INFECTION AND IMMUNITY, Dec. 1984, p. 835–838
0019-9567/84/120835-04$02.00/0

Vol. 46, No. 3

Association of Hydroxamate Siderophore (Aerobactin) with *Escherichia coli* Isolated from Patients with Bacteremia

JOHN Z. MONTGOMERIE,[1,2]* ALBRECHT BINDEREIF,[3] JOSEPH B. NEILANDS,[3] GEORGE M. KALMANSON,[4,5] AND LUCIEN B. GUZE[4,5]

University of Southern California School of Medicine, Los Angeles, California 90033[1]; Rancho Los Amigos Hospital, Downey, California 90242[2]; Department of Biochemistry, University of California, Berkeley, California 94720[3]; University of California, Los Angeles School of Medicine, Los Angeles, California 90024[4]; and Veterans Administration Wadsworth Medical Center, Los Angeles, California 90073[5]

Received 21 May 1984/Accepted 8 September 1984

Clinical isolates of *Escherichia coli* were examined for the presence of hydroxamate siderophore (aerobactin). The incidence of aerobactin-positive strains of *E. coli* from the blood was greater than the incidence of these strains isolated from other sites. The presence of aerobactin and the virulence of strains of *E. coli* in urinary tract infection were also examined in mice. The presence of aerobactin in the strains of *E. coli* correlated with virulence as measured by proportion of deaths but not with renal infection. These results suggest that the presence of aerobactin may be a significant factor in the invasion of the blood stream.

Iron compounds are known to enhance the growth of bacteria, including *Escherichia coli*. These nutrients must be obtained from the environment, where iron is usually present in an insoluble form or bound to an organic ligand. Bacteria have transport systems to obtain iron under these conditions. In *E. coli*, two iron-chelating substances, or siderophores, have been described: the catechol enterobactin (enterochelin) (12) and hydroxamate (aerobactin). Rogers (14) and other workers (7, 13) have postulated that the adequate production of siderophores by *E. coli* in vivo may be necessary for the virulence of certain microbes. Williams and Warner (18) showed that the *E. coli* mutants with aerobactin-type siderophores were more virulent than mutants without these siderophores. In the present study, we examined the frequency with which aerobactin is found in clinical isolates of *E. coli*. We also examined the relation of aerobactin to the virulence of *E. coli* in the pathogenesis of urinary tract infection and pyelonephritis in mice.

MATERIALS AND METHODS

Bacteria. Clinical isolates of *E. coli* were obtained from patients at Rancho Los Amigos Hospital, Downey, Calif.

Blood isolates. Blood isolates were from 15 patients with liver disease, 8 patients with spinal cord injury, 3 patients with diabetes mellitus, and 6 patients with other diseases. These bacteremias were associated with urinary tract infection (16 patients), spontaneous bacterial peritonitis (7 patients) and other infections (2 patients). Seven patients with liver disease had bacteremia without an identifiable source.

Ascites isolates. The 30 *E. coli* strains isolated from ascites were all associated with spontaneous bacterial peritonitis in patients with liver disease.

Urine isolates. Sixteen urine isolates were from patients with neurogenic bladders and significant bacteriuria. None of these patients had bacteremia.

Laboratory strains. Twenty additional strains of *E. coli* (called laboratory strains) had been previously examined in a murine model, and their virulence in renal infection has been previously described (6). Only 13 of these original 20 strains were available for study. These *E. coli* strains, which have been described previously (6), were stored lyophilized. Most of the strains had been maintained and used in in vitro studies in different laboratories. Their clinical sources are not known.

Rectal isolates. Nineteen rectal isolates of *E. coli* were obtained from hospital personnel at the same hospital.

Detection of aerobactin-producing strains. The strains of *E. coli* were cultured in minimal medium (Tris-succinate). The presence of hydroxamate was detected by the ferric perchlorate reaction (1). In addition, a bioassay for aerobactin was performed with *E. coli* LG1522 (obtained from Peter H. Williams). This aerobactin-requiring strain was used as a lawn in an agar plate containing 0.2 mM α,α' dipyridyl. A cell supernatant of each strain was added to a disk on the agar plate, and a halo of growth around the disk indicated the presence of aerobactin in the strain.

Survival of *E. coli* in serum. The survival of 55 *E. coli* strains in serum was examined as follows. Serum was obtained from a normal subject and frozen in small portions at −70°C within 2 hours. Overnight cultures of *E. coli* diluted in phosphate-buffered saline were added to inactivated serum (serum heated to 56°C for 30 min) to produce a final concentration of ca. 10^3 bacteria per ml and incubated for 24 h at 37°C. The number of *E. coli* cells surviving after incubation was determined by inoculation of serial dilutions of the mixture into molten nutrient agar.

