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HIGH LEVELS OF COLICIN RESISTANCE IN *ESCHERICHIA COLI*

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Abstract.—Colicins are plasmid-encoded antibiotics that are produced by and kill *Escherichia coli* and other related species. The frequency of colicinogeny is high, on average 30% of *E. coli* isolates produce colicins. Initial observations from one collection of 72 strains of *E. coli* (the ECOR collection) suggest that resistance to colicin killing is also ubiquitous, with over 70% of strains resistant to one or more colicins. To determine whether resistance is a common trait in *E. coli*, three additional strain collections were surveyed. In each of these collections levels of colicin production were high (from 15 to 50% of the strains produce colicins). Levels of colicin resistance were even higher, with most strains resistant to over 10 colicins. A survey of 137 non-*E. coli* isolates revealed even higher levels of resistance. We discuss a mechanism (pleiotropy) that could result in the co-occurrence of such high levels of colicin production and colicin resistance.

Key words.—Allelopathy, colicin resistance, colicins, *Escherichia coli*, pleiotropy.

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Colicins are commonly encountered antimicrobials produced by, and active against, *Escherichia coli* and their close relatives. Surveys of over 1000 isolates reveal that, on average, 30% of *E. coli* produce colicins (Riley and Gordon 1996). The production of colicins is believed to play a role in intraspecific competition.

Even higher levels of resistance to these same toxins were observed in one survey of *E. coli* (the ECOR collection; Riley and Gordon 1992). This study suggested that most *E. coli* are resistant to most colicins. On average, 93% of the isolates were resistant to any one colicin, and 33% were multiply resistant to all the colicins tested. There are four known mechanisms that confer colicin resistance: (1) *plasmid-encoded immunity* which results from a colicin-plasmid encoded immunity protein binding to, and rendering inactive, a specific colicin; (2) *resistance* which involves the alteration of a cell surface receptor that binds a colicin; (3) *tolerance* which results from alterations in cell membrane proteins involved in colicin translocation; and (4) *lysogen superinfection exclusion* which is a process preventing repeat infections by the same phage that also confers colicin resistance (this phenotype is very similar to the tolerance phenotype). Here we will refer to all four processes as resistance because our interest is primarily in whether a strain can survive in the presence of colicins.

The ECOR survey revealed high levels of both colicin production and resistance (Riley and Gordon 1996). Several studies have suggested that both traits can measurably lower cell fitness by growth rate reduction, cell lysis, or the general cost of carrying plasmids (Hardy 1975; Spangler et al. 1985; Luria and Suit 1987; M. Feldgarden and M. Laubichler, unpubl. data). Given the perceived ineffectiveness of colicins (with such high levels of resistance segregating) and the measurable cost of both colicin production and resistance, it is surprising that both phenotypes are maintained at such high frequencies in natural populations. Perhaps the levels of colicin resistance are abnormally high in the single published survey based upon isolates of the ECOR collection, because *E. coli* isolates generally have much lower levels of resistance. Alternatively, colicins may not act intraspecifically, but may play a role in competition between closely related species (given the limited phylogenetic range over which colicins kill). In which case the cost of carrying resistance must be considered relative to the benefit gained by killing closely related competitors. Both of these explanations could resolve the apparent paradox of the observed high levels of both colicin production and resistance.

The levels of resistance in the ECOR collection are higher than expected from exposure to the currently segregating colicins. Thirty of the ECOR strains possess a colicin, with six colicin types present. However, high levels of colicin resistance to all 20 characterized colicin types were detected. At least two hypotheses could explain this observation. First, the current resistance profiles represent historical selective pressures currently not found in the ECOR collection. In other words, resistance to a particular colicin is acquired only by exposure to that colicin and the resistance phenotype can persist after the selective pressure is removed. Alternatively, current colicin resistance patterns may result from the generation of multiple resistances from selection for resistance to a few colicin types. In this case, selection for resistance to one colicin could result in resistance to multiple colicins. After many generations, resistance could be maintained with selection by any one of a number of different colicins segregating in the population.

