COMPARATIVE DATA FOR THE DISTRIBUTION OF COLICINES AMONG DIFFERENT SPECIES OF INTESTINAL BACTERIA DETECTED BY INTRA- and INTERSPECIFIC TESTS

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The phenomenon of colicinogenicity is widespread, but is unequally distributed among the intestinal bacteria. The number of colicinogenic strains is greatest among species with well-marked parasitic properties (typhoid and paratyphoid A and B bacteria), in which interspecific antagonism is predominant and the colicines isolated are characterized by a narrow spectrum of activity; in less highly differentiated species (Escherichia coli, Shigella dysenteriae) intraspecific antagonism is more marked and the colicines have a wide spectrum and a high degree of activity.

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The phenomenon of bacteriocinogenia is widespread among different species of bacteria [1, 6, 12]. It has been studied in greatest detail in Escherichia coli. The cells of Salmonella typhi have been shown to secrete colicines [10]. Colicines have also been found in various other organisms of the Salmonella and Shigella groups and intestinal bacilli [2-4, 8, 13]. The principal object of the investigations cited above was to determine the frequency of colicine production by individual species of enterobacteria and also to use the phenomenon of colicinogenicity as a means of indicating dysentery bacteria in an epidemiologic experiment.

In the present investigation intra- and interspecific antagonistic activity between strains of different enterobacteria were studied in relation to the biological characteristics of the species.

EXPERIMENTAL METHOD AND RESULTS

Altogether 182 strains were studied and their species distribution is given in Table 1. Colicinogenicity was detected by Frédéricq's method [6]. All strains were tested in turn for their ability to produce colicine and as indicators for complete cross testing (Table 1). During cross testing, colicinogenic strains were found most frequently among the typhoid cultures and least commonly among the E. coli cultures. During intraspecific testing of the strains the opposite picture was observed. In this case, the highest percentage of active strains was found among cultures of E. coli, and they were completely absent among the typhoid and paratyphoid A strains.

Hence, during the transition from less highly differentiated, biologically less independent species to species in which these properties are well marked, we observed predominance of interspecific antagonism at the expense of intraspecific. Species with well-marked parasitic properties as a rule exhibited colicinogenic activity in relation to members of the other studied species, whereas the colincines of E. coli or of S. dysenteriae possessed a broader spectrum of activity, including intraspecific activity.

Results showing the sensitivity of enterobacteria to colicines of different origin are given in Table 2. Colicines of E. coli and S. dysenteriae possessed the widest spectrum of activity while colicines of the typhoid-paratyphoid group possessed the narrowest spectrum.

It will be noted that the dysentery microorganisms were the principal species which could be used as indicators of the colicines of the typhoid-paratyphoid group, whereas the known indicator strains (E. coli φ, E. coli B, and E. coli K12) were resistant to them in our experiments.

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TABLE 1. Frequency of Colicinogenic Strains among Different Species of Enterobacteria

Species	Total No. of strains	Salmonella typhi	Salmonella paratyphi A	Salmonella paratyphi B	Shigella dysenteriae	Escherichia coli	No. of colicinogenic strains	
							abs.	%
Salmonella typhi	54	0	0	3	54	23	54	100
Salmonella paratyphi A	13	2	0	0	8	0	8	60
Salmonella paratyphi B	44	0	1	7	14	0	21	50
Shigella dysenteriae	16	5	3	1	4	6	9	56
Escherichia coli	55	10	3	13	18	20	20	36

TABLE 2. Sensitivity of Enterobacteria Colicines of Different Origin

Species	Total No. of strains	No. of strains sensitive to colicines				
		Salmonella typhi	Salmonella paratyphi A	Salmonella paratyphi B	Shigella dysenteriae	Escherichia coli
Salmonella typhi	54	0	8	0	11	52
Salmonella paratyphi A	13	0	0	1	12	5
Salmonella paratyphi B	44	2	0	11	3	44
Shigella dysenteriae	16	11	6	5	11	16
Escherichia coli	55	3	0	0	26	53

The dysentery strains investigated included two strains of Shigella sonnei and ten strains of Shigella flexneri, and also one strain each of S. shigae, S. schmitz, S. newcastle, and S. novogorod. The best indicator for the typhoid strains was S. sonnei No. 626; strains of S. flexneri were inferior to it, but detected the majority of colicinogenic enterobacteria and all colicinogenic paratyphoid A and dysentery bacteria. The high sensitivity of the microorganisms of this species to colicines is in agreement with data reported by other workers indicating that S. sonnei strains may be used as indicators [4, 9]. Complete cross testing of the strains was carried out in 33,124 paired combinations. By this means, 1222 positive combinations of strains were detected (3.7%).

Attention was drawn to the complete absence of positive combinations during intraspecific testing of the typhoid and paratyphoid strains and to the subsequent increase in their number among the paratyphoid B, dysentery, and E. coli strains.

The colicinogenicity of 55 strains of S. typhi was detected in 186 combinations, i.e., each active typhoid strain inhibited growth on the average of 3-4 indicator strains. This figure may be used as the mean index of the spectrum of activity of the colicines, in this case typhoid. For paratyphoid A and B colicines the corresponding indices were 5.5 and 1.5. The colicines of S. dysenteriae and of E. coli, on the other hand, had higher indices of their spectrum of activity (11.6 and 42.6).

On the other hand, 53 strains of S. typhi were sensitive to colicines of different origin in 157 combinations, i.e., each of the sensitive typhoid strains was inhibited on the average by three active strains. This figure may be taken conventionally as the mean index of the spectrum of sensitivity of the strains, in this case typhoid, to colicines of different origin. For paratyphoid A and B strains the corresponding indices were 2.6 and 4.5. However, strains of E. coli (9.4) and S. dysenteriae (30.0) possessed the highest sensitivity.

Activity of the colicines was also assessed by the character of zones of inhibition of growth of the indicator strains. In this way the colicines could be subdivided into highly active, giving large (10-20 mm) zones without marked secondary growth, and weak, causing the formation of narrow zones or of zones with strong secondary growth. As a result of this analysis it was shown that the colicines of bacteria of the typhoid-paratyphoid group possessed high activity only against S. dysenteriae. The colicines of E. coli and S. dysenteriae were characterized by predominance of highly active combinations when bacteria of any studied species were used as indicators. They possessed not only a broad spectrum, but also a high level of activity.

Production of colicines may be regarded as one of the factors of intra- and interspecific antagonism. Among species with well-marked parasite properties (S. typhi, S. paratyphi A and B) interspecific antagonism is predominant, while among species which are less highly differentiated (E. coli and S. dysenteriae) and which are not distinguished by ecological stability, intraspecific antagonism is more marked. Production of colicines in the first group is characterized by secretion of antibiotic substances with a narrow spectrum of activity, but in the second group by secretion of antibiotics with a broad spectrum and high level of activity.

Hence, the results of intra- and interspecific testing of strains of enterobacteria not only confirm the fact of the widespread distribution of colicinogenicity, but also provided a basis for the hypothesis that the pattern discovered is a reflection of this sign under natural conditions.

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