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Stenotrophomonas maltophilia: Emergence of Multidrug-Resistant Strains during Therapy and in an In Vitro Pharmacodynamic Chamber Model

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Emergence of *Stenotrophomonas maltophilia* as a nosocomial pathogen is becoming increasingly apparent. Pleiotropic resistance characterizes *S. maltophilia*. Furthermore, a slow growth rate and an increased mutation rate generate discordance between in vitro susceptibility testing and clinical outcome. Despite original susceptibility, drug-resistant strains of *S. maltophilia* are often recovered from patients receiving β-lactams, quinolones, or aminoglycosides. Given the disparity among various in vitro susceptibility methods, this study incorporated a unique pharmacodynamic model to more accurately characterize the bacterial time-kill curves and mutation rates of four clinical isolates of *S. maltophilia* following exposure to simulated multidose regimens of ceftazidime, ciprofloxacin, gentamicin, and ticarcillin-clavulanate. Time-kill data demonstrated regrowth of *S. maltophilia* with all four agents. With the exception of ticarcillin-clavulanate, viable bacterial counts at the end of 24 h exceeded the starting inoculum. Ciprofloxacin only reduced bacterial counts by less than 1.0 log prior to rapid bacterial regrowth. Resistant mutant strains, identical to their parent strain by pulsed-field gel electrophoresis, were observed following exposure to each class of antibiotic. Mutant strains also had distinct susceptibility patterns. These data are consistent with previous reports which suggest that *S. maltophilia*, despite susceptibility data that imply that the organism is sensitive, develops multiple forms of resistance quickly and against several classes of antimicrobial agents. Standard in vitro susceptibility methods are not completely reliable for detecting resistant *S. maltophilia* strains; and therefore, interpretation of these results should be done with caution. In vivo studies are needed to determine optimal therapy against *S. maltophilia* infections.

In recent years, a significant increase in the incidence of *Stenotrophomonas maltophilia* has occurred, particularly in immunocompromised individuals (6, 11, 16, 18, 30). In addition to antimicrobial pressure, long-term hospitalization and catheterization are contributory factors to the increased *S. maltophilia* isolation rate evident throughout the country (6, 7, 30). Often, recovery of *S. maltophilia* tends to be associated with colonization rather than true clinical infection (12, 29, 30), yet patients colonized with *S. maltophilia* are treated with antibiotics over extended periods, creating an opportunity for accelerated resistance development. With the exception of trimethoprim-sulfamethoxazole, many posttherapy isolates of *S. maltophilia* become resistant to therapeutic agents initially tested as susceptible by disk or microdilution methods (unpublished epidemiology records).

After rigorous investigation, standards for in vitro susceptibility methods have been published by the National Committee for Clinical Laboratory Standards for rapidly growing organisms or fastidious organisms (19, 20). Criteria for testing strains of *S. maltophilia* have yet to be established. Subsequently, the clinical literature on susceptibility testing of *S. maltophilia* contains conflicting and confusing data (6, 7, 11, 16, 21, 22, 24, 28). Moreover, there is wide divergence between susceptibility reporting and clinical outcome (6, 7, 10). In particular, wide variability in results has been observed when disk diffusion zones were read at 24 versus 48 h (3, 21, 22).

Since 1989, we have accumulated several hundred *S. maltophilia* isolates. Fifty-seven strains were selected for susceptibility interpretation following extended incubation. A collection of these strains was recently used for a comparative study to evaluate various in vitro susceptibility testing methods (3).

In this paper, an in vitro pharmacodynamic modeling apparatus (PDM) was used to characterize the antimicrobial activities of four antibiotics against four clinical isolates of *S. maltophilia*.

(This work was presented in abstract form at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy [1].)