In this study we attempt to characterize more completely the levels of naturally occurring colicin resistance. We present here a survey of colicin resistance in 158 *E. coli* isolates from a variety of hosts and geographic regions. In addition, we have screened for resistance in 137 isolates of non-*E. coli* enterics, which are close relatives of *E. coli*. These data suggest that resistance levels are high, as the ECOR screen suggested (Riley and Gordon 1996). Similarly high levels of interspecific resistance to colicins is also observed. Finally, the data suggest a mechanism (involving pleiotropy) that can both produce and maintain high levels of colicin resistance in the absence of a continuous colicin selection pressure.

MATERIALS AND METHODS

Strains.—Four separate strain collections were used. For *E. coli*, all strains were acquired from T. S. Whittam (Penn

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TABLE 1. Non-*Escherichia coli* isolates included in this study.

Species	Number
Citrobacter amalonaticus	5
C. diversus	1
C. freundii	16
Enterobacter cloacae	31
E. taylorae	1
Hafnia alvei	1
Klebsiella pneumoniae	24
Kluyvera ascorbata	1
Morganella morganii	3
Porteus mirabilis	3
P. vulgaris	1
Providencia alcalifaciens	3
P. rettgeri	3
Salmonella sp.	7
Serratia liquefaciens	1
S. marcescans	8
Shigella dysenteriae	2
Xanthomonas maltophila	19
Yersinia enterocolitica group	3
Y. pseudotuberculosis	1
Untyped gut isolates	3

TABLE 2. Colicin-bearing strains used in this study (from Pugsley 1985).

Strain	Colicin type produced
BZB2101	A
BZB2102	B
BZB2103	D
BZB2104	E1
BZB2105	E2
BZB2106	E3
BZB2107	E4
BZB2108	E5
BZB2109	E6
BZB2110	E7
PAP247	E8
BZB1407	E9
CA46	G
CA58	H
BZB2114	Ia
BZB2115	Ib
BZB2116	K
PAP1	M
BZB2101	N
PAP2	S4

State University.). The Poultry collection consists of 59 strains of *E. coli* isolated from free-ranging chickens. The Pathogenic collection consists of 59 strains isolated from hospital patients. The Indian collection consists of 60 strains isolated from infants from an isolated Central American Indian community. Additionally, data from a survey of the ECOR collection were included in our analyses (Riley and Gordon 1992). For non-*E. coli*, 137 strains from twenty bacterial species (Table 1) were supplied by D.M. Gordon (Australian National University, Canberra).

Colicin Preparation.—Crude extracts were made of 18 colicins (see Table 2). 100 μl of an overnight culture was added to 10ml LB broth. Cultures were grown for 2 hr at which time 0.5 μg/ml Mitomycin C (Sigma) was added. After 8 hr cells were killed with chloroform and centrifuged to remove cell debris. The resulting supernatants were stored at −70°C and used for all experiments. For the three *E. coli* collections examined here, 14 colicin extracts were used as the other extracts did not yield reliable results. For the non-*E. coli* survey, all 20 colicins were used, although the extracts for colicins G and H did not inhibit reliably the *E. coli* control. The colicins used for screening the Indian, Pathogenic, and Poultry collections were titered to the same level of killing activity (a one-thousand-fold dilution gave clear plaques), with the exceptions of colicins Ia and Ib, which were produced at lower levels (approximately one order of magnitude).

Resistance Assays.—We added 100 μl of an overnight growth of cells to 3 ml of top agar (20 g/L LB, 7g/L Bacto-Agar) and poured on an LB plate (20g/L LB, 16g/L Bacto-Agar). Two μl of each colicin extract was then spotted onto this LB top agar lawn. Any clearing was scored as sensitivity, while absence of clearing was scored as resistance. As a control, extracts were spotted onto a lawn of BZB1011, a colicin sensitive K-12 derivative (described in Pugsley and Oudega 1987).

Statistical Analysis.—Analyses were performed using JMP 3.1.5 Statistical Discovery Software (SAS Institute). To determine if the observed frequency of strains resistant to a given percentage of colicins suggested nonindependent acquisition of resistance, a null model was constructed. For each collection, the probability of resistance to a given colicin type was based on the frequency of resistance to that colicin in that particular population. Data for colicins B, E9, Ia, Ib, and M were excluded as these data were not assayed for all four collections. A distribution of strains resistant to a given percentage of colicins was then generated and compared to the observed frequency of strains with the Kolgomorov-Smirnov test (Sokal and Rolhf 1984).