Renal infection in mice. In a previous study, we examined a number of virulence factors of *E. coli* in a murine model of renal infection (6). Ascending pyelonephritis had been produced by injecting *E. coli* cells into the exposed bladders of mice undergoing diuresis. Two measures were used to compare virulence: the ability of *E. coli* cells to produce renal infection (number of *E. coli* cells per gram of kidney), and the lethality of the infection produced by the *E. coli*. Deaths after infection with *E. coli* occurred in the first few days; the majority occurred within 24 hours. The percent mortality ranged from 0 to 79.6%. In that study, these measures of virulence were used to examine a number of bacterial factors that might influence virulence, including resistance to phagocytosis by polymorphonuclear leukocytes, growth in minimal medium and urine, presence of K antigen, resistance to serum bactericidal activity, dulcitol

* Corresponding author.

TABLE 1. Aerobactin siderophore in clinical isolates of *E. coli*

Source of *E. coli*	No. of isolates found, associated with the following disease:					No. (%) of aerobactin-positive isolates	*P* value[a]
	Liver disease	Neurogenic bladder	Diabetes mellitus	Other	None[b]		
Blood	15	8	3	6		24 (75)	
Ascites	30					14 (47)	0.04
Urine		16				6 (38)	0.03
Rectal					19	8 (42)	0.04
Laboratory					13	5 (38)	0.05

[a] Chi-square analysis of each source compared with blood isolates. For the total nonblood isolates, $P = 0.004$.
[b] Only 13 of the original 20 laboratory strains were studied. The clinical sources of these strains and the diseases associated with them are unknown. Rectal isolates were from normal subjects (hospital personnel).

fermentation, rough mutation, and motility (6). In other studies, we have examined the adherence of these *E. coli* to bladder epithelial cells (11) and enterobactin production by the *E. coli* (10).

Statistical analysis. The incidence of aerobactin-positive strains of *E. coli* isolated from different sites was compared by chi-square analysis.

In the murine model, the relation of the aerobactin-production to renal infection, proportion of deaths, growth in urine and minimal medium, serum bactericidal activity, resistance to phagocytosis, epithelial adherence, and enterobactin was examined by comparing these factors in aerobactin-positive and -negative strains with Student's *t* tests. To determine whether aerobactin correlated with K antigen, dulcitol fermentation, motility, and rough mutation, we compared the incidence of each of these factors in aerobactin-positive and -negative strains with the Fisher exact test.

RESULTS

The incidence of aerobactin-positive strains from the various sites of infection is shown in Table 1. The incidence of aerobactin-positive strains of *E. coli* from the blood (75%) was significantly higher than the incidence of these strains from other sites (38 to 47%). When the incidence of aerobactin-positive strains in blood isolates was compared with the incidence of aerobactin-positive *E. coli* from all other sites, the difference became more significant ($P = 0.004$).

In the murine model, the mean proportion of deaths in aerobactin-positive *E. coli* was significantly greater than the proportion of deaths in aerobactin-negative strains (Table 2). There were more renal infections with the aerobactin-positive strains, but the difference was not significant. Table 3 shows the results of comparing a number of measures of host-parasite interaction in aerobactin-positive and -negative strains. There was a strong correlation of aerobactin with the growth of the strains in urine (Table 3). Urine was obtained from mice undergoing diuresis and from mice without diuresis, and this same urine was diluted. The correlation was only with the urine from the mice that were diuresing or with the diluted urine. The resistance of these strains of *E. coli* to a pool of normal human serum was also examined. There was no correlation between aerobactin production and serum resistance.

E. coli strains showed a weak correlation between aerobactin production and resistance to phagocytosis and the presence of K antigen. There was no correlation with enterobactin, dulcitol fermentation, or adherence in epithelial cells. In this murine model, the aerobactin siderophore was more significant than enterobactin (Table 4). The presence of aerobactin correlated with deaths of mice and growth of strains in urine, with some questionable significance for K antigen presence and phagocytosis resistance. Enterobactin only correlated with the rough mutation and serum sensitivity.

The growth of *E. coli* in inactivated normal human serum is shown in Table 5. Aerobactin-positive strains survived better in inactivated serum than aerobactin-negative strains ($P < 0.01$).

TABLE 2. Virulence of *E. coli* in mice

Strain (*n*)	% Causing death[a]	Renal infection (log/g)
Aerobactin positive (5)	35.4 ± 12.0	4.5 ± 0.9
Aerobactin negative (8)	11.4 ± 3.4	2.9 ± 0.8
P	0.02	0.15

[a] Mean proportion expressed as percent ± standard deviation.