MATERIALS AND METHODS

In vitro pharmacodynamic model. The in vitro PDM used in this study has been previously described (8). In brief, the model consisted of a single-chamber, 300-ml vessel with individual ports for medium inflow, outflow, and sampling. On the day of each experiment, three to five isolated colonies from a 24-h subculture were placed in warmed medium and incubated for approximately 4 h at 35°C to

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† Prior to the publication of this article, Douglas M. Campbell died of complications associated with an untreatable malignancy. Doug participated in the preparation of the manuscript until he was no longer physically able. His courage, dedication, and unfailing cheerfulness recount for us our good fortune to have counted him as a collaborator and friend.

TABLE 1. Clinical characteristics of patients with sequential *S. maltophilia* infections

Patient	Clinical diagnosis	Culture source	Isolate designation	Antimicrobial therapy
A	Bone marrow transplantation	Central catheter tip	A	Ceftazidime
		Blood	AA	
H	Raynaud's disease	Soft tissue infection	H	Ceftazidime
		Soft tissue infection	H2	
W	Gunshot wound	Wound	W	Ceftazidime and ciprofloxacin
			WW	Trimethoprim-sulfamethoxazole
B	Klatskin tumor	Blood and perihepatic abscess	B	Imipenem, trimethoprim-sulfamethoxazole

a McFarland 1.0 turbidity standard. A 1:40 dilution of these exponentially growing bacteria was inoculated into the PDM to produce a starting inoculum between 10^6 and 10^7 CFU/ml. An appropriate volume of antibiotic stock solution was bolus injected into the model to produce the desired initial antibiotic concentration (C_{max}) based on the dosage regimen being simulated. The desired half-life ($t_{1/2}$) of each antibiotic was achieved with a peristaltic pump (Masterflex; Cole-Parmer, Chicago, Ill.). The pump continually delivered drug-free medium into the model, which displaced an equal volume of medium containing drug out of the PDM. The pump flow rate was set to mimic elimination characteristics of each drug in humans. Prior studies have validated the ability of the model to accurately simulate first-order elimination of antibiotic over time (8). A water bath maintained PDM temperature at 35°C, and medium was mixed continuously via magnetic stirring bars. All experiments were conducted in duplicate over 24 h to characterize bacterial time-kill curves and mutation rates associated with each drug. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was used as nutrient medium.

Antimicrobial agents. Representatives from four antimicrobial classes were selected to evaluate the antimicrobial activity against various clinical isolates of *S. maltophilia*. Antibiotic compounds were provided by their respective manufacturers. Ceftazidime was provided by Glaxo Group Research Limited, Greenford, Middlesex, United Kingdom; ciprofloxacin was provided by Miles, Inc., West Haven, Conn.; gentamicin was provided by SoloPak Laboratories, Inc., Elk Grove Village, Ill.; and ticarcillin-clavulanate was provided by SmithKline Beecham Laboratories, Philadelphia, Pa. To simulate drug disposition characteristics associated with commonly used dosing regimens of the four antibiotics, C_{max} values of 70, 2.5, 7, and 325:8 μg/ml and drug elimination half-life ($t_{1/2}$) values of 2, 3, 4, and 1.5 h were used for ceftazidime, ciprofloxacin, gentamicin, and ticarcillin-clavulanate, respectively. Fresh stock solutions of ceftazidime (4 mg/ml), ciprofloxacin (1 mg/ml), and ticarcillin-clavulanate (20 mg of ticarcillin/0.5 mg of clavulanate) were prepared, separated into single-use aliquots, and frozen at −70°C. On the day of each experiment, aliquots were thawed at room temperature and thoroughly mixed prior to use. Gentamicin was provided from a single-dose, injectable preparation (10 mg/ml). Experiments were performed over a 24-h period with intermittent bolus injections administered at 8 h (ceftazidime), 12 h (ciprofloxacin and gentamicin), and 6 h (ticarcillin-clavulanate). In addition, single-dose ceftazidime (70 μg/ml) experiments were conducted.