RESULTS

The frequency of colicin resistance to eighteen colicins is exceptionally high in all four *E. coli* collections surveyed (Table 3). On average, 75% of the strains surveyed were resistant to a particular colicin. The average resistance to a particular colicin was the highest for the ECOR collection with a frequency of 91.1% and was the lowest for the Indian collection with a frequency of 67.5%. One exception to the pattern of high frequency of resistance is colicin E4. Colicin E4 was highly effective at killing all strains, with an average resistance of 39% over the four collections surveyed.

Strains can be divided into classes based on the number of colicins they resist. The underlying distribution of colicin resistance classes for each collection was compared to that of the ECOR collection (Fig. 1). Only the Indian collection differed significantly in resistance profiles from the ECOR collection (Table 3 and Fig. 1; Pathogenic vs. ECOR: $D = 0.189$, $P = 0.196$; Poultry vs. ECOR: $D = 0.222$, $P = 0.014$; Indian vs. ECOR: $D = 0.397$, $P \ll 0.0001$), suggesting that, by this criterion, the Indian collection is less resistant than the ECOR collection. However, the other two populations had higher frequencies of resistance than did the ECOR collection.

One hundred thirty-seven non-*E. coli* isolates were screened for resistance to 20 different colicins. For 13 of the

TABLE 3. Levels of colicin resistance in four collections of *E. coli*.

Colicin	ECOR	Poultry	Path.	Indian
A	98.61[1]	83.05	86.44	56.67
B	na	98.31	91.53	90.00
D	98.61	83.05	83.05	85.00
E1	98.61	98.31	91.53	81.67
E2	88.89	67.80	57.62	58.33
E3	79.17	79.77	64.40	55.00
E4	25.00	45.76	52.54	35.00
E5	87.50	84.75	81.36	78.33
E6	97.22	72.88	61.02	60.00
E7	81.94	69.49	72.88	46.67
E8	94.44	69.49	57.63	63.33
E9	100	na	na	na
Ia	100	na	na	na
Ib	100	na	na	na
K	98.61	79.66	84.75	71.67
M	100	na	na	na
N	100	94.92	91.53	83.33
S4	100	84.75	84.75	80.00
Mean[2]	91.09	79.43	75.79	67.50

[1] Numbers are percentages of strains in each collection that are resistant to a given colicin.

[2] Mean is the average of the frequencies of resistance to all colicins.

20 colicins assayed, the non-*E. coli* isolates were as, or more, resistant to a particular colicin than *E. coli* (Table 4). Several interesting patterns emerge. Colicins A and E4 were the most likely to kill relative to other colicin types. However, resistance to colicin E4 was much more frequent in non-*E. coli* than within *E. coli*. *Citrobacter* sp. isolates were much more likely to be sensitive to colicin A and several of the E colicins than *E. coli*. Colicins G, H, Ia, and Ib did not kill the *E. coli* BZB1011 control, but were able to kill a small percentage of non-*E. coli* strains, most of which were *Xanthomonas* sp. (Table 4). Overall, 61.8% of the non-*E. coli* strains were resistant to all 20 colicins tested. This is a much higher level of resistance than is observed for *E. coli* (30.8% resistant to all colicins). Canonical correlation analysis (Dillon and Goldstein 1984) yielded a sharp difference in resistance phenotypes between *E. coli* and the combined other species (Fig. 2).

