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Influence of β-Lactam Antibiotics on Serum Resistance of K1-Positive Blood Culture Isolates of *Escherichia coli*

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The K1-positive strains of *Escherichia coli* are a group with considerable clinical importance, serum resistance being a common virulence factor of these strains. In the present paper, the influences of cephaloridine, imipenem, and ceftazidime on the serum resistance of eight serum-resistant K1-positive *E. coli* blood culture isolates with smooth-type lipopolysaccharide were studied. All strains were rendered more serum sensitive by treatment with subinhibitory concentrations of antibiotics. The amount of the reduction of serum resistance was dependent on the concentration of the antibiotic. Amounts of K1 produced under the influence of the antibiotics were measured and were found to be reduced for almost all strains tested. To further test the hypothesis that antibiotic-induced reduction of serum resistance is mediated by inhibition of K1 expression, isogenic mutants of one strain were produced by selection for resistance against infection with K1-specific bacteriophages. These mutants were found to be highly serum sensitive. We conclude from this study that β-lactam antibiotics can render K1-positive serum-resistant strains of *E. coli* highly serum sensitive and that this effect is mediated by inhibition of K1 expression.

Over the past two decades, many authors have reported that in addition to affecting primary targets like peptidoglycan or protein synthesis, antibiotics influence the composition of the bacterial cell envelope when applied at subinhibitory concentrations (3, 9, 10, 13, 27, 29). Another group of studies has demonstrated influences of antibiotics on various host-parasite interaction processes such as adherence (16, 28; for reviews, see references 24 and 25), phagocytosis (2, 18), serum resistance (4, 29, 32), and immune response to outer membrane (OM) components (13). Though a connection between the antibiotic-induced changes of bacterial cell surface and the effects of antibiotics on host-parasite interactions seemed likely, it has been very difficult to clearly demonstrate this connection. Moreover, the lack of such a connection has made it very problematic to evaluate the clinical significance of either group of data.

Escherichia coli strains expressing the K1-type capsular polysaccharide are a group with particular clinical importance, since K1-positive strains make up 80% of the *E. coli* strains isolated from neonatal meningitis, 36% of those isolated from neonatal septicemia, and 26% of those isolated from adult septicemia (20, 22, 23). In vitro experiments and animal infection studies have provided evidence that K1 is indeed a virulence factor for *E. coli* and that possible mechanisms are inhibition of opsonophagocytosis and counteraction against the bactericidal activity of serum (5, 6, 8, 12, 17, 21, 26). In a previous paper (27), we demonstrated that β-lactam antibiotics and ciprofloxacin reduced the amount of K1 produced by a K1-positive strain (BK 136) of *E. coli*, which was isolated from a patient with septicemia. This reduction was dependent on the concentration of the antibiotic. Similar observations were reported by Taylor et al. (29) for *E. coli* and by Kadurugamuwa et al. (10) for *Klebsiella pneumoniae*. In the present study, we investigated whether different β-lactam antibiotics were able to modify the serum resistance of eight K1-positive *E. coli* strains isolated from patients with adult septicemia. To test the hypothesis that the observed changes of serum resistance were mediated by inhibition of K1 expression, we measured the amounts of K1 produced by the bacteria in both the presence and absence of antibiotics. Furthermore, we produced isogenic mutants of strain BK 136, for which findings of inhibition of K1 expression by cephaloridine, imipenem, moxalactam, and ciprofloxacin had been published previously (27), by selection for resistance against infection with K1-specific bacteriophages and tested these mutants for serum resistance.

MATERIALS AND METHODS

Bacteria. The following strains of *E. coli* were used: BK 152, BK 526, BK 323, BK 136, BK 555, BK 324, BK 658, and BK 992. All were serum resistant, i.e., able to grow in 100% normal human serum (NHS). All strains were identified as K1 positive by two methods: a test of susceptibility to K1-specific phages (kindly provided by B. Rowe, Central Public Health Laboratory, London, United Kingdom) and an agar diffusion test as described by Kaijser (11) (equine meningococcus B antiserum [horse 46] was kindly provided by B. J. Robbins, Food and Drug Administration, Bethesda, Md.). Mutants were produced by selection for resistance against infection by the K1-specific phages.