Clinical isolates and patient information. *S. maltophilia* isolates were biochemically identified by API 20E Systems strips (BioMerieux Vitek, Inc., Hazelwood, Mo.) and additional biochemicals per the manufacturer's recommendation (2). Clinical isolates, obtained from four different patients, were used in all experiments (Table 1). Strain A was isolated from a transplant recipient receiving imipenem prophylaxis; a sequential isolate (AA) was grown from a blood culture following 6 weeks of ceftazidime therapy. Strain H was isolated from an autoimmune disease patient with chronic polymicrobial soft tissue infections; after 3 weeks of ceftazidime therapy, a sequential wound isolate (H2) was grown as part of the polymicrobic flora. Strain W, grown from the wound of patient W, was initially treated with ceftazidime and ciprofloxacin; a ciprofloxacin-resistant sequential strain (WW) was isolated 6 days post-initiation of therapy, and trimethoprim-sulfamethoxazole was added. Strain B was isolated from a septic immunosuppressed patient while on empiric imipenem therapy; therapy was modified to vancomycin and trimethoprim-sulfamethoxazole postculture. Various aspects of the clinical and PDM mutant strains are outlined in Tables 1 and 2.

In addition, seven isolates, which were a subpopulation of *S. maltophilia* described in a prior study (22) and further characterized by our laboratory (unpublished data), were exposed to therapeutic concentrations of ticarcillin-clavulanate in the PDM.

In vitro susceptibility testing. Prior to conducting the PDM experiments, all patient isolates were tested by established disk diffusion guidelines for aerobic gram-negative bacilli (19). Antibiotics used for patient therapy, as well as additional agents selected on the basis of disk diffusion results, were subsequently tested in the PDM. Retrospectively, all mutant strains from patients and the PDM were tested by microdilution MIC, E test, and disk diffusion as previously described (3).

Quantification of viable bacteria. To quantitate bacteria over time, 150-μl

TABLE 2. Characteristics of *S. maltophilia* clinical and PDM strains following exposure to various antimicrobial agents

Drug	Strain designation	Source of isolation	Molecular typing	Antibiotic susceptibility		
				Microdilution MIC (μg/ml)	E-test MIC (μg/ml)	Disk diffusion (mm)
Ceftazidime	A	Blood[a]	D	32	24	26
	Am1	PDM	D	64	64	19
	Am2	PDM	D	64	≥256	10
	Am3	PDM	D	512	≥256	No zone
	AA	Blood	D	64	≥256	No zone
	H	Wound	B	4	2	29
	Hm1	PDM	B	32	32	17
	H2	Wound[b]	C	512	≥256	No zone
Gentamicin	H	Wound	B	1	1	25
	Hm2	PDM	B	4	6	17
	Hm4	PDM	B	≥32	48	No zone
Ciprofloxacin	W	Wound	A	8	3	22
	Wm2	PDM	A	16	8	15
	Wm1	PDM	A	≥128	≥32	No zone
	WW	Wound	A	≥128	≥32	No zone
Ticarcillin-clavulanate	B	Blood[c]	G	64/2	24/2	29
	Bm3	PDM	G	64/2	64/2	19
	Bm4	PDM	G	≥512/2	≥256/2	No zone

[a] Isolated from an aseptically removed catheter tip.
[b] Nonrelated strain obtained from patient H following ceftazidime therapy.
[c] Identical isolates obtained from two blood cultures and liver abscess.

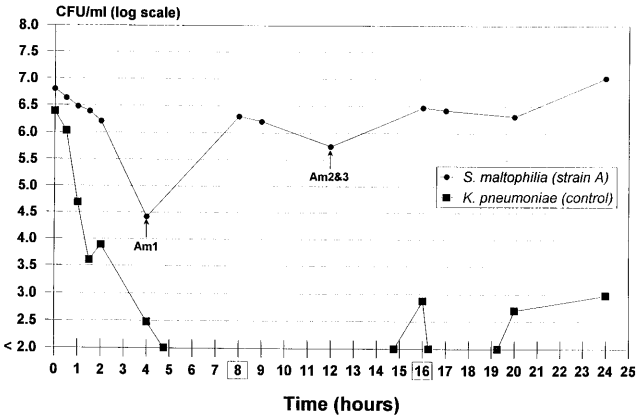

FIG. 1. Average bacterial time-kill curves for *S. maltophilia* A and *K. pneumoniae* control, after exposure to ceftazidime (70 μg/ml) administered every 8 h. Dosage administration is indicated by a square on the *x* axis, and the appearances of specific mutants are indicated by arrows.

