ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1993, p. 1377–1379
0066-4804/93/061377-03$02.00/0

Vol. 37, No. 6

Elimination of Quinolone Antibiotic Carryover through Use of Antibiotic-Removal Beads

RICHARD A. ZABINSKI,[1] ALISON J. LARSSON,[2] KARLA J. WALKER,[2] SUSAN S. GILLILAND,[2] AND JOHN C. ROTSCHAFER[2]*

Miles Inc., West Haven, Connecticut 06516-4175,[1] and College of Pharmacy, University of Minnesota, and the Antibiotic Pharmacodynamic Modeling Institute, St. Paul-Ramsey Medical Center, St. Paul, Minnesota 55101-2595[2]

Received 24 August 1992/Accepted 15 March 1993

To prove the utility of antibiotic-removal beads in separating antibiotics from bacterial samples, *Escherichia coli* ATCC 25922 was exposed to five separate quinolones before and after each was exposed to antibiotic-removal beads. Plates treated with antibiotic solutions that were exposed to beads demonstrated antibiotic removal, and plates treated with antibiotic solutions that were not exposed to beads demonstrated antibiotic carryover. After exposure to beads, fluoroquinolone concentrations decreased from 5 μg/ml to 0.14 μg/ml (ciprofloxacin), 0.04 μg/ml (temafloxacin), <0.01 μg/ml (ofloxacin), <0.01 μg/ml (sparfloxacin), and 0.02 μg/ml (clinafloxacin). These data indicate that antibiotic carryover can be successfully circumvented through the use of antibiotic-removal beads.

Antibiotic carryover is the compromised ability to accurately quantitate viable bacterial numbers in broth or serum cultures because of the transference or carryover of antibiotic with a bacterial sample (3, 10). Researchers commonly encounter this problem when performing pharmacodynamic studies that involve postantibiotic effect, time-kill kinetics, inoculum effect, protein binding, stationary- versus log-phase killing, and synergism (1, 10).

Experiments designed to evaluate the exposure of members of the family *Enterobacteriaceae* to the fluoroquinolones pose a particular problem, because most *Enterobacteriaceae* are extremely susceptible to this class of antimicrobial agents. Several methods that have attempted to eliminate antibiotics from culture samples have been developed, but until now, a simple, inexpensive, and noncumbersome method has yet to be devised. The purpose of the study described here was to validate the efficacy of antibiotic-removal beads in removing fluoroquinolones from bacterial culture samples.

Antibiotics. Ciprofloxacin hydrochloride was provided by Miles Pharmaceuticals (New Haven, Conn.), temafloxacin hydrochloride was provided by Abbott Pharmaceuticals (Abbott Park, Ill.), ofloxacin was provided by Ortho Pharmaceuticals (Raritan, N.J.), and sparfloxacin (CI-978) and clinafloxacin hydrochloride (CI-960) were provided by Parke-Davis Pharmaceuticals (Ann Arbor, Mich.).

Organism. *Escherichia coli* ATCC 25922 was used as the test organism. *E. coli* was reconstituted from frozen stock (−80°C) and was subcultured twice before use. Standardized cultures were prepared by inoculating a 10-ml volume of cation-supplemented Mueller-Hinton broth with two colonies of *E. coli* that were grown for less than 24 h on a Trypticase soy blood agar plate. This suspension was diluted 1:100 and was grown to a turbidity equivalent to that of a McFarland no. 1 standard. This standardized suspension was diluted fivefold so that a 100-μl inoculum would yield 100 to 200 colonies per plate.

Media. Cation (Mg^{2+}, Ca^{2+})-supplemented Mueller-Hinton broth (Difco, Detroit, Mich.) was used for test cultures, controls, and antibiotic stock solutions; Trypticase soy blood agar plates (Dimed, Roseville, Minn.) were used for colony count determinations.

Antibiotic-removal beads. Polymeric binding resin, Amberlite XAD-4/1090 (Fig. 1), was supplied by Rohm and Haas (Philadelphia, Pa.). Antibiotic-removal beads were divided into 1-g aliquots, poured into Pyrex glass test tubes (16 by 125 mm), and sterilized by autoclaving for 15 min at 121°C.

Antibiotic assay. Concentrations of ciprofloxacin, temafloxacin, ofloxacin, sparfloxacin, and clinafloxacin were quantified by high-pressure liquid chromatography (HPLC) (5). The lower limit of detectability was 0.01 μg/ml. The intraday coefficient of variation was 7.4% over a concentration range of 0.31 to 5.63 μg/ml.

