SHORT COMMUNICATION

Distribution of Group E Colicin Types in Shiraz, Iran

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(Received 19 January 1981; revised 4 June 1981)

Seventy-six (7.5%) of 1007 clinical isolates of *Escherichia coli*, *Shigella* and *Salmonella* from patients in Saadi Hospital, Shiraz, Iran, produced colicins of group E. The majority of isolates produced colicin E1 and none produced colicins E2 or E3. This is the first report of the production of colicin E4_{Horak} by strains of *Salmonella* and of colicins E5 and E6 by strains other than *Shigella sonnei*, and only the second report of the isolation of any strain producing colicin E7.

INTRODUCTION

Until recently, the known group E colicins were divided into subgroups E1, E2 and E3 on the basis of their E specific colicinogenic immunity (Fredericq, 1956). In recent investigations, Horák (1975) and Males & Stocker (1980, 1982) have identified new colicinogenic immunity groups called E4_{Horak}, E5, E6 and E7. In the investigation reported here, over 1000 strains of *Escherichia coli*, *Shigella* and *Salmonella*, obtained from clinical specimens, were tested for the production of E colicins, in particular of the newly recognized E colicin types. These strains were also tested for the production of non-E colicins.

METHODS

Bacteria. A total of 1007 strains of *Escherichia coli*, *Shigella* spp. and *Salmonella* spp., isolated during a 1 year period, from the urine, faeces, blood and other specimens from patients in the various wards of Saadi Hospital, Shiraz, Iran, were examined for the production of colicin. The colicin-producer strains of the same species and colicin type came from unrelated sources. The Col⁺ strains isolated in outbreaks were not included in this study. Standard colicin-producer and colicin-indicator strains were obtained from B. A. D. Stocker (Department of Medical Microbiology, Stanford University, U.S.A.).

Media and cultures. Blood agar base (Oxoid) was used for the cultivation and maintenance of the strains and for the determination of their production of or sensitivity to colicins. Cultures were incubated at 37 °C for 24 h and were stored at 4 °C. Stock cultures were kept frozen in nutrient broth (Oxoid) with 10% (v/v) glycerol at –70 °C.

Colicin testing. Strains were tested for the production of or sensitivity to colicins by the method of Ozeki *et al.* (1962). *Escherichia coli* strain CL142 (=K12-Row) was used as the indicator strain sensitive to all colicins. For a preliminary classification of the colicin(s), strains identified as colicinogenic were tested for activity on two indicator strains, AG041 and 023BM, of known susceptibility patterns. Strain AG041, a *tonB* deletion mutant of *E. coli* B, is unaffected by all colicins of group B (Davies & Reeves, 1975*a*), namely, colicins B, D, G, H, M, I, S1, Q and V, but sensitive to colicins of group A (Davies & Reeves, 1975*b*), namely, A, E1, E2, E3, K, L, N, S4 and X. Strain 023BM, presumably a *btuB* mutant of *E. coli* K12, is resistant to all E colicins and to phage BF23 and is almost completely resistant to colicin A. It was inferred that colicinogenic strains totally inactive on indicator strain 023BM but active on indicator strain AG041 produced one or more group E colicins. Conversely, it was inferred that strains inactive on strain AG041 but active on strain 023BM produced one or more colicins of group

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B. Strains active on both indicators were considered as producing either one or more non-E colicins of group A or both E and non-E colicins. Group E colicins were typed by their activity, or lack of it, on seven E-immune indicator strains, each immune to one type of E colicin, i.e. E1 to E7.

Colicin transfer. Transfer of the multiple E colicins (or a new type of E colicin) produced by *E. coli* strains 44 and 310 to *E. coli* CL142 was performed using strain Stm470, which carries an incomplete form of R Utrecht (van Embden *et al.*, 1976), as 'helper'. The mixture of organisms, which contained 1.0 mg trypsin ml⁻¹, was incubated at 37 °C for 24 h. Transconjugants were then selected on blood agar base (Oxoid) containing 1 mg streptomycin ml⁻¹.

RESULTS AND DISCUSSION

Among the clinical isolates examined, 15% of *E. coli* strains, 15.5% of *Shigella* strains and 10% of *Salmonella* strains produced one or more colicins (Table 1). This incidence is similar to previous reports of 20 to 40% among *E. coli* strains from clinical material (Fredericq, 1948; Vosti, 1968) but differs somewhat from the reported 50% or more among *Shigella* strains (Papavassiliou *et al.*, 1964) and 5% among *Salmonella* strains (Males, 1978).