If one makes a simplifying assumption that resistance to each colicin type arises independently, then the probability that a strain is resistant to a given number of colicin types can be predicted from the individual frequencies of resistances within a population. The expected distribution of multiply resistant strains can be compared to the number observed in each population. If the distribution is not signifi-

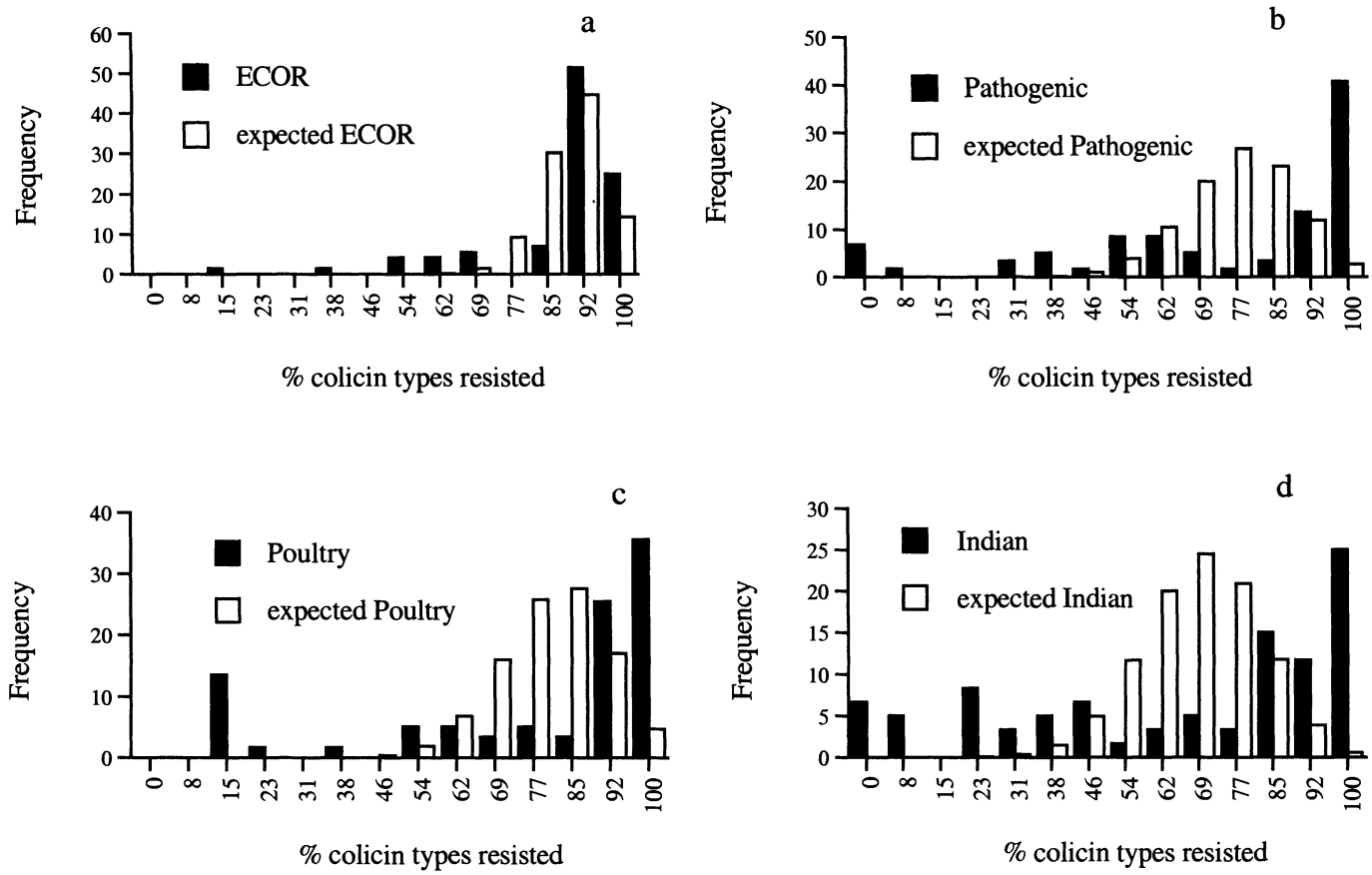

FIG. 1. (a–d) The observed (black shading) and expected (light shading) frequencies of strains resistance to a given percentage of colicin types in the ECOR, Pathogenic, Poultry, and Indian *Escherichia coli* collections. All four observed distributions were significantly different than the expected distributions ($P < 0.01$). Only the data for the 13 colicins scored in all four surveys are shown (colicins B, G, H, Ia, Ib, M, and V were excluded as they were not scored in all surveys).

