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Effectiveness of the Antimicrobial Removal Device, BACTEC 16B Medium, and Thiol Broth in Neutralizing Antibacterial Activities of Imipenem, Norfloxacin, and Related Agents

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The Antimicrobial Removal Device (ARD), BACTEC 16B medium, and Thiol broth were evaluated for their effectiveness in reducing the activity of imipenem (IPM), cefoxitin, moxalactam, and ceftazidime in blood samples. In addition, the capability of the ARD and Thiol broth to bind norfloxacin and the ARD to bind oxolinic and nalidixic acids in urine samples was investigated. At the highest concentrations of the drugs tested (32 μg/ml for the four β-lactams and 256 μg/ml for the three quinolinecarboxylic acids), there was at least a 95% reduction in the in vitro activity of each of the antibacterial agents for treated versus untreated samples. Of the compounds tested in the ARD system, the organic acids were more completely removed than were the β-lactams. The Thiol broth was more effective than the ARD and the BACTEC 16B medium in inactivating imipenem, but it had no effect on the antibacterial activity of norfloxacin.

Proper management of bacteremia is highly dependent upon prompt isolation of the causative organism. Basically, this is also true of infections associated with other body fluids. The empiric initiation of antibiotic therapy before specimen collection is known to prevent or delay the recovery of the offending organism (3). For this reason, there are presently available at least three systems that are capable of inactivating or removing most of the antimicrobial agents currently used in septicemic patients. The Antimicrobial Removal Device (ARD; Marion Scientific, Kansas City, Mo.) and BACTEC 16B medium (Johnston Laboratories, Cockeysville, Md.) are two such systems that utilize resins to bind up the antibiotic (1, 2, 4, 6, 7). Thiol broth (Difco Laboratories, Detroit, Mich.) inactivates a variety of antimicrobial agents by a mechanism that still remains to be defined (5; the promotional literature of the manufacturer). The ARD requires processing of the blood sample before culturing in a regular blood culture medium. No such prior manipulation of the specimen is needed with either Thiol broth or BACTEC 16B medium; both of these are essentially blood culture media which have been supplemented with appropriate chemical inactivators or removers.

Both imipenem (IPM) and norfloxacin are remarkably potent broad spectrum antibacterial agents that will soon become available clinically for a wide range of therapeutic indications (IPM) or for prophylaxis and therapy of urinary tract infections (norfloxacin). The present study was undertaken to determine the effectiveness of Thiol broth, BACTEC 16B medium, and the ARD in neutralizing the antibacterial activities of IPM, norfloxacin, and related drugs contained in blood or urine samples.

MATERIALS AND METHODS

Antimicrobial agents and test devices. Antimicrobial agents were supplied as follows: IPM, norfloxacin, and cefoxitin from Merck & Co., Inc., Rahway, N.J.; moxalactam from Eli Lilly & Co., Indianapolis, Ind.; ceftazidime from Glaxo Group Research, Ltd., Middlesex, England; nalidixic acid from Aldrich Chemical Co., Milwaukee, Wis.; and oxolinic acid from Parke-Davis, Morris Plains, N.J.

The test devices were purchased from their respective manufacturers. The ARD bottles (Marion Scientific) contained a saline suspension of 15 ml of polymeric-adsorbent resin, 10 ml of cationic-exchange resin, and 5 mg of sodium polyanethole sulfonate. BACTEC 16B culture vials (Johnston) contained non-ionic-adsorbing resin (13.3% [wt/vol]), cationic-exchange resin (0.8% [wt/vol]), and sodium polyanethole sulfonate (0.025% [wt/vol]) in 30 ml of tryptic soy broth. Blood culture bottles (Difco) consisted of 50 ml of Thiol broth and sodium polyanethole sulfonate.

