JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1980, p. 690-694
0095-1137/80/11-0690/05$02.00/0

Vol. 12, No. 5

Aberrant Forms of *Escherichia coli* in Blood Cultures: In Vitro Reproduction of an In Vivo Observation

C. RICHARD MAGNUSSEN[1,2*] AND JEROME F. HRUSKA[1,3]

University of Rochester School of Medicine and Dentistry[1] and Department of Medicine, St. Mary's Hospital,[2] Rochester, New York 14611, and Department of Medicine, Strong Memorial Hospital, Rochester, New York 14642[3]*

Aberrant filamentous forms of *Escherichia coli* were observed on direct Gram stain of blood cultures from a patient being treated with the beta-lactam antibiotic cephalexin. After the institution of an alternative antibiotic regimen which included a different cell wall-active agent, *E. coli* of normal morphology was detected in blood cultures for an additional 48 h. Filamentous forms of *E. coli* could be reproduced reliably in vitro by incubating the organism in Mueller-Hinton broth containing various concentrations of cephalexin. Both supra- and subinhibitory concentrations of cephalexin resulted in filament formation after 4 h of incubation, whereas 24 h of incubation yielded intact filaments at only a narrow range of subinhibitory concentrations of cephalexin. In vitro comparison of the ability of cephalexin, cephalothin, ampicillin, and gentamicin to cause filamentous forms of *E. coli* showed that cephalexin and cephalothin produced pure filament formation after 4 h of incubation at subinhibitory concentrations of as low as one-fourth the minimum inhibitory concentration of the antibiotic. Ampicillin was not associated with pure filament formation at concentrations below the minimum inhibitory concentration, and gentamicin produced no filaments at any concentration. The effect of preincubation of *E. coli* with subinhibitory concentrations of cephalexin on subsequent minimum inhibitory concentrations of ampicillin was examined in an effort to develop an explanation for the persistent sepsis exhibited by the patient. No diminution of the activity of ampicillin by preincubation with cephalexin could be demonstrated. Other possible clinical implications of filamentous forms of gram-negative bacilli are discussed.

Subinhibitory concentrations of antibacterial agents which affect cell wall synthesis can produce a wide variety of aberrant morphological forms of gram-negative bacilli (7). Aberrant forms have been observed in cultures of urine (2), cerebrospinal fluid (11), and blood (9) of patients treated with cell wall-active antibiotics. The clinical significance of the aberrant morphology is unclear (14), but these bacteria have been implicated in persistent infection during antibiotic therapy (10). Cephalexin (Keflex) has exhibited a special predilection to cause filamentous forms of gram-negative bacilli in vitro (5). Detection of aberrant filamentous *Escherichia coli* in blood cultures from a patient receiving cephalexin is described. The unique ability of cephalexin to produce filamentous *E. coli* in vitro is demonstrated and correlated with the patient presentation.

CASE REPORT

A 58-year-old man was admitted to the hospital because of the sudden onset of shaking chills and a temperature of up to 104°F (ca. 40°C). The patient had been treated with cephalexin (Keflex) at a dosage of 250 mg orally four times daily for 5 days before admission for presumed prostatitis. Cephalexin at a dosage of 500 mg orally every 6 h was continued for the first 24 h of hospitalization and then discontinued. Three blood cultures had been obtained in brucella broth with 10% sucrose (Pfizer, Inc., New York, N.Y.) on day 1, and direct Gram stain of two of the blood cultures after 24 h of incubation revealed filamentous organisms suggestive of fungi (Fig. 1). These organisms were subsequently proven to be aberrant morphological forms of *E. coli* by the API system (Analytab Products, Inc., Plainview, N.Y.). On day 2, three new blood cultures were positive for *E. coli* of normal morphology, and therapy was instituted with ampicillin and gentamicin. No clinical improvement occurred initially, and five of seven blood cultures obtained over the next 48 h were positive for *E. coli* of normal morphology. Extensive diagnostic evaluation failed to reveal a source for the persistent *E. coli* sepsis. The minimum inhibitory concentrations (MICs) of the *E. coli* to cephalothin, ampicillin, and gentamicin were 12.5, 1.6, and 0.4 μg/ml, respectively. These MIC values are within the normal range for community-ac-