DISCUSSION

There are numerous studies indicating that infections may be enhanced by iron, and there are many suggested mechanisms by which iron may influence host-parasite interaction (2, 4, 17). Iron compounds increase the growth of bacteria, including *E. coli* in vitro and in vivo (3). Parenteral iron has been shown to enhance various experimental infections, including pyelonephritis (5, 17). It has been suggested that human serum may be bactericidal or bacteriostatic because

TABLE 3. Factors influencing virulence of *E. coli* in mice: comparison of aerobactin-positive and negative strains

Test and factor	*P*[a]
Student test	
Growth in urine (diuresis)	0.01
Growth in urine	NS
Growth in diluted urine	0.05
Serum resistance	NS
% Phagocytosis	0.07
Enterobactin	NS
Fisher exact test	
Epithelial adherence	NS
Presence of K antigen	0.07
Dulcitol fermentation	NS
Motility	NS
Rough mutation	NS

[a] Association of each factor with 5 aerobactin-positive and 8 aerobactin-negative strains of *E. coli*. NS, Not significant.

TABLE 4. Factors associated with siderophores, aerobactin, and enterobactin

Factor	Significance (*P*) of the presence of:	
	Aerobactin	Enterobactin
Deaths in mice	0.03	NS
Renal infection	0.15	NS
Growth in urine	NS	NS
Growth in urine (diuresis)	0.01	NS
Growth in urine (diluted)	0.05	NS
Growth in minimal medium	NS	NS
Presence of K antigen	0.07	NS
% Phagocytosis	0.07	NS
Dulcitol fermentation	NS	NS
Epithelial adherence	NS	NS
Serum sensitivity	NS	0.05
Rough mutation	NS	0.01

of the presence of iron-binding proteins (transferrins) in the serum which reduce the availability of iron to bacteria (15, 17). It has also been postulated that bacteria which can multiply in mammalian serum may possess iron-binding siderophores which remove iron from transferrin complexes in the serum (7, 13, 14). Mutants of *E. coli* with aerobactin siderophores were found to be more virulent than mutants without aerobactin in a murine model of bacteremia (18). Bacteria possessing greater quantities of aerobactin may have an advantage in the serum. The present studies, by showing that the presence of aerobactin was associated with bacteremia and that aerobactin-positive strains survived better in heat-inactivated serum, support this hypothesis. In a study of hydroxamate-positive *E. coli* from different sources, Stuart et al. (16) also observed a high incidence of hydroxamate-positive strains in isolates from the blood of humans and poultry.

The bowel is the probable reservoir of *E. coli* for human infection, and an alternative explanation for our results might be that patients with bacteremia had more aerobactin-positive strains in the stool than patients with other infections. Further studies will be necessary to resolve this question.

Isolates of *E. coli* from ascites were from patients with spontaneous bacterial peritonitis. Since this infection probably follows bacteremia, it is surprising that a greater proportion of these strains were not aerobactin positive. It is possible that patients with liver disease have altered host defenses as a result of changes in iron metabolism. Some indication that this is so is seen in the incidence of aerobactin-positive *E. coli* in bacteremic patients with liver disease. Of 15 bacteremic patients with liver disease, 9 had *E. coli* that were aerobactin positive. Of 17 *E. coli* isolated from patients with bacteremia associated with other diseases, 15 were aerobactin positive.

TABLE 5. Growth of *E. coli* in inactivated human serum

Source of *E. coli*	Proportion of isolates multiplying in serum[a]	
	Aerobactin positive	Aerobactin negative
Blood	3/6	0/1
Urine	2/2	1/6
Ascites	4/5	1/7
Stool	6/8	5/10
Laboratory	5/5	2/4

[a] Multiplication is defined as recovery of >100% of inoculum after 24 h in inactivated serum.

In mice, the aerobactin-positive strains were associated with a higher proportion of early deaths which were probably the result of bacteremia. We previously examined the siderophore enterobactin, which did not correlate with virulence in *E. coli* in the development of renal infection or the proportion of deaths (10). Miles and Khimji (9) were also unable to show a relationship between enterobactin production and virulence in *E. coli*. Our results suggest that aerobactin may be a more significant factor than enterobactin in bacteremia in mice. All wild-type *E. coli* produce enterobactin, and the aerobactin siderophore may confer advantage on *E. coli* strains that also synthesize enterobactin. Konopka et al. (8) found that in serum, aerobactin had a greater ability than enterobactin to sequester iron from transferrin.