Of the 1007 strains tested for colicin production, 144 (14.3%) were found to be colicinogenic (Table 1): 76 (7.5%) strains produced only colicin(s) of group E, 54 (5.4%) produced only group B colicin(s) (Davies & Reeves, 1975*a*) and 9 (0.9%) produced one or more non-E colicins of group A (Davies & Reeves, 1975*b*) or combinations of E and non-E colicins.

Two of the *E. coli* isolates, strains 44 and 310, produced only E colicin(s) which were active on all the seven E-immune indicator strains. The results of the conjugation crosses between strain 310 and strain CL142 indicated that strain 310 produced colicin E6 and at least one additional E colicin, probably E1, E2 or E7. Attempts to transfer the colicin plasmid(s) carried by *E. coli* strain 44 were unsuccessful.

Six strains of *E. coli*, two strains of group A *Salmonella* and one strain of *Sh. flexneri* produced colicin E4_{Horák} and one *E. coli* strain produced E4 and Ia. This finding is of interest since Barker (1980) in a study on colicinogeny in *S. typhimurium* found only E1 and E2 colicins among the 4481 strains that she examined. Colicin E4_{Horák} has been reported as being only produced by strains of *Sh. sonnei* (Horák, 1975) and two strains of *E. coli* (Males & Stocker, 1982).

Colicins E5 and E6 have also been reported as being produced only by strains of *Sh. sonnei* (Horák, 1975; Males & Stocker, 1982). Among the clinical isolates examined were found, for the first time, strains of *E. coli* which produced colicins E5 and E6 and strains of *S. typhimurium* which produced colicin E5. The two E7 colicins, E7-212 and E7-KBB, produced by *E. coli* strains 212 and KBB, are only the second and the third reported colicins of type E7. The first identified E7 colicin, E7-K317, is produced by Fredericq's strain CL137 (=K12-317). This colicin, formerly thought to be E2, has recently been shown by Males & Stocker (1980) to be an E colicin of a previously undescribed type.

Most of the colicinogenic isolates examined were either poor indicators showing only narrow or faint zones of inhibition with any E-producing strains, or were totally insensitive to all E colicins and phage BF23. The two E7-producing strains, *E. coli* strains 212 and KBB, however, were sensitive to all the E colicins except colicin E7-K317 produced by *E. coli* K12-317. Unexpectedly, however, these two strains were insensitive to phage BF23. Some *E. coli* K12 derivatives carrying ColIb-P9 (Strobel & Nomura, 1966) or other ColIb plasmids (Stocker *et al.*, 1963) or pRES-K317 (Males & Stocker, 1980) restrict phage BF23 in the sense that they interfere with the multiplication of the phage even though adsorption and penetration are normal. Although the presence of a restricting plasmid could not be demonstrated in strains 212 and KBB, it may well be that these strains also carry such plasmids.

Table 1. *Distribution of colicin types among colicinogenic E. coli, Shigella spp. and Salmonella spp.*

Colicin(s) produced	Number of strains			
	<i>E. coli</i>	<i>Shigella</i> spp.	<i>Salmonella</i> spp.	Total
E1	35	16	5	56
E2	0	0	0	0
E3	0	0	0	0
E4 ^{Horak}	6	1*	2†	9
E4 ^{Horak} + Ia	1	0	0	1
E5	1	0	3‡	4
E6	1	0	0	1
E6 + Ib	1	0	0	1
E7§	2	0	0	2
E6 + other E or new E	1	0	0	1
Multiple E¶ or new E	1	0	0	1
E + non-E or multiple non-E	4	3	2	9
Non-E	40	11	3	54
Lost before typing	4	1	0	5
Total Col ⁺	97	32	15	144
Total tested	653	206	148	1007

* *Sh. flexneri*.† Group A *Salmonella*.‡ *S. typhimurium*.

§ Produced by strains 212 and KBB.

|| Produced by strain 310. ¶ Produced by strain 44.

Colicin E2 and E7 (but not other E colicins) produce 'double zones' of inhibition against K12 *btuB*⁺ indicator strains carrying plasmids of types ColE4, ColE5 or ColE6. Males & Stocker (1982) have attributed the 'double zone' phenomenon to increased sensitivity of these indicators to colicins E2 and E7. *Escherichia coli* strains 212 and KBB, both isolated in Shiraz, producing colicin E7, likewise produced 'double zones' on K12 *btuB*⁺ strains carrying ColE4, ColE5 or ColE6. Thus the two new E7 colicins behave like the original E7 colicin, E7-K317, in this respect.

The author wishes to thank Professor B. A. D. Stocker for valuable discussions and for providing the standard colicin producer and indicator strains. The technical assistance of Nilofar Subhashini is appreciated.

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