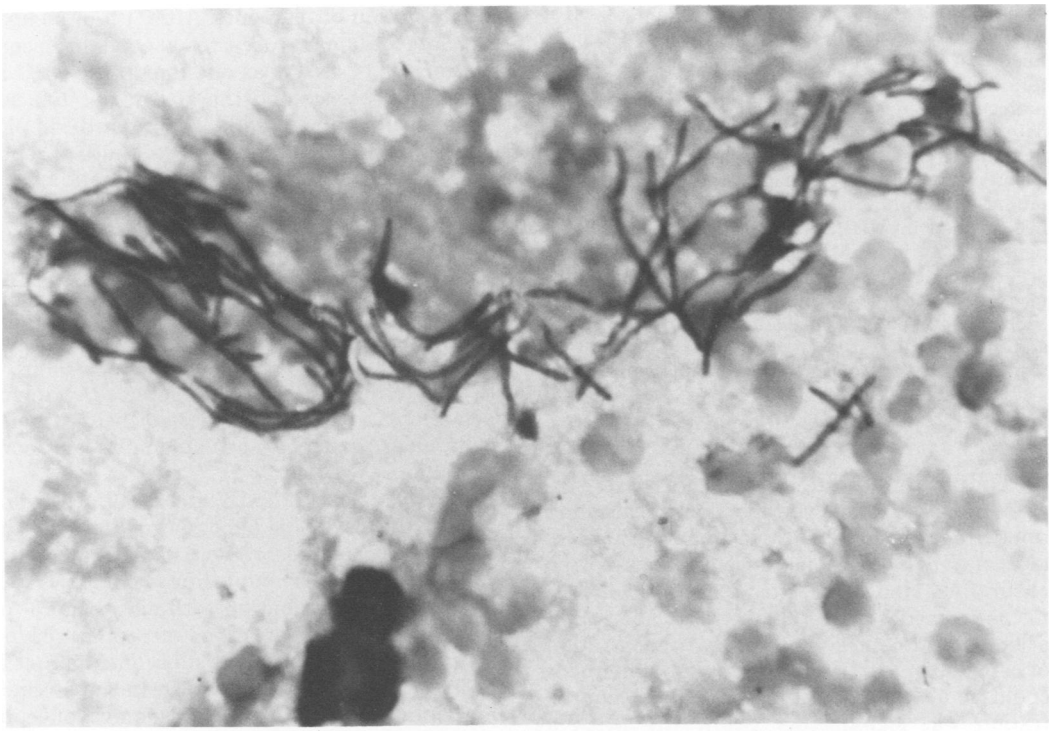

FIG. 1. *Filaments of E. coli in blood culture at 24 h (Gram stain, ×1,600).*

quired blood culture isolates of *E. coli* (12). Gentamicin was discontinued, and the patient gradually defervesced and clinically improved on ampicillin alone over the next week. Six additional blood cultures were negative, and the patient recovered completely.

MATERIALS AND METHODS

Aberrant morphology in vitro. A variation of the microtiter broth dilution method was used to determine the MIC of cephalexin to *E. coli* and to examine the production of aberrant morphology by cephalexin, cephalothin, ampicillin, and gentamicin. The *E. coli* blood isolate from the patient described herein (patient *E. coli*) was tested concurrently with two recent *E. coli* isolates from blood cultures by the hospital microbiology laboratory (control *E. coli* no. 1 and control *E. coli* no. 2). All three organisms had been stored at −70°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing 15% glycerin.

The *E. coli* isolates were initially transferred from the thawed Trypticase soy broth-glycerin to a blood agar plate and grown to confluency at 35°C. Five colonies from the blood agar plate were inoculated into 2 to 3 ml of Trypticase soy broth and incubated overnight at 35°C. Samples of the overnight broth were added to Mueller-Hinton broth (MHB; BBL) to yield 62 ± 2% transmittance at 625 nm in a Bausch & Lomb Spectronic 20 spectrophotometer. The MHB was diluted 1:100 to yield an inoculum of approximately 10^6 colony-forming units per ml. To determine the effect of the presence of 10% sucrose on cephalexin-induced *E. coli* aberrant morphology, an *E. coli* inoculum was similarly prepared in brucella broth with 10% sucrose (Pfizer, Inc.).

Stock solutions of cephalexin, cephalothin, ampicillin, and gentamicin (1 mg/ml) stored at −70°C were thawed and diluted 1:10 in MHB to yield a working solution of 100 μg/ml. A working solution of cephalexin was similarly prepared in brucella broth with 10% sucrose. Serial dilutions of each antibiotic were made in MHB in sterile glass tubes, and serial dilutions of cephalexin were also made in brucella broth with 10% sucrose. A 0.5-ml sample of the *E. coli* inoculum was added to each tube to yield serial twofold concentrations of antibiotic from 0.025 to 50 μg/ml in a total volume of 1.0 ml of broth. The patient *E. coli* only was added to serial dilutions of cephalexin prepared in brucella broth with 10% sucrose. One set of tubes was incubated at 35°C for 4 h, and another set was incubated for 24 h. The MIC was read as the lowest antibiotic concentration revealing no visible growth after 24 h of incubation. At 4 h and 24 h, a 0.01-ml sample was removed from each tube, dried on a clean glass slide, and stained with Gram stain to observe the morphology of the bacteria.