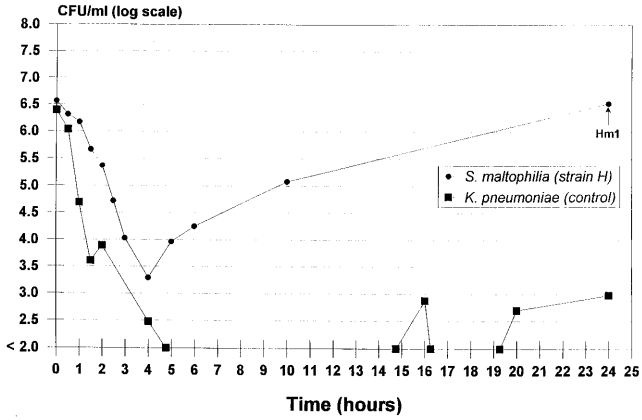

FIG. 2. Average bacterial time-kill curves for *S. maltophilia* H, after exposure to a single dose of ceftazidime (70 μg/ml), and *K. pneumoniae* control, after exposure to ceftazidime (70 μg/ml) administered every 8 h. The appearance of a specific mutant is indicated by an arrow.

samples were aseptically taken initially and at selected intervals throughout the 24-h experiments. Viable bacteria were determined by serially diluting each sample and immediately plating 100-μl aliquots of each dilution onto blood agar plates (tryptic soy agar with 5% sheep blood; PML, Tualitin, Oreg.). Plates were incubated 24 to 48 h at 35°C. Viable bacteria (CFU per milliliter) were logarithmically plotted over time for each experiment to determine the bacterial time-kill curve.

Isolation of in vitro resistant mutants. Blood agar plates supplemented with low and high antibiotic concentrations were used to isolate resistant mutants. Plates were prepared by evenly flooding the agar surface with concentrated antibiotic solution to provide the approximate final antimicrobial levels: (i) ceftazidime, 8 and 200 μg/ml; (ii) ciprofloxacin, 4 and 64 μg/ml; (iii) gentamicin, 1 and 64 μg/ml; and (iv) ticarcillin-clavulanate, 8:0.2 and 200:5 μg/ml. A 100-μl sample, diluted 1:10, was placed onto the low- and high-antibiotic-supplemented plates and incubated at 35°C for 48 h. Colonies growing on the plates were tested by disk diffusion, microdilution, and E test (3, 20).

Molecular typing. Molecular typing of all isolates was performed by pulsed-field gel electrophoresis as described by Pfaller and colleagues (23). Whole chromosomal DNA in agarose was digested by *Spe*I and *Xba*I, and the restriction fragments were separated in a contour-clamped homogeneous electric field DRII apparatus (Bio-Rad, Richmond, Calif.). After electrophoresis, gels were stained with ethidium bromide, illuminated under UV light, and photographed. Two observers, blinded to the results of one another, examined the photographs of the ethidium bromide-stained gels to detect similarities and differences in banding patterns. All bands had to match exactly to classify isolates as identical. Banding patterns with ≥90% but <100% of the bands matching were considered similar and designated as subtypes. Generally, this meant that patterns differed by one to three bands. Isolates with <90% of bands matching (>3 bands different) were considered different pulsed-field gel electrophoresis types. Final molecular typing was determined based upon the results obtained with both restriction enzymes. Arbitrary letter designations were used to describe strain identity.

