NOTES

Abnormal Morphology of Bacteria in the Sputa of Patients Treated with Antibiotics

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Filaments of Klebsiella pneumoniae were observed by Gram stain in the sputum of a patient with a respiratory infection who was treated with half the usual dose of cefazolin. Identical filaments were observed in vitro when this strain was incubated with subminimum inhibitory concentrations of cefazolin. Large gram-positive cocci containing multiple cross walls were observed by electron microscopy in the sputum of a patient with a respiratory infection who was treated with ampicillin and gentamicin. Antibiotic administration was suspended the night before the sputum was obtained. The ultrastructure of these cocci was very similar to the ultrastructure of Staphylococcus aureus incubated with subminimum inhibitory concentrations of cephalexin or oxacillin. It was suspected that the low dose of cefazolin and the intermittent therapy with ampicillin resulted in a subminimum inhibitory concentration of antibiotic in the respiratory tract which induced the abnormal morphology of the bacteria observed in the sputum of both patients. The presence of abnormal forms of bacteria in the specimen of a patient, rather than in the culture of a specimen, has clinical significance.

Since the initial observations by Gardner that low concentrations of penicillin change Escherichia coli into filaments (3), it has now been well established that beta-lactam antibiotics which bind predominantly to penicillin-binding protein no. 3 produce filaments in vitro from Enterobacteriaceae (14). Abnormal forms of bacteria have been seen in cultures of specimens such as blood cultures (8, 10) and a pleural effusion (5) which were obtained from patients who were receiving antibiotic therapy. Filaments of E. coli have been seen in the urine of a patient receiving ampicillin (5). Salmonella filaments have been observed in the tissues of a patient who was also treated with ampicillin (5). “Bizarre” forms of E. coli were observed in the spinal fluid of a child who was treated with antibiotics (11). Filaments of Pseudomonas aeruginosa were observed in the sputum as well as in the spinal fluid of a patient who was treated with carbenicillin (12). The presence of abnormal forms of bacteria in the specimen of a patient, rather than in the culture of a specimen, has clinical significance; these abnormal forms have an abnormal susceptibility to defense mechanisms (7, 13) and are indicative of low antibiotic concentrations at the site of infection.

Case reports. An 89-year-old female was admitted for coughing and shortness of breath. She had 19,700 leukocytes with 90% polymorphonuclear neutrophil cells and was afebrile. The day after admission an X ray was taken which showed a density in the right lower lobe, suggesting a pneumonic infiltrate. The patient was started on cefazolin (2 g per day intravenously), and 2 days later a sputum was obtained. The X ray was repeated three times for the next 12 days but showed no change. Cefazolin was then discontinued, and the patient was started on gentamicin and penicillin.

The Gram stain of the sputum showed a moderate number of polymorphonuclear cells, no epithelial cells, rare gram-negative bacilli, and a moderate number of gram-negative filaments 10 to 30 μm long as well as filaments with a central enlargement. Some of the filaments were surrounded by an unstained zone, suggesting a capsule (Fig. 1A and B). The culturing of the sputum on blood and MacConkey agars yielded numerous colonies of Klebsiella pneumoniae and a few colonies of Proteus mirabilis. The minimum inhibitory concentrations of cefazolin were determined by the twofold broth dilution method (4) and were 6 μg/ml for K.
FIG. 1. (A) Sputum with a filament and a few short filaments with central enlargements. (B) Sputum with encapsulated filaments. (C) Filaments of *K. pneumoniae* with enlargements from area containing subminimum inhibitory concentrations of cefazolin in the agar. Gram stain ×2,130.
pneumoniae and 30 μg/ml for P. mirabilis. K. pneumoniae was tested with a disk containing 30 μg of cefazolin. A cover glass was dropped over the edge of the zone of inhibition, which was the area containing subminimum inhibitory concentrations of cefazolin (5). The print of K. pneumoniae showed filaments and filaments with enlargements identical to those observed in the sputum (Fig. 1C).

A 77-year-old male was admitted to the hospital because of acute gastrointestinal symptoms. He developed a spiking fever (101°F [ca 38°C]) and a leukocytosis (14,900 leukocytes) 13 days after admission, and antibiotic therapy consisting of ampicillin (4 g per day intravenously) and gentamicin (240 mg per day intravenously) was prescribed. The patient developed a harsh productive cough with purulent sputum. X-ray studies showed an indistinct bronchial vascular pattern suggestive of congestion without consolidation. The antibiotic administration was suspended the night preceding the collection of the sputum specimen. This sputum was the only specimen obtained from this patient, and it was processed within 1 h from the time it was obtained.

The Gram stain of the sputum showed polymorphonuclear cells and many large gram-positive cocci as well as some cocci of normal size and a few small gram-negative bacilli (Fig. 2A). The sputum was cultured on blood, chocolate, and MacConkey agars. After 24 h of incubation, only a few colonies of P. aeruginosa were isolated. After 48 h of incubation, no additional growth was observed.

The sputum was fixed in 5% cacodylate-buffered glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in a series of graded alcohols, and embedded on Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens electron microscope.

Electron microscopy showed numerous cocci, most of which were oval and much larger than normal (1.8 to 2.6 μm in diameter) with multiple thick cross walls (Fig. 2B). These large cells were surrounded by a peripheral cell wall of normal structure (Fig. 3A). Some normal-size cocci and some lysed organisms were also present. The ultrastructure of Staphylococcus aureus exposed to cephaloridine was similar to the structure of the cocci observed in the sputum (Fig. 3B).

Discussion. The recommended dose per day for cefazolin is 4 g, but patient no. 1 received only one-half of this dose. Considering that tissue concentrations are usually lower than serum concentrations, it is possible that the cefazolin concentration in the lung of this patient who was treated with one-half the normal dose was below 6 μg/ml, which is below the minimum inhibitory concentration of this strain of K. pneumoniae. Subminimum inhibitory concentrations of cefazolin produced in vitro filaments of K. pneumoniae which were identical to those observed in the sputum.

FIG. 2. Sputum with large gram-positive cocci and some normal-size cocci. (A) Gram stain. (B) Electron microscopy showing three large cells with multiple cross walls and three normal cocci (arrows). ×10,050. Bar = 1 μm.
FIG. 3. (A) Gram-positive coccus from the sputum. (B) S. aureus grown for 4 h in the presence of cephaloridine at the concentration equal to one-third the minimum inhibitory concentration of this strain. ×40,000. Bar = 1 μm.
The Gram stain of the sputum from patient no. 2 showed large gram-positive cocci. Because there was no growth of gram-positive cocci, possibly due to the effect of gentamicin, it was not possible to determine their species. The ultrastructure of these gram-positive cocci as observed by electron microscopy was, however, very similar to the structure of *S. aureus* exposed to subminimum inhibitory concentrations of cephaloridine or oxacillin (Fig. 3B) (6). Because the patient did not receive antibiotics the night preceding the sputum collection, it is possible that the abnormal cocci were the result of subminimum inhibitory concentrations of ampicillin in the lung.

Abnormal forms of bacteria have been reported from patients who did not receive antibiotic therapy (2, 9). Antibacterial agents remain, however, a major cause of the abnormal forms of bacteria in specimens or cultures submitted to the clinical laboratories. Such abnormal forms may appear by the Gram stain to be quite different from normal forms, thus making the interpretation of a Gram stain quite difficult. Nevertheless, their presence may indicate a sublethal antibiotic concentration at the site of infection which resulted from a low dose of antibiotic or intermittent, possibly unsuspected, antibiotic therapy.

**LITERATURE CITED**