TABLE 4. Levels of colicin resistance in five genera of enteric bacteria.

Colicin	All *E. coli*	Non-*E. coli*	*Serratia*	*Citro-bacter*	*Entero-bacter*	*Xantho-monas*
A	80.00[1]	80.15	100.00	36.36	80.00	94.74
B	93.26	99.27	100.00	100.00	100.00	94.74
D	87.60	96.35	100.00	95.46	100.00	94.74
E1	92.80	94.89	100.00	95.46	93.55	94.74
E2	69.20	85.40	100.00	63.64	93.55	89.47
E3	69.60	97.81	100.00	95.46	100.00	94.74
E4	38.80	78.10	87.50	36.36	93.55	84.21
E5	83.20	85.40	100.00	59.09	93.55	94.74
E6	71.20	97.81	100.00	95.46	100.00	94.74
E7	72.80	81.75	100.00	54.55	93.55	84.21
E8	68.80	97.81	100.00	54.55	93.55	84.21
E9	100.00	94.81	87.50	100.00	100.00	89.74
G	na	2.19†	100.00	100.00	100.00	94.74
H	na	2.19†	100.00	100.00	100.00	94.74
Ia	100.00	3.65†	100.00	100.00	100.00	89.74
Ib	100.00	5.11†	100.00	100.00	96.77	89.74
K	84.40	94.16	100.00	95.46	96.77	94.74
M	100.00	97.81	100.00	100.00	100.00	94.74
N	92.80	94.89	100.00	100.00	96.77	84.21
S4	88.00	95.62	100.00	95.46	100.00	94.74

[1] Percentages of strains in each species that are resistant to a given colicin.
† Indicates the percentage of strains killed by a colicin that was ineffective against an *Escherichia coli* control.

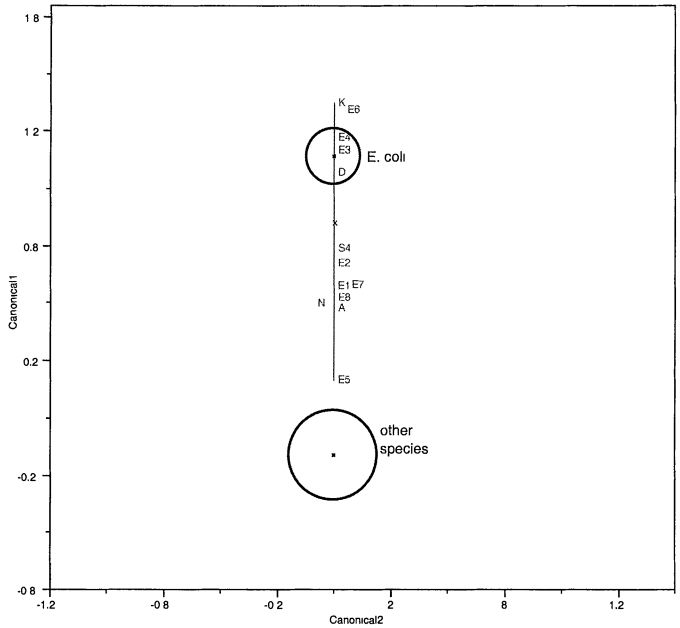

FIG. 2. Canonical analysis of resistance phenotypes comparing all *Escherichia coli* against all other species. Thirteen colicins were used (colicins B, G, H, Ia, Ib, M, and V were excluded as they were not scored in all surveys). The circles correspond to the 95% confidence region for *E. coli* and non-*E. coli*, with the **x** marking the center of the distribution. The vectors indicate the directions of the original response variables in the test space, and the length of the ray indicates the relative importance of each response variable.

cantly different, this suggests that each colicin resistance arose independently. If the distribution is significantly different, this would suggest that the origin of multiple colicin resistance patterns is more complex than this simple model assumes. Resistance patterns in all four *E. coli* collections were significantly different from the expected distribution (Fig. 1; ECOR: $D = 0.403$, $P < 0.0001$; Pathogenic: $D = 0.406$, $P = 0.0001$; Poultry: $D = 0.415$, $P \ll 0.0001$), suggesting that in these populations at least some resistances did not arise independently.