Control experiments. As a mutant control and to demonstrate typical bacterial time-kill kinetics, an isolate of *Klebsiella pneumoniae* was subjected to similar concentrations of ceftazidime, ciprofloxacin, and ticarcillin-clavulanate in the PDM.

Antimicrobial assays. Declining antibiotic concentrations were verified by various assay methods. Samples containing ceftazidime or ticarcillin-clavulanate were measured by a *Micrococcus luteus* bioassay (4). Ciprofloxacin concentrations were determined via high-pressure liquid chromatography (9), and gentamicin concentrations were measured by a polarized immunofluorescence assay (TDx; Abbott Laboratories).

RESULTS

The pharmacokinetics (C_{max} and $t_{1/2}$) of each agent fell within an acceptable therapeutic range for each PDM experiment (data not shown). Table 2 contains susceptibility data for the four parent clinical *S. maltophilia* isolates and their respective mutant strains; all parent isolates were susceptible by disk diffusion and usually one or more of the other in vitro methods. As determined by molecular typing (Table 2), all mutant strains obtained from the PDM had a molecular type identical to their cognate parent strains.

Bacterial time-kill curves associated with the four initially susceptible parent isolates are shown in Fig. 1 to 5. These curves represent an average of two separate experiments. On average, variation in the datum points from the duplicate experiments did not exceed 3%. Actual starting inoculum averaged 4.0×10^6 CFU/ml (range, 1.7×10^6 to 6.3×10^6 CFU/ml). The *K. pneumoniae* control strain demonstrated typical kill-curve responses (Fig. 1, 2, 4, and 5), and no resistant mutants were isolated.

In all experiments, except the ticarcillin-clavulanate studies (Fig. 5), viable cell counts at the end of 24 h equaled (Fig. 2) or exceeded (Fig. 1, 3, and 4) the starting inoculum. Ciprofloxacin was the only agent unable to initiate a 2-log kill (Fig. 4). Bacterial time-kill curves for all strains of *S. maltophilia* subjected to ticarcillin-clavulanate (including the seven additional isolates evaluated) demonstrated similar kill patterns: i.e., following a pronounced initial reduction, bacterial regrowth was evident but cell counts never exceeded 10^6 CFU/ml (only data for strain B shown).

When subjected to interval ceftazidime dosing in the PDM,

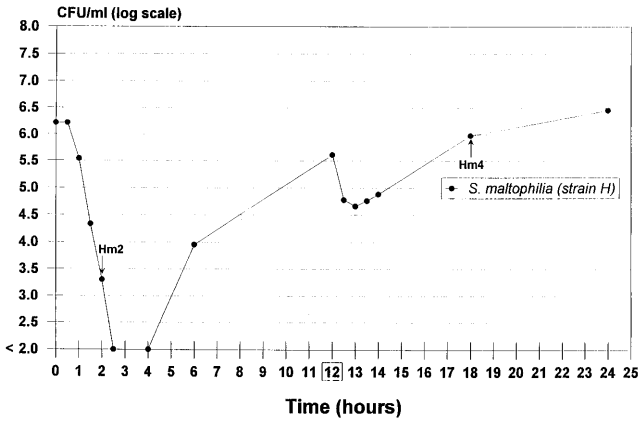

FIG. 3. Average bacterial time-kill curves for *S. maltophilia* H, after exposure to gentamicin (7.0 μg/ml) administered every 12 h. Dose administration is indicated by a square on the *x* axis, and the appearances of specific mutants are indicated by arrows.

