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Survey of Drug and Phage Resistance and Colicin and Hemolysin Production Among Coliforms Isolated in the Ivory Coast

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Analysis of 178 strains isolated as total and fecal coliforms in the Ivory Coast revealed that (i) hemolytic activity was scarce (0.6%) among this bacterial population; (ii) the most prevalent colicins detected were, in decreasing order, E, I, A, and G; (iii) the frequency of coliphage and drug resistance was similar to that observed in other countries, except for those of drug-resistant strains in animal feces, which were lower than in countries where animals are antibiotic fed; and (iv) one of the drug resistance plasmids seemed to possess a restriction-modification system and another seemed to code for capsular material.

Many bacterial properties, especially among the family of the *Enterobacteriaceae*, have been shown to be plasmid determined. It is well known that multiple-drug resistance (25), colicinogeny (21), and hemolysin production (23) are plasmidic properties. It is also known that some plasmids can cause modification of the phage sensitivity pattern (3).

The growing interest in plasmids and type II restriction endonucleases, which are often coded by plasmids (8), prompted us to look for plasmidic properties among strains isolated in remote areas. In this paper, we present the results of the distribution of some of these properties among coliforms isolated in the Republic of Ivory Coast and the partial characterization of some of the plasmids found.

The coliform strains were isolated from water samples and human and animal feces in the region of Korhogo (northern Ivory Coast) as total or fecal coliforms and identified with API 20E (API Products, St. Laurent, Quebec) strips as previously described (12). Colicinogenic strains and colicin-resistant mutants are listed in Tables 1 and 2, respectively. *Escherichia coli* K-12 strain BM21 (from Y. A. Chabbert, Institute Pasteur) was used as the recipient in the transfer of R factors and as a point of comparison for the determination of the phage resistance patterns. This strain is resistant to penicillin, rifampicin, oxacillin, and nalidixic acid. Nutrient agar and Mueller-Hinton media were purchased from Difco Laboratories (Detroit, Mich.), and Trypticase soy agar and brain heart infusion broth and agar were purchased from BBL Microbiology Systems (Cockeysville, Md.). The bacteriophages used in this study were coliphages 121Q, T2, T4, E920g, pt1, T5, T6, T7, β4Q, λvir, HK243, N4, Esc 7-11, and φX174. They were kindly provided by H. W. Ackerman from the Félix d'Hérelle Reference Centre for Bacterial Viruses (Département de Microbiologie, Faculté de Médecine, Université Laval, Quebec). The preliminary tests for the determination of the phage sensitivity patterns among the coliform strains were performed by the technique described by Hancock and Reeves (7) but a Steers replicator (Craft Machine Inc., Chester, Pa.) was used instead of a multiple-syringe device to deposit the drop of phage suspension on the preseeded plates. For the strains that appeared resistant at 10^4 PFU/ml and sensitive at 10^6 PFU/ml, the efficiency of plating was determined as described by Hancock and Reeves (7) with BM21 as the reference strain.

Resistance to antibiotics was assessed on Mueller-Hinton agar by the standard Kirby-Bauer technique (2), except that intermediate strains were considered sensitive. The antibiotic disks used were: ampicillin, 10 μg; bacitracin, 10 U; chloromycetin, 30 μg; colimycin, 10 μg; furadantin, 300 μg; gentamicin, 10 μg; kanamycin, 30 μg; nalidixic acid, 30 μg; neomycin, 30 μg; penicillin G, 10 U; polymyxin B, 300 U; streptomycin, 10 μg; tetracycline, 30 μg; triple sulfa, 300 μg. All disks were purchased from Difco. Transfer of drug resistance was assayed by three different mixed culture techniques: (i) simple, mixed culture in brain heart infusion broth (4); (ii) in brain heart infusion broth to which 0.2% agar was added (17); and (iii) on a membrane filter which was placed on top of brain heart infusion agar to which the selective antibiotics were added (15). We obtained the best results with the brain heart infusion broth plus 0.2% agar.