MIC determinations. MICs were determined in cation-supplemented Mueller-Hinton broth according to the guidelines of the National Committee for Clinical Laboratory Standards (8) (by microtiter broth dilution by using a final inoculum of approximately 10^5 bacteria per ml).

Determination of antibiotic carryover. Each set of experiments was performed in triplicate. One set of blood agar plates was plated with 100 μl of one of five fluoroquinolone solutions (concentration, 5 μg/ml). A second set of plates was plated with the fluoroquinolone solutions after 1 ml of the 5-μg/ml solutions was vortexed together with 1 g of antibiotic-removal beads for less than 15 s. One gram of beads was chosen so that 1 ml of fluid would saturate the beads and leave approximately 300 μl for sampling. Antimicrobial agents were allowed to absorb into the agar for 15 min. Each plate in sets one and two was then inoculated with a standardized suspension of *E. coli*. A third set of plates was inoculated with the standardized suspension of *E. coli* after a 1-ml sample was exposed to 1 g of antibiotic-removal beads. This set served as a control which was used to evaluate bacterial adsorption to the beads. A fourth set of plates was inoculated with 100 μl of the broth culture of *E. coli*. This set served as a control which was used to compare colony counts from the other three sets of plates.

Plates were incubated at 37°C for 24 h. Bacterial counts were enumerated for all plates. Differences between colony

* Corresponding author.

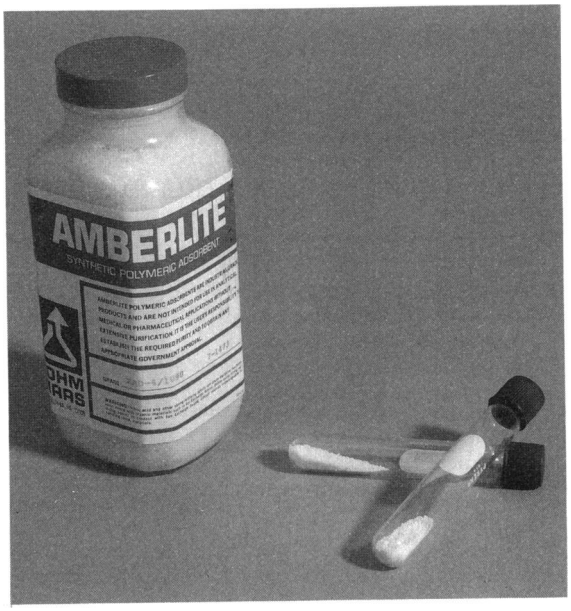

FIG. 1. One-gram aliquots of antibiotic-removal beads (Amberlite XAD-4/1090 resin) in Pyrex glass test tubes.

counts from the growth control, antibiotic-treated and non-bead-exposed, and antibiotic-treated and bead-exposed plates were statistically compared by an independent Student's t test. Statistical significance was defined as a P value of <0.05. Statistical analyses were performed by using the computer program Statistical Package for the Social Sciences (SPSS Inc, Chicago, Ill.).

To further validate antimicrobial removal, bacterial samples containing antibiotic were quantitatively analyzed by HPLC after exposure to antibiotic-removal beads for less than 15 s.

Susceptibility testing. The MICs of ciprofloxacin, temafloxacin, ofloxacin, sparfloxacin, and clinafloxacin for *E. coli* ATCC 25922 were 0.015, 0.03, 0.125, 0.03, and ≤ 0.008 μg/ml, respectively.

Colony count determinations. The mean colony counts of the growth controls which were exposed to antibiotic-removal beads were not significantly different from those of growth controls that were not exposed to beads ($P > 0.05$; 95% confidence interval, −14.8 to 18.8). Means ± 1 standard deviation for growth control plates with and without exposure to antibiotic-removal beads were 69.7 ± 8.1 and 71.7 ± 6.7 colonies per plates, respectively.

Plates treated with antibiotic solutions that were not exposed to beads demonstrated antibiotic carryover. Colony counts for these plates were significantly less than those for growth control plates. However, for plates treated with antibiotic solutions that were exposed to beads, antibiotic removal was demonstrated. Colony counts for these plates were not significantly different from those for growth control plates. Additionally, colony counts for plates treated with antibiotic solutions that were exposed to beads were significantly different from those for plates treated with antibiotic solutions that were not exposed to beads.

Results of colony counts (mean ± 1 standard deviation) from the growth control and antibiotic-exposed plates that were and were not exposed to antibiotic removal beads are summarized in Table 1.