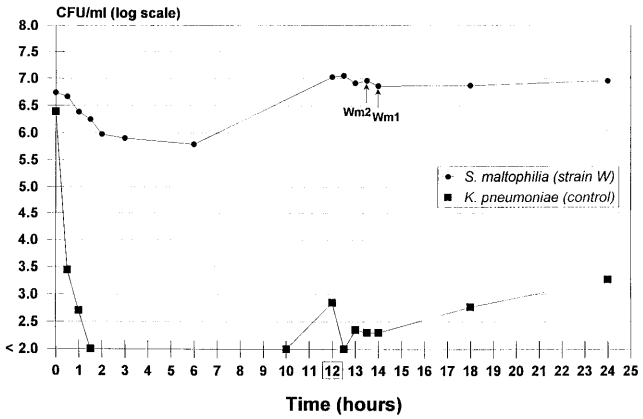

FIG. 4. Average bacterial time-kill curves for *S. maltophilia* W and *K. pneumoniae* control, after exposure to ciprofloxacin (2.5 μg/ml) administered every 12 h. Dose administration is indicated by a square on the *x* axis, and the appearances of specific mutants are indicated by arrows.

parent strain A, initially susceptible by disk diffusion, yielded three resistance phenotype mutants: Am1 was obtained after the initial dose and Am2 and Am3 were obtained after the second infusion of ceftazidime (Table 2; Fig. 1). Also from patient A, clinical isolate AA was obtained from a blood culture after 6 weeks of ceftazidime therapy; this sequential clinical isolate (AA) was ceftazidime resistant.

Based on the three independent susceptibility methods, each mutant isolate within each drug class revealed a distinct susceptibility phenotype and molecular typing identical to each parent strain (Table 2). From patient H, the parent strain (H) was exposed to a single bolus of ceftazidime (70 μg/ml), and a single mutant phenotype (Hm1) was isolated (Table 2; Fig. 2). A ceftazidime-resistant clinical isolate unrelated to the original strain by pulsed-field gel electrophoresis analysis (H2) was isolated from the patient following 3 weeks of ceftazidime therapy (Table 2).

A similar selection of resistant mutants was observed with the three parent strains H, W, and B subjected to gentamicin, ciprofloxacin, and ticarcillin-clavulanate, respectively, in the PDM. For each agent, intermediate- and high-level-resistance phenotype strains were found during the experiments (Table 2 and Fig. 3 to 5). Strain WW (ciprofloxacin resistant, trimethoprim-sulfamethoxazole susceptible) was isolated from patient W following a 9-day course of ciprofloxacin and ceftazidime therapy; molecular typing of this clinical isolate was identical to parent strain W (Table 2). Of the seven additional *S. maltophilia* strains subjected to ticarcillin-clavulanate in the PDM, five produced mutants with variable resistance phenotypes comparable to those derived from experiments involving strain B. Molecular typing was not performed for the additional *S. maltophilia* isolates or the derived mutants. In vitro testing of the seven original isolates revealed MICs (E test) of ticarcillin-clavulanate ranging from 3 to ≥256 μg/ml.

Resistant clinical or PDM mutants tested by disk diffusion were characterized as follows. Mutant strains selected in the PDM with an intermediate phenotype (Table 2) had reduced zone diameters for all bactericidal agents as well as the specific antibiotic used in the model experiments; trimethoprim-sulfamethoxazole and doxycycline were typically unaffected. The term "intermediate" is used to differentiate these strains from the National Committee for Clinical Laboratory Standards designation of indeterminate. That is, the isolates' phenotypic expression revealed zone diameters (or MICs) between the parent isolates' original zone diameter and "no zone." This term does not imply resistance or susceptibility. Clinical or model mutants expressing high-level resistance (Table 2) revealed reduced diameters or no zones only with the specific class of agent used for therapy or the PDM experiments; e.g., mutant strains arising after exposure to ceftazidime or ticarcillin-clavulanate were resistant only to other β-lactam agents.