Colicin resistance profiles within the four *E. coli* populations were compared using canonical correlation analysis (Dillon and Goldstein 1984) (Fig. 3). The resistance patterns of the Poultry and Pathogen collections were indistinguishable from each other. The Indian and ECOR collections were markedly different from all other collections. The Indian collection strains were much less resistant to colicins A, E1, and E7. The ECOR collection was distinct in large part because most ECOR strains were resistant to colicins D and E8, and few were resistant to colicin E4 (see Table 3).

DISCUSSION

A survey of 72 isolates of *E. coli* suggested that levels of naturally occurring resistance is high (Riley and Gordon 1996). Data presented here confirm that observation. Greater than 70% of the *E. coli* isolates surveyed here, on average, are resistant to any one colicin. Roughly 30% of the isolates are multiply resistant to all eighteen colicins tested. Surprisingly, levels of colicin resistance here are much higher than levels of resistance to classical antibiotics (Levy 1986).

It has been suggested that colicins play a role in mediating intraspecific interactions (Reeves 1972; Frank 1994). This role seems unlikely given the high levels of co-occurring resistance observed in this survey. It may be that colicins serve in interspecific competition. However, our survey of non-*E. coli* isolates reveals that resistance to colicins among close relatives to *E. coli* is even greater with 61.8% of the non *E. coli* strains resistant to all 20 colicins tested. *Citro-*

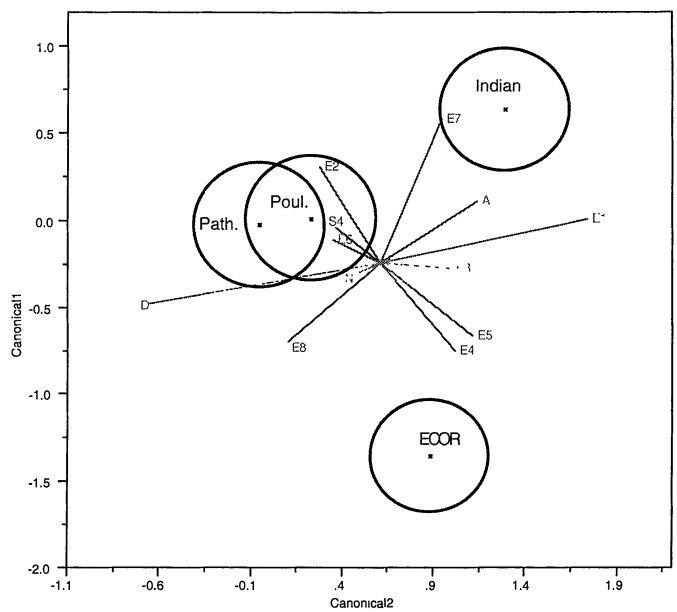

FIG. 3. Canonical analysis of resistance phenotypes among the four *Escherichia coli* collections to the 13 colicins scored in all four surveys: Poul (Poultry), Path (Pathogenic), Indian, and ECOR).

bacter sp. appears to be an exception in that it has the same frequencies of colicin resistances as *E. coli*. *Citrobacter* is closely related to *E. coli*. In fact, some *Citrobacter* sp. isolates fall within *E. coli* clades (Lawrence et al. 1991). On the other hand, the genera *Serratia* and *Klebsiella* are more closely related to *E. coli* than is *Xanthomonas* (Maidak et al. 1996), yet *Serratia* and *Klebsiella* isolates are more resistant to colicins than are *Xanthomonas* isolates. Thus, there does not appear to be a simple phylogenetic trend with respect to the levels of resistance observed among species. Canonical analysis suggests that the colicin resistance profiles of *E. coli* are very different than those of the non-*E. coli* enteric isolates assessed here. This might result from the higher percentage of non-*E. coli* strains that are completely resistant to all colicins.