TABLE 1. Colony counts of growth control and antibiotic-exposed plates that were and were not exposed to antibiotic-removal beads

Plate	CFU/plate (mean ± 1 SD) after:		P value
	No exposure to beads	Exposure to beads	
Growth control	71.7 ± 6.7	69.7 ± 8.1	>0.05
Ciprofloxacin	24.7 ± 6.4	62.7 ± 3.8	<0.01
Temafloxacin	45.0 ± 12.5	75.3 ± 6.8	<0.05
Ofloxacin	54.0 ± 3.0	83.0 ± 11.5	<0.05
Sparfloxacin	47.7 ± 6.7	69.0 ± 8.7	<0.05
Clinafloxacin	11.0 ± 6.6	72.7 ± 2.1	<0.01

Antibiotic removal determined by HPLC. Antibiotic concentrations in Mueller-Hinton broth after exposure to antibiotic-removal beads for less than 15 s were 0.14, 0.04, <0.01, <0.01, and 0.02 μg/ml for ciprofloxacin, temafloxacin, ofloxacin, sparfloxacin, and clinafloxacin, respectively. Although the antibiotic-removal beads bound more than 97% of each fluoroquinolone, the data revealed that certain fluoroquinolones were bound more efficiently than others. For example, ciprofloxacin was less efficiently bound by the beads than the four other fluoroquinolones were.

A number of methods have been used to remove antibiotics from bacterial samples (2). Enzymatic inactivation of β-lactam antibiotics is commonly carried out with β-lactamases. This method is both simple and efficient. However, inactivating enzymes are not available for most other antimicrobial classes. Filtration through a 0.25-μm-pore-size filter has been used, but this method is both cumbersome and costly (2, 4, 6, 11). Repeated washing of microorganisms is a method that is commonly used for drug removal. However, washing requires approximately 30 min and therefore does not lend itself for use when rapid sampling is necessary (2). A fourth method, serial dilution, diminishes the sensitivity of the bacterial assay. Although serial dilution is simple and inexpensive, antibiotics as well as bacteria are diluted, thereby compromising the investigator's ability to accurately quantitate bacteria that are present in low numbers.

Antibiotic-removal beads, identical to those used in BACTEC blood culture bottles (1, 7, 9), are a rapid, simple, inexpensive, noncumbersome, and efficacious tool which can be used to eliminate the problem of antibiotic carryover. The method described here can dramatically improve the investigator's ability to quantify bacteria in undiluted samples which contain physiologic concentrations of fluoroquinolones.

Mean colony counts for growth control plates were not significantly different from those for growth control cultures which were exposed to antibiotic-removal beads. Furthermore, colony counts for plates treated with antibiotic solutions that were exposed to beads were significantly different from those for plates treated with antibiotic solutions that were not exposed to the beads. However, HPLC analysis of bead-exposed samples of ciprofloxacin, temafloxacin, and clinafloxacin revealed that inhibitory concentrations of these antibiotics were still present. Antibiotic carryover was, therefore, effectively eliminated, even though the plates were covered with concentrations of antibiotic that were in excess of the MICs for the bacteria. The most plausible explanation for this is that the bead-exposed antibiotic solutions were diluted beyond inhibitory concentrations as they were spread across the surface of the blood agar plates.

These results demonstrate that the fluoroquinolones can be effectively removed from a culture of *E. coli* without affecting bacterial recovery and assay sensitivity. However, the method is not without limitations. First, some quinolones seem to be more efficiently adsorbed by the beads than others, and second, only approximately 300 ml of a 1-ml sample is recovered after exposure to beads. Therefore, the use of this method may not be appropriate in certain situations. In vitro performance studies done with antibiotic-removal beads have demonstrated successful removal of aminoglycosides, penicillins, cephalosporins, macrolides, sulfonamides, glycopeptides (vancomycin), and tetracyclines from cultures of a variety of gram-positive and gram-negative bacterial species (1, 7, 9). Therefore, this method can likely be used to eliminate antibiotic carryover that may occur with many classes of antimicrobial agents.

We are grateful to Charles Stratton, David R. P. Guay, Julia A. Moody, Diann Clarens, Varen K. Herman, David H. Wright, and Frank N. Konstantinides for support in developing the research set forth in this report.

At the time that the study was conducted, R. A. Zabinski was a postdoctoral fellow at the University of Minnesota and the St. Paul-Ramsey Medical Center. This study was supported by research grants from the ASHP Foundation and Abbott Laboratories.

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