Antibiotics. The following antibiotics were used: ceftazidime (Hoechst AG, Frankfurt, Federal Republic of Germany), cephaloridine (Glaxo Pharmaceuticals, Ltd., Greenford, United Kingdom), and imipenem (Merck Sharp & Dohme, Rahway, N.J.).

MIC determinations. The MICs were determined by microdilution plate assay DIN 38940. Values are the means from triplicate independent assays. The following MICs (micrograms per milliliter) were determined: imipenem, 0.125 for all strains except BK 152 (not done); ceftazidime, 0.25 for all strains except BK 658 (0.5) and BK 136 (not done); and cephaloridine, 2.0 for BK 152, BK 323, and BK

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TABLE 1. Influence of antibiotics on serum resistance of K1-positive, serum-resistant *E. coli* strains

Antibiotic[a]	Reduction of serum resistance[b] of strain:							
	BK 136	BK 152	BK 323	BK 324	BK 526	BK 555	BK 658	BK 992
Cephaloridine	1.3	0.2	0.5	1.9	0.6	2.0	3.9	5.8
Ceftazidime	ND[c]	1.7	1.5	0.9	2.1	1.4	3.4	1.1
Imipenem	2.0	ND	4.3	3.4	3.2	3.0	3.7	4.5

[a] Administered at one-fourth of the MIC.
[b] Decrease of log CFU after 2 h of incubation in 50% NHS compared with value for control in unsupplemented ISB.
[c] ND, Not done.

526, 4.0 for BK 136, BK 324, and BK 555, 8.0 for BK 658, and 16.0 for BK 992.

Serum resistance assay. Bacteria were inoculated from a nutrient agar slant into 0.9% NaCl to a concentration of 0.5 McFarland standards and diluted 1:1,000 in Iso-Sensitest broth (ISB) (Oxoid Ltd., Wesel, Federal Republic of Germany). One milliliter of the bacterial suspension was added to 1 ml of ISB containing twice the intended final concentration of the antibiotic. These cultures were incubated overnight, diluted 1:100 with ISB containing the desired concentration of the antibiotic, and incubated with shaking for 3 h at 37°C. For the serum resistance assay, a sample of this culture was again diluted 1:100 with ISB containing the same concentration of antibiotic. Next, 0.5 ml of this sample was mixed with 0.5 ml of freshly thawed human serum and incubated with shaking at 37°C. Growth kinetics were monitored over the next 2 h by the CFU method. All experiments included three control growth curves: in ISB alone, in ISB plus one-fourth of the MIC without serum, and in ISB plus 50% serum without antibiotic. These control growth curves were identical within limits of variability. Representative assays were performed repeatedly, and variation was always lower than ±1 log step. All experiments were performed with samples from the same pool of serum.

Determination of K1 capsular polysaccharide. Bacteria were inoculated from an overnight culture into Mueller-Hinton broth containing one-fourth of the MIC of ceftazidime, cephaloridine, or imipenem or no antibiotic. Bacteria were grown to late log phase, harvested, washed, and disrupted by sonication. Protein was determined by the method of Markwell et al. (15), and a volume corresponding to 5 μg of protein was analyzed by rocket immunoelectrophoresis by the method of Weeke (31). The monoclonal antibody against meningococcal group B polysaccharide was kindly provided by M. Frosch, Universität Hannover, Hannover Federal Republic of Germany. K1 extracted and purified from *E. coli* BK 152 by the method of Pelkonen et al. (19) and quantitated by the method of Barry et al. (1) was used as a standard.

RESULTS

Influence of β-lactam antibiotics on serum resistance of K1-positive strains of *E. coli*. Table 1 shows the reduction of CFU counts for the different K1-positive strains and antibiotics tested compared with the count for the control in unsupplemented ISB. All strains were rendered more serum sensitive by antibiotic treatment. The reduction of serum resistance was most pronounced for strain BK 992 with cephaloridine. The antibiotics which induced the most pronounced reduction of serum resistance differed from strain to strain.