The interspecific survey was conducted on a collection of isolates from Australian mammals. This represents a quite different set of hosts (and geography) than is represented in the *E. coli* collections surveyed here. However, *E. coli* were isolated from the Australian mammal collection and were shown to have levels of colicin production and resistance similar to those observed in the four *E. coli* collections analyzed here (D. Gordon, pers. comm.). Thus, geography and host do not appear to play a major role in determining colicin and resistance profiles (D. Gordon, pers. comm.).

Escherichia coli resistance profiles suggest that resistance to different colicin types probably did not arise independently. Three lines of evidence argue against the independent generation of multiple resistances. First, all colicins enter a cell by recognizing and binding to a specific cell surface receptor. Loss of the receptor can confer resistance to all colicins that recognize that receptor. For example, mutations in the *btuB* locus can result in resistance to all nine E colicins (Pugsley 1985; James et al. 1996). Alternatively, other receptor mutations can provide specific resistance to a single colicin or a subset of colicins that use a certain receptor (Braun et al. 1994). Mutations at loci involved in colicin translocation can result in resistance to some or all colicins that share the same pathway (Webster 1991). For example, mutations in the *tonB* locus confer resistance to colicins B, D, G, H, Ia, Ib, M, and V (Davies and Reeves 1975). Thus, numerous mechanisms exist for the generation of colicin resistance that would result in a multiply resistant strain due to one mutational event.

Second, our very simple model suggests the resistance profiles observed in nature do not conform to a one mutation-one resistance model. One possible explanation is that multiple resistances arise after exposure to a single colicin. When colicin sensitive *E. coli* K-12 is exposed to one colicin, resistance to multiple colicins is ubiquitous (M. Feldgarden and M. A. Riley, unpubl. data). The generation of multiple resistances from a single colicin exposure suggests that the lack of fit between our resistance data and the one mutation-one resistance model found among the natural isolates could be due to a similar pleiotropic effect of resistance generation.

A third line of evidence for the generation of multiple resistance from a single mutation is provided by a comparison of the patterns and levels of resistance between the Poultry and Pathogenic collections. The patterns of resistance are virtually identical in these two collections in terms of the frequencies of resistance to particular colicin types and the number of colicin types resisted. This contrasts sharply with the disparate patterns of colicin production in the two populations (Table 5). Although we can not rule out the possibility that these two populations experienced essentially identical colicin exposures in their pasts, the pleiotropic generation of multiple resistances appears to be a more plausible explanation for the similarity of current colicin resistance patterns in these two collections.

TABLE 5. Colicin types produced in the Pathogenic and Poultry *Escherichia coli* collections.

Poultry isolates*	Pathogenic isolates
Col A-1 (16.67%)	Col A-1 (10%)
Col N:V-1 (16.67%)	Col B:M-3 (30%)
Col V-4 (66.67%)	Col Ia-2 (20%)
	Col Ib-3 (30%)
	Col N-1 (10%)

* Other strains in this collection that were not assayed for colicin resistance were assayed for colicin production. Twenty-four percent of isolates from this collection produced colicin V.

The ECOR and Indian collections possess distinct resistance profiles from each other and from all other populations (refer to Table 3 and Fig. 3). The differences between these two collections in terms of resistance patterns and frequencies might be related to their quite different levels of genetic diversity. The ECOR collection is a world-wide sample collected over two decades, designed to encompass the genetic diversity of *E. coli* (Ochman and Selander 1984). On the other hand, the Indian collection is a genetically depauperate group (Rodrigues et al. 1996) with strains collected at one time point from sick infants in an isolated village in Mexico (T. Whittam, pers. comm.). These two collections represent extremes in terms of sampling strategies.

Although pleiotropy may explain much about resistance profiles in nature, there is one striking exception where the *absence* of pleiotropy might explain a colicin resistance pattern. There is a significant lack of resistance to colicin E4 in natural populations. These lower levels of colicin E4 resistance are surprising since levels of resistance to other E colicins (E1-E9) that use the same receptor (*btuB*) and translocation pathway (*tolQRA*) are much higher. Colicin E4 plasmids are extremely rare, having been documented only once (Pugsley and Oudega 1987), unlike colicins E1, E2, E5, and E8 which are relatively common (Riley and Gordon 1992; Afrasiabi and Waleh 1986; D. Gordon, pers. comm.). Colicin E4 resistance might be infrequent because exposure to colicin E4, resulting in selection for E4 resistance, is rare. On the other hand, the rarity of E4 resistance might have less to do with ecological factors, and more to do with the low frequency of mutations conferring resistance to colicin E4. Preliminary data from our lab suggest that colicin E4 resistance is difficult to generate under selection on a wide array of colicins (M. Feldgarden, unpubl. data). Thus, an absence of pleiotropy with respect to colicin E4 might explain the lower frequency to resistance to this colicin.