In the present experiments, we examine the sensitivity of *E. coli* to heat-inactivated serum and observed that aerobactin-positive strains survived significantly better than aerobactin-negative strains. The previous results with normal human serum with retained complement activity did not show any correlation with the presence of aerobactin to serum sensitivity. How these results relate to the high incidence of aerobactin-positive strains in bacteremia is not clear, but it is possible that there are sites in the bloodstream where complement may be less active or inactive.

In urinary tract infections in mice, aerobactin-positive strains multiplied more readily in diluted urine than did aerobactin-negative strains. This may reflect the presence of lactoferrin or low iron levels in the urine, further reduced by dilution or diuresis. This increased rate of multiplication in the urine was not reflected in an increase in renal infection. We are unable to show significant differences in renal infection between aerobactin-positive and aerobactin-negative strains, although there was a trend in favor of the aerobactin-positive strains. Further studies will be necessary to examine the relation of aerobactin to urinary tract infection.

LITERATURE CITED

1. **Atkin, C. L., J. B. Neilands, and H. J. Phaff.** 1970. Rhodotorulic acid from species of *Leucosporidium*, *Rhodosporidium*, *Rhodotorula*, *Sporidiobolus*, and *Sporobollmyces*, and a new alanine-containing ferrichrome from *Cryptococcus melibiosum*. J. Bacteriol. **103**:723–733.
2. **Bullen, J. J.** 1981. The significance of iron in infection. Rev. Infect. Dis. **3**:1127–1138.
3. **Bullen, J. J., L. C. Leigh, and H. J. Rogers.** 1968. The effect of iron compounds on the virulence of *Escherichia coli* for guinea-pigs. Immunology **15**:581–588.
4. **Finkelstein, R. A., C. V. Sciortino, and M. A. McIntosh.** 1983. Role of iron in microbe-host interactions. Rev. Infect. Dis. **5**:S759–777.
5. **Fletcher, J., and E. Goldstein.** 1970. The effect of parenteral iron preparations on experimental pyelonephritis. Br. J. Exp. Pathol. **51**:280–285.
6. **Guze, L. B., J. Z. Montgomerie, C. S. Potter, and G. M. Kalmanson.** 1973. Pyelonephritis XVI. Correlates of parasite virulence in acute ascending *Escherichia coli* pyelonephritis in mice undergoing diuresis. Yale J. Biol. Med. **46**:203–211.
7. **Kochan, I., J. T. Kvach, and T. I. Wiles.** 1977. Virulence-associated acquisition of iron in mammalian serum by *Escherichia coli*. J. Infect. Dis. **135**:623–632.
8. **Konopka, K., A. Bindereif, and J. B. Neilands.** 1982. Aerobactin mediated utilization of transferrin iron. Biochemistry **24**: 6503–6508.
9. **Miles, A. A., and P. L. Khimji.** 1975. Enterobacterial chelators of iron: their occurrence, detection, and relation to pathogenicity. J. Med. Microbiol. **8**:477–490.

10. **Montgomerie, J. Z., G. M. Kalmanson, and L. B. Guze.** 1979. Enterobactin and virulence of *Escherichia coli* in pyelonephritis. J. Infect. Dis. **140:**1013.
11. **Montgomerie, J. Z., S. Turkel, G. M. Kalmanson, and L. B. Guze.** 1980. *E. coli* adherence to bladder epithelial cells of mice. Urol. Res. **8:**163–165.
12. **O'Brien, I. G., and F. L. Gibson.** 1970. The structure of enterochelin and related 2, 3 dihydroxy-*N*-benzoylserine conjugates from *Escherichia coli*. Biochim. Biophys. Acta **215:** 392–402.
13. **Payne, S. M., and R. A. Finkelstein.** 1978. The critical role of iron in host-bacterial interactions. J. Clin. Invest. **61:**1428–1440.
14. **Rogers, H. J.** 1973. Iron-binding catechols and virulence in *Escherichia coli*. Infect. Immun. **7:**445–456.
15. **Schade, A. L., and L. Caroline.** 1946. An iron-binding component in human blood plasma. Science **104:**340–341.
16. **Stuart, S. J., K. T. Greenwood, and R. K. J. Luke.** 1982. Iron-suppressible production of hydroxamate by *Escherichia coli* isolates. Infect. Immun. **36:**870–875.
17. **Weinberg, E. D.** 1978. Iron and infection. Microbiol. Rev. **42:**45–66.
18. **Williams, P. H., and P. J. Warner.** 1980. ColV plasmid-mediated, colicin V-independent iron uptake system of invasive strains of *Escherichia coli*. Infect. Immun. **29:**411–416.