DISCUSSION

Several studies have demonstrated variable susceptibility of *S. maltophilia* isolates and the unpredictability of in vitro susceptibility tests, including agar dilution as the "gold standard" method (6, 7, 11, 16, 21, 22, 24, 28). Moreover, investigation has demonstrated that prolonged incubation during disk diffusion testing enhances detection of in vitro resistance (3, 21, 22). Our findings support the prior reports of susceptibility inconsistencies and provide a partial explanation for the variable and improved predictability when extended incubation (48 h) is used for disk diffusion testing. In a separate *S. maltophilia* study (3), we noted the appearance of multiple inner zone colonies (often exceeding 50 colonies) at 48 h which were not apparent at 24 h. Congruent with our PDM data, the inner colonies most likely reflect a high rate of de novo resistance development analogous to that described for type I β-lactamase producers (27). However, in contrast to the inducible to constitutive synthesis mutations typified by type I organisms, *S. maltophilia* isolates develop resistance to multiple classes of antibiotics (i.e., β-lactams, aminoglycosides, and quinolones) and express phenotypic diversity. The resistant mutants identified in our study were either pleiotropic or agent and class specific. Although we did not identify specific mechanisms of resistance, disk diffusion data obtained from resistant mutants provide inferential evidence of resistance classification. Strains with intermediate-resistance phenotypes (Am1, Hm1, Hm2, Wm2, and Bm3) had reduced zone diameters for all bactericidal agents as well as the specific antibiotics used in the model; this characteristic suggests diminished permeability. Expression of high-level resistance (Am2, Am3, AA, Hm4, Wm1, WW, and Bm4) corresponded only to resistance to agents of the same class as the therapeutic or model agents. Mutation to constitutive synthesis of antimicrobial agent-inactivating enzymes (β-lactams and aminoglycosides), alterations in target sites (quinolones), or efflux may characterize these high-level-

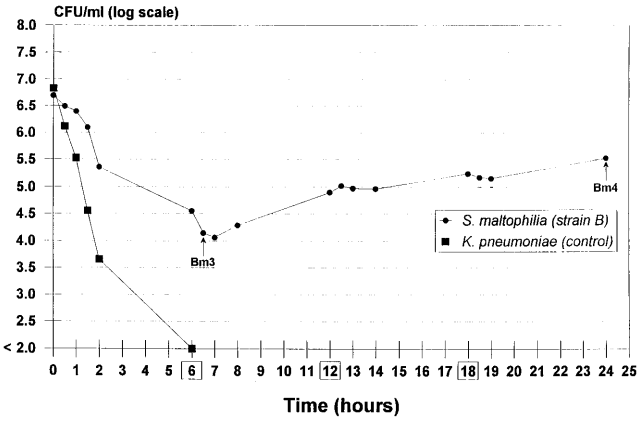

FIG. 5. Average bacterial time-kill curves for *S. maltophilia* B and *K. pneumoniae* control, after exposure to ticarcillin-clavulanate (325:8 μg/ml) administered every 6 h. Dose administration is indicated by a square on the *x* axis, and the appearances of specific mutants are indicated by arrows.

resistant mutants. Thus, the slow growth rate of *S. maltophilia*, accompanied by increased mutation rates to multiple agents, and perhaps other unidentified factors, contributes to the unique resistance and unreliability of in vitro methods in detection of susceptibility.

With all agents evaluated in our study, growth patterns of *S. maltophilia* in the PDM contrasted significantly with typical time-kill curves involving *K. pneumoniae*. With the exception of ciprofloxacin, all agents produced at least an initial 2-log growth decline in *S. maltophilia*. However, bacterial regrowth occurred rapidly and exceeded starting inoculum within 24 h. In contrast to experiments with other antimicrobial agents, regrowth of *S. maltophilia* observed with ticarcillin-clavulanate never exceeded the starting inoculum. Our observations are consistent with another report which demonstrated bacterial regrowth in time-kill experiments involving ticarcillin-clavulanate and *S. maltophilia* (22).