Processing of blood samples. Aqueous stock antibiotic solutions were diluted in human whole blood in volumes of 4 ml for testing with BACTEC 16B medium or 6 ml for testing with Thiol broth and ARD. The range of concentrations tested for each antibiotic included the clinically significant plasma levels. The ARD bottles were injected with 5 ml of the antibiotic-containing blood sample and allowed to rotate for 15 min at room temperature. The BACTEC 16B medium vials were injected with 3 ml of the blood sample and agitated at room temperature for 30 min. As for the Thiol broth, each bottle received 5 ml of the blood sample and was mixed briefly before being incubated at 37°C for 60 min. After these various treatments, 1-ml portions of treated and untreated samples were centrifuged, and supernatants were assayed in 20-μl quantities for antibiotic contents by the microbiological disk diffusion method, with 1/4'' (0.635 cm) blank paper disks (Schleicher & Schuell; no. 740E) and *Bacillus subtilis* ATCC 6633 for IPM, *Staphylococcus aureus* ATCC 29608 (MB 2786) for cefoxitin, and *Klebsiella pneumoniae* ATCC 10031 for moxalactam and ceftazidime. Antibiotic concentrations (in micrograms per milliliter) in pre- and posttreatment samples were determined by reference to standard curves.

Removal of antibiotics from urine. Norfloxacin, nalidixic, and oxolinic acids were dissolved in 0.1 N NaOH and diluted serially in 6-ml volumes of human urine to within levels achievable clinically. The urine used was tested for antimicrobial activity before the experiment. Samples (5 ml) of the drug-containing urine test solutions were treated in the ARD for 15 min or the Thiol broth for 60 min. The amount of antibiotic per milliliter of untreated and ARD-treated samples was determined as described previously, using *K. pneumoniae* ATCC 10031 as the assay organism.

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TABLE 1. Removal of antibiotics from blood samples[a]

Antimicrobial agent	Concn attempted	ARD		BACTEC 16B medium		Thiol broth	
		Concn achieved	Concn after ARD	Concn achieved	Concn after BACTEC 16B medium	Concn achieved	Concn after Thiol broth
IPM	4	5.2	<0.6	4.2	<0.2	4.2	<0.3
	8	8.3	0.8	9.5	0.3	8.5	<0.3
	16	16.0	1.1	21.5	0.5	15.0	<0.3
	32	30.8	1.4	37.2	1.9	36.0	<0.3
Cefoxitin	8	8.0	<0.5	4.9	<0.4	9.5	<0.3
	16	16.0	<0.5	14.5	<0.4	22.0	0.4
	32	29.0	<0.5	31.0	<0.4	42.0	0.8
Moxalactam	8	5.0	<0.05	7.5	0.2	11.0	0.4
	16	17.5	0.3	18.5	0.5	15.5	0.5
	32	30.0	1.1	45.0	1.9	40.0	1.4
Ceftazidime	8	10.5	<0.1	5.5	<0.05	10.0	<0.05
	16	22.5	0.2	15.5	<0.05	15.0	0.3
	32	35.0	0.5	32.0	0.05	40.0	0.7

[a] Concentrations are expressed in micrograms per milliliter.

RESULTS AND DISCUSSION

The results presented here (Table 1) show all three antibiotic-removing or -inactivating devices (ARD, BACTEC 16B medium, and Thiol broth) to be capable of causing a significant reduction in the antibacterial activity of each of the four β-lactam agents tested. A simple computation of these data would show reductions of at least 90% in activity at each of several concentrations of the drugs tested.