TABLE 1. Colicinogenic strains used[a]

Strain no.	Species[b]	Colicin produced	Origin
CA31	*Citrobacter freundii*	A	P. Fredericq
AG097	*E. coli*	B	B. A. D. Stocker
K-12 W3110 (CA23)	*E. coli*	D	K. Timmis
TR75	*E. coli*	E1	Y. Hamon
K-53	*E. coli*	E1	P. Fredericq
CA42	*E. coli*	E2	P. Fredericq
CL124	*E. coli*	F1	Y. Hamon
CL1375	*C. freundii*	F2	Y. Hamon
1903	*Klebsiella ozaenae*	F5	Y. Hamon
CA46	*E. coli*	G	P. Fredericq
CA58	*E. coli*	H	P. Fredericq
K-12 167	*E. coli*	Ia	Y. Hamon
CA53	*E. coli*	Ia	P. Fredericq
K-235	*E. coli*	K	P. Fredericq
398	*K. ozaenae*	L	Y. Hamon
284	*C. freundii*	N and E	Y. Hamon
CA7	*C. freundii*	V	Y. Hamon
P15	*Shigella sonnei*	S4	P. Fredericq
K-12 185 (K-235)	*E. coli*	X	I. Smarda

[a] These strains were kindly provided by L. G. Mathieu and A. Sassarman from the Department of Microbiology and Immunology, University of Montreal.

[b] The strains were identified with API 20E strips.

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TABLE 2. Specific mutants resistant to the colicin used in this study

Resistant mutants[a]	Colicinogenic strain against which the mutant was selected	Colicin resistance pattern	Origin[c]
R-A	CA31	A, (L, E2)	1
R-B	AG097	B, D	2
R-D	K-12 W3110(CA23)	D, (B)	2
R-E1	K-53	E1	2
R-F5	1903	F1, F2, F5	2
R-G	CA46	G, (V)	3
R-H	CA58	H, B	2
R-Ia	CA53	Ia	2
R-L	398	L	2
R-N	284	N, (F5)	2
R-V	CA7	V	2
R-X	K-12 185 (K-235)	X	2

[a] All mutants were spontaneous mutants of *E. coli* HfrH 180.
[b] Colicinogenic strains are listed in Table 1. Partial resistances are given in parenthesis,
[c] Mutants were from the following sources: 1, Lavoie and Mathieu (1975); 2, M. Arriaga-Alba, this study; 3, G. Lavoie, our laboratory.

The production of colicins and the colicin sensitivity tests were performed by the deferred antagonism test as described by Fredericq (6). Spontaneous mutants resistant to the different colicins were obtained from the sensitive strain of *E. coli* HfrH 180 (kindly provided by A. Sassarman, Université de Montréal) and purified as previously described (11). The hemolytic activity of the coliform isolates was tested on blood agar base (Difco) to which defibrinated sheep blood (5% [vol/vol]) was added.

The analysis of plasmid DNA was essentially as described by Maniatis et al. (14). Molecular weight standards of pBR322 and pBR322 with inserts of chloroplastic DNA were kindly provided by G. Bellemare (Département de biochimie, Faculté des sciences et de génie, Université Laval, Quebec).

One of 178 strains appeared to be hemolytic. Of the 178 coliforms tested, 51 (29%) inhibited strain HfrH 180. The number of colicinogenic strains detected varied with the medium used: 43 on brain heart infusion agar plus blood, 32 on Trypticase soy agar, 21 on brain heart infusion agar, and 14 on nutrient agar. Colicinogenic strains were from the following sources: 40% (30 of 75) of the animal strains, 21% (16 of 76) of the human strains, and 19% (5 of 27) of the strains isolated from well-water samples. The number of isolates producing colicins E, I, A, G, B, D, K, N, F, S, and V were, respectively, 18, 10, 8, 6, 4, 2, 2, 2, 1, 1, and 1. A total of 12 colicins could not be identified, but 5 could be assigned to group B as described by Davies and Reeves (5).

The percentage of coliform strains resistant to the different phages varied from 53 to 97. The proportion of antibiotic-resistant strains showing resistance to phages N4, β4Q, T2, T3, T4, pt1, T5, and T6 was particularly elevated (>90%).

All isolates were resistant to penicillin G and bacitracin. Among the 178 isolates tested, 41 (23%) were resistant to at least one of the other antibiotics used, and 20 (11%) were resistant to two or more other antibiotics. Of 41 resistant strains, 34 were identified as *E. coli*, 4 as *Enterobacter* sp., 2 as *Citrobacter* sp., and 1 as a *Klebsiella* sp. According to their origin, the drug-resistant strains represent 8% (6 of 75) of the animal isolates, 34% (26 of 76) of the human strains, and 33% (9 of 27) of the strains isolated from well-water samples. A total of 19 strains were resistant to tetracycline only, 10 strains were resistant to streptomycin-triple sulfa-tetracycline; the resistance patterns to ampicillin, ampicillin-kanamycin, streptomycin-triple sulfa, streptomycin-tetracycline, and ampicillin-chloromycetin-streptomycin-triple sulfa-tetracycline were observed in two isolates each, and the patterns ampicillin-streptomycin-tetracycline and ampicillin-chloromycetin-kanamycin-neomycin-streptomycin-triple sulfa-tetracycline were observed in one isolate each.