TABLE 2. K1 capsular polysaccharide content of *E. coli* blood culture strains treated with subinhibitory concentrations of antibiotics

Strain	Antibiotic[a]	K1 content[b]	% of K1 expressed
BK 152	None	78	100
	Ctz	66	85
	Imp	54	69
	Ceph	78	100
BK 324	None	74	100
	Ctz	62	84
	Imp	48	65
	Ceph	44	60
BK 526	None	62	100
	Ctz	52	84
	Imp	52	84
	Ceph	58	93
BK 555	None	62	100
	Ctz	56	90
	Imp	38	61
	Ceph	36	59
BK 658	None	36	100
	Ctz	36	100
	Imp	22	61
	Ceph	ND[c]	ND
BK 992	None	62	100
	Ctz	50	81
	Imp	36	58
	Ceph	32	52

[a] Administered at one-fourth of the MIC. Ctz, Ceftazidime; Imp, imipenem; Ceph, cephaloridine.
[b] Micrograms of K1 per milligram of cell protein.
[c] ND, Not done.

For BK 992 and cephaloridine, the experiment was performed with four different concentrations of cephaloridine (0.5 to 4 μg/ml). Reduction of serum resistance was dependent on the concentration of the antibiotic.

Influence of β-lactam antibiotics on amounts of K1 capsular polysaccharide expressed by *E. coli* blood culture strains. The influence of ceftazidime, imipenem, and cephaloridine on K1 expression of six of the *E. coli* blood culture strains was investigated by measuring K1 by rocket immunoelectrophoresis (Table 2). Except for ceftazidime with strain BK 658 and cephaloridine with strain BK 152, all antibiotics reduced the amounts of K1 expressed by the strains tested. The maximum effect observed was the reduction of K1 expressed to 52% by strain BK 992 in the presence one-fourth of the MIC of cephaloridine.

Serum resistance of K1-specific-phage-resistant mutants of *E. coli* BK 136. In order to test whether K1 was responsible for the serum resistance of BK 136, isogenic mutants of strain BK 136 were selected for resistance against infection with K1-specific phages and tested for serum resistance. The growth curves are shown in Fig. 1. The isogenic mutants were highly serum sensitive, in contrast to the serum-resistant wild-type strain.

DISCUSSION

Over the past two decades, evidence that antibiotics in subinhibitory concentrations have influences on the composition of the cell envelopes of gram-negative bacteria has

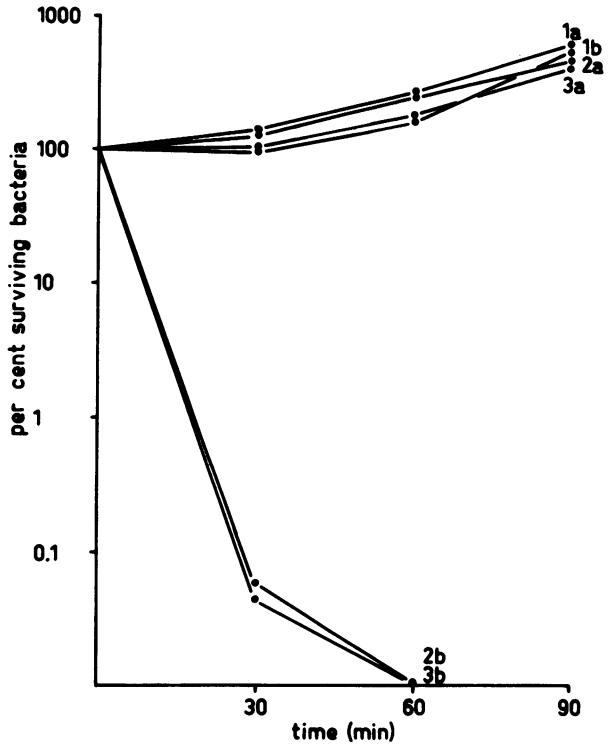

FIG. 1. Growth curves of two K1-specific-phage-resistant mutants of strain BK 136 and wild-type BK 136 during serum-resistance assay. Wild-type strain BK 136 (1a and b) and mutant strains BK 136/3 (2a and b) and BK 136/10 (3a and b) were cultured in ISB with 50% inactivated (a) or active (b) NHS.

accumulated (3, 7, 9, 10, 13, 27, 29). There have also been reports about an influence of antibiotics on serum resistance. Wiemer et al. (32) reported that imipenem made serum-resistant *Enterobacter cloacae* serum sensitive, and Taylor et al. (29) reported the same about one K1-positive strain of *E. coli*. However, the phenomenon has not yet been investigated for larger groups of strains, nor has the mechanism of this effect been studied.