IPM was more completely inactivated by the Thiol broth (Difco) than by the ARD and BACTEC 16B medium. From an initial blood concentration of ca. 32 μg/ml, the bioassays of IPM show a reduction to less than 0.3 μg/ml (>99.2% reduction), 1.4 μg/ml (95.5% reduction), and 1.9 μg/ml (94.9% reduction) after proper treatment in the Thiol broth, ARD, and BACTEC 16B medium, respectively. In comparison with the ARD and BACTEC 16B medium, the Thiol broth was somewhat less effective in neutralizing the antibacterial activity of cefoxitin. Moxalactam was inactivated or was bound equally well in all three systems, from an initial concentration of ca. 32 μg/ml to 1.1, 1.9, and 1.4 μg/ml for the ARD, BACTEC 16B medium, and Thiol broth, respectively. BACTEC 16B medium was more effective than the other two devices in inactivating ceftazidime in blood specimens as shown by a reduction in the bioassays of this compound from ca. 32 to 0.05 μg/ml for BACTEC 16B medium, 0.5 μg/ml for the ARD, and 0.7 μg/ml for Thiol broth. The efficacy of the resin-containing devices (ARD and BACTEC 16B medium) in removing β-lactam antibiotics from blood samples was at least twofold greater for cefoxitin and ceftazidime than for IPM and moxalactam. The low-binding capability of resins in the ARD for both IPM and moxalactam has been noted by other investigators (S. R. Snavely, W. Brabender, K. Ooe, G. R. Hodges, and W. G. Barnes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C413, p. 167). IPM inhibits most clinically significant bacterial species at concentrations of less than 1 μg/ml. This observation plus the lower binding property of this drug for the resins in the ARD system would suggest that IPM-containing blood samples should be subjected to two ARD treatments before culturing. It should be pointed out, however, that after ARD treatment, samples are routinely diluted 10-fold when subsequently inoculated into regular blood culture media, thus reducing the potential need to reprocess the sample to achieve a subinhibitory level of the drug. For economical considerations, one should be reminded that in addition to the added cost of the routine media, the ARD is considerably more expensive than the Thiol broth and is theoretically more prone to bacterial contamination.

The ARD was evaluated for its ability to reduce to below therapeutic levels the amount of norfloxacin, nalidixic, and oxolinic acids (Table 2) contained in urine samples. At the highest urinary drug concentration tested (ca. 256 μg/ml), the ARD was capable of binding up to 99.9% of norfloxacin and oxolinic acid and at least 98.8% of nalidixic acid initially present in the specimen. These results also seem to suggest that the ARD was more effective in removing the organic acids from urine than the β-lactam antibiotics from blood. In addition to the ARD, the Thiol broth also was examined for its ability to neutralize the antibacterial activity of norfloxacin in urine. After the effect of a 10-fold dilution of the original urine sample into the Thiol broth was accounted for,

TABLE 2. Removal of antibiotics from urine samples by ARD[a]

Concn attempted	Norfloxacin			Nalidixic acid			Oxolinic acid		
	Concn achieved	Concn after ARD	% Reduction	Concn achieved	Concn after ARD	% Reduction	Concn achieved	Concn after ARD	% Reduction
4	4.8	<0.13		3.5	<3.0		5.5	<0.14	
16	12.5	<0.13		12.0	<3.0		16.5	<0.14	
32	22.5	<0.13		25.0	<3.0		30.0	<0.14	
256	300.0	0.15	99.9	240.0	<3.0	>98.8	260.0	<0.15	99.9

[a] Concentrations are expressed in micrograms per milliliter.

the Thiol broth system appeared not to contain ingredients capable of affecting the antibacterial activity of norfloxacin.

It can be concluded on the basis of this investigation that clinically achievable blood levels of IPM, cefoxitin, moxalactam, and ceftazidime can be significantly reduced through the use of the BACTEC 16B medium, the Thiol broth, or the ARD. Furthermore, it has been demonstrated that the ARD can substantially reduce therapeutic levels of norfloxacin, oxolinic, and nalidixic acids in urine specimens, whereas the Thiol broth was found to have no effect on norfloxacin. Except for the obvious greater effectiveness of the Thiol broth over the ARD and BACTEC 16B medium in inactivating IPM (Table 1), the observed differences among the three devices in neutralizing the in vitro activity of the other β-lactam antibiotics tested may be too small to be statistically significant.

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