Effect of cephalexin preincubation on ampicillin MIC. The patient *E. coli* (approximately 3×10^6 colony-forming units per ml) was incubated with twofold dilutions of cephalexin from 0.05 to 50 μg/ml in a total volume of 2.0 ml of MHB for 1 h at 35°C. Preincubation was also performed for 4 h at cephalexin

concentrations of 50, 25, 12.5, 6.25, 3.12, and 0 μg/ml. At the end of the 1- and 4-h preincubations, a standard microtiter broth dilution ampicillin MIC was performed for each concentration of cephalexin with the preincubated organisms as the inoculum for the MIC determination. After 1 h of preincubation, ampicillin MICs were performed either in the presence of cephalexin or after the *E. coli* had been centrifuged for 20 min at 2,000 rpm in a centrifuge (Damon/IEC Div.), resuspended in MHB, centrifuged again, and resuspended in 2.0 ml of MHB. No wash followed the 4-h preincubation. Ampicillin MIC's were performed in a microtiter plate (Cooke Engineering Co., Alexandria, Va.) in a total volume of 0.05 ml of MHB in each well and incubated at 35°C for 18 to 24 h. The MIC was read as the lowest concentration of ampicillin with no visible growth.

RESULTS

Production of filamentous morphology in *E. coli*. Table 1 lists the morphological alterations produced in vitro by incubating three isolates of *E. coli* in various concentrations of cephalexin for 4 and 24 h. After 4 h, all three organisms formed striking filamentous aberrations at both supra- and subinhibitory concentrations of cephalexin. After 4 h of incubation, filaments could be observed until the cephalexin concentration was significantly below the MIC, at which point increased numbers of single forms and decreased numbers of filaments were detected. After 24 h of incubation, intact filaments could be observed over only a narrow range of subinhibitory cephalexin concentrations (Table 1). After 24 h, intact filaments were no longer present at suprainhibitory cephalexin concentrations, and no single forms had appeared. The filaments present after 4 h of incubation at subinhibitory cephalexin concentrations were replaced by single forms after 24 h of incubation (Table 1).

Incubation of the patient *E. coli* in broth supplemented with 10% sucrose did not enhance the formation of filaments. After 4 h of incubation, intact filaments occurred at cephalexin concentrations of as low as one-fourth the MIC in both the presence and the absence of 10% sucrose. Incubation for 4 and 24 h in broth supplemented with 10% sucrose in the absence of cephalexin did not result in filament formation.

Ampicillin, cephalothin, and gentamicin were also tested for their ability to cause filamentous morphology in the patient *E. coli* isolate in vitro. Cephalexin and cephalothin induced pure filament formation in the absence of any single forms after 4 h of incubation at subinhibitory concentrations of as low as one-fourth the MIC of the antibiotic, ampicillin induced filament formation above the MIC but not below it, and gentamicin produced no filaments at any concentration.

Effect of cephalexin preincubation on ampicillin MIC. The effect of a 1-h preincubation of the patient *E. coli* with cephalexin on the subsequent MIC of ampicillin is demonstrated in Table 2. Preincubation with either supra- or subinhibitory concentrations of cephalexin did not cause an increase in the subsequent ampicillin MIC over the MIC observed in the absence of a cephalexin preincubation. Removal of cephalexin by an MHB wash before the ampicillin MIC determination had no effect. Also, preincubation with cephalexin for 4 h (data not shown) did not increase the subsequent ampicillin MIC.

DISCUSSION

The ability of subinhibitory levels of beta-lactam antibiotics to produce aberrant bacterial morphology has been known for more than 40 years (4). However, the biochemical events responsible for the production of aberrant morphology have only recently begun to be understood as knowledge of the mechanism of action

TABLE 1. *Effect of various cephalexin concentrations and incubation times on E. coli morphology in vitro*

E. coli blood isolate	Effect[a] at cephalexin concn (μg/ml) of:											
	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0.10	0.05	0.025
4-h incubation												
Patient[b]	F	F	F	F	F	F + S	F + S	S	S	S	S	S
Control no. 1[b]	F	F	F	F + S	F + S	S	S	S	S	S	S	S
Control no. 2[c]	F	F	F	F	F	F + S	S	F + S	S	S	S	S
24-h incubation												
Patient[b]	D	D	D	S	S	S	S	S	S	S	S	S
Control no. 1[b]	D	D	D	D	F + S	S	S	S	S	S	S	S
Control no. 2[c]	D	D	F + D	S + D		S	S	S	S	S	S	S

[a] F, Intact filaments, S, single forms of normal morphology, D, degenerating forms of variable morphology.
[b] Cephalexin MIC, 12.5 μg/ml.
[c] Cephalexin MIC, 25.0 μg/ml.

