

Colicin Production and Colicin Typing of Uropathogenic *Escherichia coli*

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Abstract: Colicins are bactericidal macromolecules in protein structures and they are produced by some strains of *Escherichia coli* and Shigella, which are members of the Enterobacteriaceae. They have a bactericidal effect on the same species or closely related bacteria, but have no effect on the strains which produce them. In this study, 129 *E. coli* strains isolated from urine samples of patients in Izmir and Manisa were examined for colicin production, and the colicinogenic strains were examined for their colicin types by means of Horak specific indicator strains. Indicator

strains were obtained from the National Type Culture Collection Hygiene and Epidemiology Institute, Prague. Colicin production was determined by a modified version of the method of Djønne. Out of 129 uropathogenic *E. coli* strains, 33 (25.5%) were colicinogenic. Horak's typing scheme was used for the determination of colicin types. *E. coli* strains produced 20 different types of colicin. Most of the colicin types were of group E.

Key Words: *Escherichia coli*, colicin typing, urinary tract infections

Introduction

Colicins are bactericidal macromolecules which have narrow spectrum activity and they are produced by some strains of *Escherichia coli* and related Enterobacteriaceae (1). They occur in protein structures and their molecular weights range from about 12000 to 90000. They are specified by col plasmids. Each col plasmid confers immunity to the particular type of colicin which it encodes (1,2). Colicins are divided into two groups according to their cross-resistance patterns (3). Group A: A, E1, E2, E3, K, L, N, S4, and X; Group B: B, D, G, H, I, Ia, Ib, M, Q, S and V.

They are synthesized in the cytoplasm of colicinogenic cells and produced in large amounts and, in general, released into the extracellular medium (4). After being released into the medium, colicins kill sensitive bacteria in 3 defined steps (2,4):

1. Adsorption onto a specific receptor at the surface of the bacterium.
2. Translocation across the outer membrane.
3. Killing activity.

E. coli have been the most common causative agents for urinary tract infections for some years. Therefore, in this study we investigated the colicin production

properties and colicin types of *E. coli* strains isolated from the urine of patients in Izmir and Manisa who had urinary tract infections.

Materials and Methods

One hundred and twenty-nine *E. coli* strains isolated from the urine of patients in Izmir and Manisa were studied. The *E. coli* strains were identified by standard microbiological methods (5). The indicator strains, which were used for determining colicin production and colicin typing, are shown in Table 1. Indicator strains were obtained from the National Type Culture Collection Hygiene and Epidemiology Institute, Prague. Colicin production was determined by a modified version of the method of Djønne (6). Spot cultures were grown on plates of blood agar base no. 2 (CM 27, Oxoid) at 37°C for 48 hours and killed by exposure to chloroform vapour and left at room temperature for 30 min to remove residual chloroform. The universal indicator strain "a1" was cultured on blood agar. One colony of indicator strain "a1" from this medium was mixed with 5 ml 0.9% NaCl and then overlaid on sterilized plates; excess material was removed. After leaving them for 1-2 hours at room temperature, they were incubated for 24 hours at 37°C. If the inhibition zone was larger than 1mm around the spot

culture, colicin production was considered to be positive. All colicinogenic strains were tested with each indicator strain. The indicator strains are shown in Table 1.

Results

Out of 129 uropathogenic *E. coli* strains, 33 (25.5%) produced colicin. The results are shown in Table 2. The strains obtained from Izmir and Manisa produced 10 identical types of colicin in common; the strains from Izmir and Manisa produced 3 and 7 different types of colicin respectively. Among these colicin types, the group E was more than 50%. None of the strains produced colicin E6 (Table 3). Out of 129 *E. coli* strains, 22 (17%) produced colicin V (Table 4).

Table 1. Indicator strains used for determining colicin types.

No	Immune to colicin or colicins	Resistant to colicin or colicins
a1	Universal indicator	
a2	---	E,I,V
a4	Ib	---
a5	---	E
7	E2	---
10	E1, Ia	---
11a	E1, Ib	---
17a	E6, Ib	X
19	K	---
23	---	D
31	E4,Ia	---
33	V	---
35	B, M, E6	---
41	K	X, E, I, V
44	K	E
45	B, M, Ia	---
47	E1	D
50	E1	---
52	E2,K	---
58	B,M	E
59	---	X, I, V
65	K	I, V
68	B, M	I, V
70	---	IV
74	E1	X
75	E2	X
76	---	X, E
80	E3	---
84	Ib	D
85	Ia	D
88	---	E, I, V, X
90	V, M, Ia	---
95	B, M	---
96	V, B, M	---
97	V, M, D	---
99	V,M,K	---
101	D	E,I,V

Discussion

Colicin typing is used to show the epidemiological relation in *E. coli* and Shigella infections. In this study, the colicin production was detected and colicin types were determined in 129 uropathogenic *E. coli* strains. Thirty-three (25.5%) strains produced colicin, and these colicinogenic strains produced 10 different and 10 identical colicin types. Twenty-two (17%) of all the strains produced colicin V.

In previous studies performed in Izmir with uropathogenic *E. coli* strains, the rate of colicin production were found to be 23% and 22% (7,8). The results of these studies are similar to those of the present study (25.5%).

Table 2. Colicin production rates of uropathogenic *E. coli*.

City	Total number	Colicinogenic strain (%)
Izmir	66	14 (21.2)
Manisa	63	19 (30.1)
Total	129	33 (25.5)

Table 3. The types of colicins produced.

Cities		
Izmir	Manisa	Izmir and Manisa
E	K	E,I,V
D	E4, Ia	Ib
X,I,V	B,M,Ia	E1,a
	E2, K	E1,b
	B, M, E	V
	E1, X	K,X,E,I,V
	E2, X	E1,D
		E1
		K,I,V
		I,V

Table 4. Percentages of colicin V production in uropathogenic *E. coli*.

City	Total number	Number of colicin V producing strains (%)
Izmir	66	8 (12.12)
Manisa	63	14 (22.22)
Total	129	22 (17)

McGeachie (9) reported that out of 534 *E. coli* strains isolated from the urine samples of patients 194 (36.3%) produced colicins. Davies et al. (10) found that the colicinogenity of 107 *E. coli* strains isolated from the urine samples of hospitalized patients was 43%, and that for 215 nonhospitalized patients was 41%. Nowickie (11) studied 400 uropathogenic *E. coli* strains and found that 42.7% were colicinogenic. The colicin production percentages reported for uropathogenic *E. coli* strains in those studies were higher than the results of the present study.

Waleh (12) determined colicin production to be 15% in 653 uropathogenic *E. coli* strains. This value is lower than the value in this study.

The difference in the reported frequencies of colicin-producing strains may be due to differences in the

characteristics of the *E. coli* strains found in different countries during various periods (6).

Previous studies have demonstrated differences in the incidence of colicin V production of uropathogenic *E. coli*. Davies et al. (10) found a rate of 26.2%, Minshew et al. (4) 11.8% and Coşar et al. (13) 16%. In the present study, the rate of colicin V production was 17%.

In this study, 20 colicin types were identified. Eleven of these are similar to the results of a study carried out in Izmir (15). Eight of these 20 colicin types are similar to the results of another study carried out in Izmir (8). According to these results, some of the *E. coli* strains which caused urinary tract infection in Izmir and Manisa produced certain types of colicin. Probably these colicin types are endemic for Izmir and Manisa.

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