+	-
KH1041	-	-	-	-	-	+	+	-	-	+	-	-	+	-
KH1043	-	+	-	-	-	+	-	-	-	+	-	-	-	-
KH1044	-	+	-	-	-	+	+	-	-	+	-	-	-	-
KH1100 <sup>a</sup>	-	-	-	-	-	-	-	-	-	-	-	-	I <sup>a</sup>	-

<sup>a</sup> KH1100 is immune to colicin V-K94 but is not necessarily immune to other type V colicins.

TABLE 4. Colicin V production by clinical isolates of *E. coli*

Source	No. of strains that were:			ColV <sup>+</sup> strains as a percentage of:	
	Examined	Col <sup>+</sup> <sup>a</sup>	ColV <sup>+</sup>	All strains	All Col <sup>+</sup> strains
Feces	213	67 (31.4)	29	13.6	43.3
Urine (patients not hospitalized)	215	88 (40.9)	43	20.0	48.9
Urine (hospitalized patients)	107	46 (43.0)	28	26.2	60.9
Blood	196	100 (51.0)	62	31.6	62.0

<sup>a</sup> Numbers within parentheses are the percentage of Col<sup>+</sup> strains.

all strains ( $P < 0.01$ ) or between ColV<sup>+</sup> strains as a percentage of all colicinogenic strains ( $P < 0.02$ ). The proportion of ColV<sup>+</sup> strains (expressed as a percentage of all Col<sup>+</sup> strains) from the urines of hospitalized patients was not significantly greater ( $P > 0.05$ ) than the percentage of ColV<sup>+</sup> strains among Col<sup>+</sup> fecal isolates, but only 107 isolates from hospitalized-patient urine samples were examined as opposed to 196 strains from blood. There was not a significant difference between the percentage of ColV<sup>+</sup> bacteria isolated from feces and those isolated from urines of patients who were not hospitalized. There were no significant differences in the percentages of Col<sup>+</sup> or ColV<sup>+</sup> bacteria among enteropathogenic serotypes in comparison with other fecal strains.

The colicin V immunity determinant of ColV,I-K94 (present in strain KH1100) conferred immunity to 26% (42 of 162) of the type V colicins which were produced by clinical isolates. Of the strains producing V colicins in the same immunity group as V-94, nine were isolated from feces, five from the urine of nonhospitalized patients, nine from the urine of hospitalized patients, and 19 from blood.

Of 162 ColV<sup>+</sup> strains, 103 (63.6%) produced at least one other type of colicin. Of these strains, 22 were isolated from feces, 27 from the urine of nonhospitalized patients, 14 from the urine of hospitalized patients and 40 from blood.

## DISCUSSION

These results show that, in comparison with *E. coli* strains isolated from feces, a significantly greater proportion of *E. coli* strains isolated from blood or from the urine of hospital patients with urinary tract infections produce colicin V. However, the percentage of ColV<sup>+</sup> strains isolated from blood (31.6%) was not as high as the percentages of colicin V producers found among *E. coli* strains responsible for generalized infections in livestock. Smith and Huggins (22) found that 25 of 31 strains of serotype 078:K80 isolated from cattle were ColV<sup>+</sup>, 36 of 44 chicken strains of serotype 078:K80 were ColV<sup>+</sup>, 40 of 54 chicken strains belonging to serotype 02:K1 were ColV<sup>+</sup>, and 50 of 68 untyped chicken strains and 10 out of 45 human strains were ColV<sup>+</sup>.

The frequent occurrence of virulence determinants on ColV plasmids is apparently responsible for the high proportion of ColV<sup>+</sup> strains among isolates causing certain types of disease. Many ColV plasmids increase the virulence of *E. coli* (21, 22), but there is no evidence that either colicin V or immunity to colicin V is responsible for the increased virulence of ColV<sup>+</sup> bacteria (2, 16). Two virulence determinants have been found on ColV plasmids. A determinant (*iss*) which increases the resistance of *E. coli* to the bactericidal effects of normal serum is known to be present on ColV,I-K94 (2). This

determinant causes an approximately 100-fold reduction in the 50% lethal dose of *E. coli* for chicks. A second virulence determinant present on certain ColV plasmids facilitates the uptake of iron by *E. coli* and involves the formation of an iron-chelating hydroxamate (24, 27).