The apparent paradox of populations of *E. coli* segregating both high levels of colicinogeny and high levels of colicin resistance might be resolved with a better understanding of both the role of pleiotropy in the generation of colicin re-

sistance and in the selective pressures that maintain colicin plasmids. In fact, colicin killing might not be the primary selective force maintaining colicin plasmids. Colicin plasmids might be maintained by selection for other functions (Todd and Hurst 1961; Cooke et al. 1972; Wadolkowski et al. 1988; Feldgarden et al. 1995). For example, it has been shown that half of the characterized colicin plasmids promote a significant defense against bacteriophage predation (Feldgarden et al. 1995). Further, colicin V presence is often correlated with increased strain virulence. This appears to be due to the presence of an aerobactin gene on the colicin plasmid (Waters and Crosa 1991).

If resistance to multiple colicins arises frequently after exposure to a single colicin (M. Feldgarden, pers. obs.), the pleiotropic effect of selection for resistance to a single colicin might explain the current high levels of resistance. Even with very few segregating colicins, high levels of multiple colicin resistance will be continually selected. Further, colicin resistance may provide additional benefits to the cell, beyond avoiding the killing effects of colicins. For example, many colicin resistance mutations confer phage resistance (Hancock et al. 1976; Webster 1991). In the ECOR collection, 96% of strains possess lysogenic phage (Riley and Gordon 1992). What we have characterized as colicin resistance may have been selected for by phage presence.

Given the high levels of phage in natural populations (Furuse 1987) and the pleiotropic effects of resistance generation, why is resistance not fixed in natural populations? Colicin sensitive cells are more tolerant to environmental perturbations, including detergents and bile salt activity (Webster 1991; Clavel et al. 1996), and these traits might be more advantageous under some circumstances than colicin resistance. For example, the pathogenic strain O157:H7 in its nonhuman host (where it does not cause disease) is described as a "shedder" (Faith et al. 1996). Such strains typically are transient gut inhabitants, and spend a considerable amount of time outside the gut. Given the alternatives of high tolerance to environmental perturbations and colicin resistance, colicin sensitivity might be favored in strains which face harsh environmental challenges outside the gut.

Murinda et al. (1996) showed that diarrheagenic *E. coli* strains (DEC), which include serotype O157:H7, are sensitive to most colicins. On the other hand, among many DEC strains, resistance to classical antibiotics has increased rapidly. Over a period of three years, the fraction of *E. coli* isolates with multiple resistances to antibiotics has increased from 0% to 7.4% (Kim et al. 1994). It is believed that classical antibiotic resistance has spread rapidly throughout *E. coli* via gene transfer, due to intense selection resulting from the high levels of antibiotics released into the environment (Levy 1986). However, colicin resistance, which our results indicate is nearly ubiquitous among non-DEC strains, is very scarce among DEC strains. Additionally, if horizontal gene transfer plays a similar role in the evolution of colicin resistance as it does in classical antibiotic resistance, the high number of multiply colicin resistant strains suggests that a source of genetic information encoding colicin resistance is not a limiting factor. Despite the high potential for lateral transfer and a currently strong selective pressure, DEC strains still have not acquired colicin resistance, even though these strains have acquired multiple antibiotic resistance rapidly. This suggests that the cost of colicin resistance for *E. coli* that spend considerable time outside the gut selects against the maintenance of colicin resistant strains. Due to this tradeoff, we propose that the use of colicins as antibiotics against this class of strains might be a successful therapy that may not result in increased levels of colicin resistance among pathogenic *E. coli*.

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