TABLE 2. *Effect of 1-h cephalexin preincubation on ampicillin MIC*

Preincubation cephalexin concn (μg/ml)[a]	Ampicillin MIC (μg/ml)	
	No wash	Wash
50	0.025	1.56
25	0.025	1.56
12.5	0.10	1.56
6.25	0.20	3.12
3.12	1.56	3.12
1.56	3.12	3.12
0.78	3.12	3.12
0.39	3.12	3.12
0.20	3.12	3.12
0.10	3.12	3.12
0.05	6.25	3.12
0	3.12	3.12

[a] Cephalexin MIC, 25.0 μg/ml.

of beta-lactam antibiotics increases (13). Antibiotic inhibition of bacterial penicillin-sensitive enzymes is considered central to interference with normal cell wall synthesis, and bacteria lysis or survival with aberrant morphology depends on which penicillin-sensitive enzymes are inhibited. Two basic growth processes, elongation and septation, are each subject to inhibition by beta-lactam antibiotics. Presumably, abnormal elongation results when the antibiotic concentration is sufficient to inhibit the enzymes which initiate septation, but not sufficient to inhibit the enzymes involved in elongation (7). This process allows "unrestricted" elongation to occur at subinhibitory antibiotic concentrations (3). Other morphology can occur dependent on which combination of penicillin-sensitive enzymes is affected by the antibiotic (13).

The clinical significance of aberrant morphology remains unclear (14). Spheroplasts and protoplasts have been implicated in occult chronic infectious processes or recrudescence of overt infection when antibiotic therapy is discontinued (10). The clinical significance of filamentous forms is even less clear (6). It has been suggested that filaments may be precursors to spheroplast and protoplast formation, and there is some evidence for a diminished bactericidal effect of serum or blood on filaments (8).

The filamentous forms of gram-negative bacilli which have been observed in urine (2), spinal fluid (11), and blood cultures (9) presumably result from the presence of subinhibitory levels of antibiotic in the particular specimen. This is the first report of cephalexin-induced aberrant bacterial morphology in a clinical specimen, and there has been only one patient previously reported to have filamentous *E. coli* in blood cultures (9). The unique ability of subinhibitory concentrations of cephalexin to produce *E. coli* filaments has been demonstrated in vitro (5), and the patient presented herein indicates that the same phenomenon may occur in clinical settings. Cephalexin probably has a specific affinity for interaction with the penicillin-sensitive enzyme responsible for initiation of septation in *E. coli*, and the observations in this case report may be in vivo confirmation of that characteristic of cephalexin.

The persistence of *E. coli* sepsis demonstrated by the patient after cephalexin was discontinued and adequate doses of ampicillin and gentamicin were begun was perplexing. The organism was sensitive to ampicillin and gentamicin by MIC criteria, and radiological and ultrasound studies indicated no intraabdominal abscess. It was postulated that aberrant *E. coli* morphology produced by exposure to subinhibitory levels of cephalexin before ampicillin therapy interfered with the inhibitory action of ampicillin. Acar et al. have presented evidence for potential antagonism between combinations of beta-lactam antibiotics and specifically demonstrated an eightfold increase in the MIC of carbenicillin for a strain of *E. coli* in the presence of subinhibitory concentrations of cephaloridine (1). However, preincubation of the patient *E. coli* did not result in an increased ampicillin MIC by the methods described herein.

The clinical implications of filamentous gram-negative bacilli remain to be clarified, but microbiology laboratories must be made aware of the potential appearance of such bacilli in clinical specimens to avoid confusion with fungi and other naturally filamentous organisms. Physicians should consider the possibility that filamentous forms may indicate that subtherapeutic concentrations of antibiotic are being achieved at the site of infection (6). Further experience may also indicate a role for aberrant morphology in predisposing to chronic sepsis.

ACKNOWLEDGMENTS

We appreciate the secretarial assistance of Wendy Kurnath.

J.F.H. is an American College of Physicians Research and Teaching Scholar.

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