Derived essentially from in vitro analyses, ticarcillin-clavulanate is reported to be one of the most active agents against *S. maltophilia* (22, 24, 28). However, the intermediate growth suppression followed by regrowth in the model (Fig. 5) raises a potential concern as to the therapeutic efficacy of this inhibitor-antibiotic compound against *S. maltophilia*. Moreover, the appearance of both early and late mutant phenotypes from strain B and five of the seven additional isolates verifies the genetic instability of *S. maltophilia* with respect to β-lactam antibiotics. Two additional observations made during the ticarcillin-clavulanate model experiments are noteworthy. The two isolates which did not yield resistant mutants generated time-kill curves similar to those in all of the other ticarcillin-clavulanate studies (i.e., initial suppression followed by regrowth). Secondly, three isolates for which MICs were ≥256 μg/ml generated identical suppression-regrowth profiles. Inconsistent in vitro activity of ticarcillin-clavulanate has been described for *Pseudomonas aeruginosa* (15) and members of the family *Enterobacteriaceae* (26). The combination of ticarcillin and clavulanic acid appears to possess, at least with certain classes of organisms, an unidentified synergism which may lead to erroneous conclusions regarding susceptibility. Our study does not exclude the potential clinical utility of ticarcillin-clavulanate in *S. maltophilia* infections, but it does suggest the strong need for additional controlled studies, preferably in an animal model.

Therapeutic use of quinolones (25) for *S. maltophilia* on the basis of in vitro analysis is debatable. One study involving ciprofloxacin proposed that in vitro testing had unacceptable major or very major error rates with all methods except agar dilution (21). More significantly, another investigation detailed the isolation of mutant strains expressing multiple resistance phenotypes at high frequency following exposure of susceptible isolates to nalidixic acid and fluoroquinolones (13); at least some of the resistant strains were attributed to changes in outer membrane proteins as had been previously described (5, 17). In our PDM experiments evaluating ciprofloxacin, two mutant phenotypes were identified (Table 2). In a separate study involving a levofloxacin-susceptible strain of *S. maltophilia* (MIC of 0.5 μg/ml and zone diameters of 26 mm), similar results were observed following exposure to levofloxacin in the PDM; an initial decline in growth followed by regrowth and the appearance of resistant mutants (unpublished data).

Trimethoprim-sulfamethoxazole has been advocated as an effective agent for use in patients with *S. maltophilia* infection. We did not test trimethoprim-sulfamethoxazole in the model because the agent is bacteriostatic with most isolates (28), and clinical experience indicates that the organism rarely undergoes resistance development during therapy. Furthermore, the technical aspects associated with accurately simulating the pharmacokinetic profile of this two-drug combination are complex; trimethoprim-sulfamethoxazole evaluations in this type of modeling apparatus have not been reported. Also, the therapeutic effectiveness of trimethoprim-sulfamethoxazole is based primarily on in vitro activity and anecdotal clinical reports (10–12, 24, 28). Patients receiving prophylaxis with trimethoprim-sulfamethoxazole have become culture positive for *S. maltophilia* (6, 11, 14), and in vitro data indicate that many isolates have inhibitory concentration clustering near the resistance breakpoints (28). In addition, both patients W and B in our study had persistent *S. maltophilia* despite long-term trimethoprim-sulfamethoxazole therapy and continued in vitro susceptibility.

Therapy for *S. maltophilia* is largely empiric; however, the need for effective therapy in significantly immunocompromised patients is critical. Given the rapid emergence of resistant *S. maltophilia* strains to all classes of bactericidal agents, it is unlikely that single-drug therapy will be effective in managing neutropenic patients with severe *S. maltophilia* infections. Present susceptibility methods may be able to detect high-level resistance but are inadequate in predicting true susceptibility and ultimately therapeutic efficacy. Until this information is available, in vitro susceptibility data for *S. maltophilia* should be cautiously interpreted. We agree with Pankuch and colleagues (21, 22) that all susceptibility testing be considered with skepticism, and there is in fact no gold standard method currently available for testing *S. maltophilia*. In vitro testing will not resolve the issue of clinical adequacy in immunocompromised patients, and an in vivo model to evaluate combination therapies will greatly assist in evaluating promising antimicrobial agents.

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