Because the determinants for colicin V and for virulence are frequently present together on the same plasmid (2, 21, 22), the results reported here indicate that a significant proportion of strains causing human extraintestinal infections have virulence plasmids. Other characteristics, in addition to colicin V synthesis, are found more commonly in strains causing extraintestinal infections than in other strains. Strains responsible for extraintestinal infections are more likely to be hemolytic than are strains from the fecal flora of healthy people (4, 5, 14, 20, 26). For example, Minshew et al. (14) found the percentages of hemolytic strains isolated from the following sources to be 35% (18 of 51) from blood, 49% (29 of 59) from urine, and 56% (18 of 32) from other extraintestinal sites. Only 1 strain of 20 (5%) from the fecal flora of healthy individuals was hemolytic. In addition, many strains causing extraintestinal infections bind specifically to epithelial cells, Svanborg-Eden et al. (25). This is reflected in their ability to agglutinate erythrocytes in a reaction which is insensitive to D-mannose (14, 18). In comparison with strains from the normal enteric flora of healthy people, a higher proportion of *E. coli* strains responsible for neonatal meningitis, neonatal septicemia, and pyelonephritis in children produce the K1 capsular polysaccharide (11, 13, 17). The ColV-associated virulence determinants are therefore only one of several factors which can contribute to the virulence of *E. coli* strains causing human extraintestinal infections.

The criteria for determining colicin V production adopted here were (i) inhibition of an indicator strain which was resistant to all tested colicins with the exception of colicin V and (ii) ineffectiveness against a colicin V-tolerant (*cvt*) strain. Strain KH1038 is sensitive only to colicin V when tested against the set of standard colicinogenic strains listed in Table 1. The exact number of colicin types is unclear (see 6, 7), but it seems unlikely that there are more than four other types of colicin which were not included in the set of colicinogenic bacteria used here. Strain KH1038 had been made resistant to many colicins and, bearing in mind that many mutations to colicin-resistance confer resistance to several colicin types (6, 7), the strain may be resistant to the few types of colicin which were not included in the set of Col<sup>+</sup> strains used here. In addition, it was possible to check that colicins which were active against strain KH1038 were

inactive against a colicin V-tolerant strain. Finally, the fact that all of the colicins which were judged to be colicin V from their effects on these two indicators produced inhibition zones larger than those of any other tested colicins also indicates that the method is reliable. Colicin V is a highly diffusible colicin which forms large inhibition zones (19). There seems to be no basis for separating colicins Q and V into two groups; they are indistinguishable from their effects on indicator strains and they have very similar electrophoretic mobilities (6).

The colicin V immunity determinant of ColV,I-94 conferred immunity to 26% of the V colicins produced by clinical isolates. Type V colicins appear to resemble type E and I colicins in being divisible into a number of immunity groups (10). Seven immunity groups of E colicins have been distinguished (12), and there are two immunity groups of type I colicins (23). We have not examined cross-immunity relationships among V colicins to determine the number of V immunity groups. Because there is more than one V immunity group, not all V producers are detected by comparing the effects of colicinogenic strains on a ColV<sup>+</sup> and ColV<sup>-</sup> form of an indicator strain (14). Strains which produce colicin V together with another type of colicin also remain undetected by this method because both the ColV<sup>+</sup> and the ColV<sup>-</sup> indicators will be inhibited by the other colicin(s) produced by the colicinogenic strain.

The different method for detecting colicins used here may, in part, explain why the proportion of colicin V producers found among isolates from blood and urine samples are greater than those reported by Minshew et al. (14), who found that colicin V producers formed 12% of blood culture isolates, 16% of isolates from other extraintestinal sources, and 7% from urine isolates. No colicin V producers were found among 20 fecal isolates.

#### ACKNOWLEDGMENTS

We thank C. T. Keane for his helpful suggestions and the medical and technical staff of the Federated Dublin Voluntary Hospitals for their cooperation.

This work was supported in part by a grant from the Medical Research Council, United Kingdom.

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