In the present work, we have studied the influence of three β-lactam antibiotics on a panel of eight K1-positive serum-resistant strains of *E. coli*, including strain BK 136, for which data about the effects of antibiotics on K1 expression have been published previously (27). In the presence of antibiotics, all strains became more sensitive to the bactericidal action of serum, with the amount of CFU reduction varying between 0.2 and 5.8 log after 2 h of incubation in 50% NHS. (The definitions of the terms serum resistance and serum sensitivity have been the subject of considerable debate. In this paper, we call strains that are capable of growing in undiluted NHS serum resistant and determine changes of serum resistance by giving the concentration of serum tested and the reduction of CFU after 2 h of growth in the given concentration of serum.) The reduction of serum resistance was dependent on the concentration of the antibiotic.

The data from the literature made a connection between antibiotic-induced changes of serum resistance and inhibition of K1 expression seem likely. To further test this hypothesis, two experiments were performed. We measured the amounts of K1 produced by the wild-type strains in the presence of antibiotics, and we studied the serum resistance properties of isogenic mutants of strain BK 136, which had been selected for resistance against infection with K1-specific phages. Also, we found an antibiotic-induced decrease in K1 production for the strains and antibiotics used in this study. There was no linear correlation between amount of K1 and degree of serum resistance. This is consistent, however, with the findings of Vermeulen et al. (30), who found that the quantitative relationship between amount of K1 (which was modified by changes of growth conditions) and serum resistance was characterized by the existence of a threshold level of K1 that was necessary to protect cells from serum killing.

Since antibiotics also have effects on other cell surface properties such as the quantitative composition of the OM or cross-linking between the OM and peptidoglycan (7, 27, 29), effects which could also be responsible for alterations of serum resistance, we examined isogenic K1-specific-phage-resistant mutants of one strain for their serum resistance properties in order to establish more firmly the connection between antibiotic-induced changes of K1 expression and serum resistance. The finding that these mutants were highly serum sensitive supports the concept that β-lactam antibiotics interfere with serum resistance via modification of K1 expression, though it cannot be ruled out that antibiotic effects other than the reduction of K1 expression may contribute to the observed effects on serum resistance. Our data and interpretation are consistent with reports that have demonstrated that K1 alone can confer complete serum resistance to bacteria. Opal et al. (17) and Gemski et al. (5) have shown that isogenic, K1-negative mutants of serum-resistant, rough-type strains of *E. coli* are serum sensitive. We have recently examined groups of such mutants derived from serum-resistant, smooth-type, K1-positive blood culture strains and found that almost all of these mutants were more serum sensitive than the wild-type strains. Changes of serum resistance correlated well with K1 content but not with the few changes of OM protein or lipopolysaccharide patterns observed (14). Until now, there have not been any studies concerning the mechanism of antibiotic action on capsule formation. The few data that are available suggest that antibiotics other than β-lactam antibiotics, like the gyrase inhibitor ciprofloxacin, can exert effects on the bacterial cell envelope that are similar to those of β-lactam antibiotics (3, 13, 27), indicating that a target different from penicillin-binding proteins might be involved in those effects.

It is also unknown whether antibiotics can affect the serum resistance of other bacteria with capsules from polysialic acid (like group B meningococci), bacteria with different capsules, or unencapsulated bacteria.

The difficulty of evaluating the clinical significance of data is common to studies about influences of antibiotics on host-parasite interactions. However, it seems likely that the finding that a commonly used group of antibiotics extensively influences a major virulence factor of gram-negative bacteria is of importance in the therapeutic setting.

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