Only 7 (20%) resistances could be transferred to the recipient strain BM21 under our conditions. The patterns of resistance transferred were as follows: T^r, S^r Su^r T^r, A^r S^r Su^r T^r, A^r C^r K^r N^r T^r, and A^r C^r K^r N^r T^r S^r Su^r. The properties transferred by conjugation to the recipient strain BM21 and the corresponding DNA bands observed on agarose gels are summarized in Table 3. The acridine orange treatment of BM21 (A^r C^r K^r N^r T^r S^r Su^r) gave three types of segregant phenotypes: sensitive, S^r Su^r only, and A^r C^r K^r N^r T^r only. The strains completely cured of the antibiotic resistance markers did not show any plasmid DNA bands on agarose gels nor any resistance to phages E920g, β4Q, and pt1. From the segregants resistant to S-Su, only a 3.8-megadalton DNA band could be seen on agarose gels. These strains were still resistant to the three above-mentioned phages. Paradoxically, they seemed to acquire a resistance to T4. Segregants showing A^r C^r K^r N^r T^r revealed only the >13.6-megadalton DNA band on agarose gels, were still resistant E920g, β4Q, and pt1, and they also seemed to become resistant to T4.

Although the hemolytic activity is found in high proportion (35 to 94%) among pathogenic *E. coli* (9, 24), the frequency of this property among *E. coli* isolated from healthy individuals (13, 22) and from the environment (10) was found to be low (3 to 30%). The low frequency observed in this study compared well with these reported observations.

All the colicinogenic strains isolated in the present work were identified as *E. coli*. In our study, the most prevalent colicins detected were, in decreasing order of frequency: E, I, A, and G. A similar frequency distribution was observed in Greece (20) and in England (1). On the other hand, Obi (18) and Obi and Campbell (19), working in Zaire, found the colicins G, K, E, and A to be the most prevalent. We have no explanation for this discrepency. Among the colicins studied in the present work, 12 could not be identified precisely. Five of these colicins could be placed in group B as defined by Davies and Reeves (5), which included colicins B, D, H, Ia, Ib, M, Q, S1, and V. The difficulties in the identification of these colicins could be attributed to the fact that certain colicinogenic strains produce more than one colicin (6) and to the high frequency of cross-resistance

TABLE 3. Properties transferred by conjugation to recipient strain BM21

Donor strain	Antibiotic resistance	Phage resistance[a]	DNA bands (megadaltons) on agarose gels
MB11	T^r S^r Su^r	N4, β4Q, T4	3.5 and 5.6
MB11	T^r S^r Su^r	pt1, T3, T5, T6	>13.6
BB18	T^r	None	ND[b]
PB33	T^r	φX174, T3	ND
MB56	T^r	N4, T4, pt1	>13.6
MB103	A^r C^r K^r T^r N^r S^r Su^r	E920g, β4Q, and pt1	3.8 and >13.6
MB103	A^r C^r K^r T^r N^r	None	>13.6

[a] These phage resistances were assessed by the determination of the efficiency of plating of the phages, with the recipient strain BM21 as a reference.
[b] ND, Not done.

among the colicins of group B (5). Unfortunately, no new colicins have been identified so far from the strains of *E. coli* isolated in the Ivory Coast. Work is presently in progress in our laboratory to further characterize the seven unknown colicins.

Although antibiotics are not theoretically as extensively used in the Ivory Coast as in more industrialized countries, we could always find a wide variety of antibiotics sold at the market in Korhogo without prescription (M. C. Lavoie, personal observation). This uncontrolled use of antibiotics could maintain a proportion of R^+ bacteria in the population similar to those observed in more developed countries where antibiotics are more extensively used (16). Our results with animal isolates favor the established principle that drug resistance is maintained among bacterial populations by the selective pressure of antibiotic use. None of the animals sampled in this study were antibiotic fed, and only 8% of the coliforms isolated from their feces were drug resistant.

Two DNA bands have been observed from the extract of strain BM21 which acquired resistance to S-Su-T after conjugation with MB11 (Table 3). So far we cannot assign any phenotypic properties to either of these two bands because we have not been able to observe them independently in different strains.

Preliminary results seem to indicate that the acquired resistance to most of the phages used (N4, β4Q, PT1, T3, T4, T5, T6) accompanying the acquisition of antibiotic resistance (S^r Su^r T^r) could be attributed to the production of more capsular material by the exconjugant strain.

The plasmid pMLT56 seems to be the only candidate for the presence of a restriction-modification system as judged by the increase in the EOP after the subculture of the phage N4 on BM21 (pMLT56).

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