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Brief reports

Heat stability of the antimicrobial activity of sixty-two antibacterial agents

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Sixty-two antimicrobial agents, including several combinations, were examined for stability at 56°C for 30 min and 121°C for 15 min, respectively. A microtiter broth dilution MIC test and an agar disk diffusion test served to test each chemo-agent for residual antimicrobial activity. Eleven drugs were partially heat-labile (MICs raised four- to eight-fold after autoclaving) and 26 drugs were heat-labile (MICs raised $\geqslant$ 16-fold following autoclaving); the remainder proved heat-stable (MICs raised $\leqslant$ two-fold after autoclaving). Surprisingly, the β-lactams, azlocillin, aztreonam, mezlocillin, and oxacillin, were remarkably heat-stable.

Introduction

In a recent study sera from patients in early convalescence from systemic *Acinetobacter baumannii* infection were tested for opsonizing antibodies against homologous isolates (Traub, Leonhard & Bauer, 1994). Most sera contained antibacterials as determined by a simple agar diffusion method (Traub & Leonhard, 1992) and conventional heat-inactivation at 56°C for 30 min failed to inactivate the antibacterial activity in the majority of cases. Therefore, a systematic study was undertaken to determine the heat-lability as opposed to heat-stability of a large number of antimicrobials.

Materials and methods

Bacterial strains

The following strains were employed: *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Bacillus subtilis* ATCC 6633.

Media

Mueller-Hinton agar (MHA) and broth (MHB), brain heart infusion broth (BHIB), and Tryptic Soy agar (TSA) were purchased from Difco Laboratories (Detroit, MI). The bacterial strains were maintained on TSA slants at 4°C (bimonthly transfers) and, for backup purposes, in a mixture of 1 volume of BHIB plus 1 volume of bovine serum without additives (Behringwerke AG, Marburg, Germany) at −65°C.

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Table I. MICs of antibacterials after heat-treatment at 50°C for 30 min or 121°C for 15 min

Antimicrobial	*S. aureus* ATCC 25923 MIC			*S. aureus* ATCC 29213 MIC			*E. coli* ATC 25922 MIC			*E. coli* ATCC 35218 MIC			*B. subtilis* ATCC 6633 MIC			*B. subtilis* ATCC 6633 ϕ inhibition zones (mm)		
	RT	56	121	RT	56	121	RT	56	121	RT	56	121	RT	56	121	RT	56	121
Amikacin	0·5	0·5	0·5	2	4	2	2	4	2	1	1	1	⩽0·125	⩽0·125	⩽0·125	21	21	21
Amoxycillin	0·125	0·25	2	1	1	8	4	4	32	—[b]	—	—	⩽0·125	⩽0·125	0·5	27	26	19
Amoxycillin in 4 mg/L clavulanic acid	⩽0·125	⩽0·125	2	⩽0·125	⩽0·125	2	4	4	64	4	4	⩾256	⩽0·125	⩽0·125	0·5	26	24	17
Ampicillin	⩽0·125	⩽0·125	0·5	1	1	4	4	4	16	—	—	—	⩽0·125	⩽0·125	⩽0·125	27	26	22
Ampicillin in 10 mg/L sulbactam	⩽0·125	⩽0·125	0·5	0·125	⩽0·125	1	2	2	8	16	8	⩾256	⩽0·125	⩽0·125	⩽0·125	27	26	23
Azlocillin	0·5	0·5	1	2	2	4	8	16	16	—	—	—	16	16	16	21	20	18
Aztreonam	—	—	—	—	—	—	⩽0·125	⩽0·125	0·25	⩽0·125	⩽0·125	⩽0·125	—	—	—	—	—	—
Carbenicillin	1	1	1	4	4	4	16	8	128	—	—	—	0·25	0·25	4	20	20	22
Cefamandole	0·25	0·5	128	0·25	0·5	128	0·5	1	⩾256	8	16	⩾256	⩽0·125	⩽0·125	16	34	32	9
Cefazolin	0·5	0·5	64	0·5	0·5	64	2	1	⩾256	4	2	⩾256	⩽0·125	⩽0·125	32	27	26	9
Cephalothin	0·25	0·25	32	0·25	0·25	64	16	16	⩾256	32	32	⩾256	⩽0·125	⩽0·125	4	33	32	9
Cefixime	8	8	128	16	16	128	0·5	0·5	8	0·25	⩽0·125	1	4	2	32	9	9	9
Cefotaxime	1	1	128	1	2	128	⩽0·125	⩽0·125	16	⩽0·125	⩽0·125	4	0·25	1	32	20	19	9
Cefotiam	1	1	128	0·5	1	128	⩽0·125	⩽0·125	⩾256	⩽0·125	0·25	⩾256	0·25	0·25	32	23	20	9
Cefoxitin	2	2	⩾256	4	4	⩾256	4	4	⩾256	2	2	⩾256	1	0·5	⩾256	18	18	9
Cefpirome	2	2	⩾256	2	2	⩾256	⩽0·125	⩽0·125	64	⩽0·125	⩽0·125	32	0·5	0·5	⩾256	18	18	9
Ceftazidime	16	16	⩾256	8	8	⩾256	⩽0·125	0·25	32	⩽0·125	⩽0·125	16	4	4	⩾256	9	9	9
Ceftizoxime	1	1	128	2	2	⩾256	⩽0·125	⩽0·125	8	⩽0·125	⩽0·125	2	0·25	0·25	16	17	17	9
Ceftriaxone	4	4	⩾256	4	4	⩾256	⩽0·125	⩽0·125	32	⩽0·125	⩽0·125	16	1	1	64	17	16	9
Cefuroxime	1	1	⩾256	1	1	⩾256	4	4	⩾256	2	2	⩾256	0·5	0·5	128	15	14	9
Chloramphenicol	8	8	16	16	16	16	4	4	4	128	64	128	4	4	4	13	13	12
Ciprofloxacin	0·125	0·125	0·25	0·25	0·25	0·25	⩽0·125	⩽0·125	⩽0·125	⩽0·125	⩽0·125	32	⩽0·125	⩽0·125	⩽0·125	32	32	32
Clarithromycin	0·25	0·25	0·25	0·25	0·25	0·25	32	32	32	32	32	32	⩽0·125	⩽0·125	⩽0·125	27	26	26
Clavulanic acid	16	16	⩾256	32	32	⩾256	32	32	⩾256	32	32	⩾256	16	32	⩾256	9	9	9
Clindamycin	⩽0·125	⩽0·125	0·25	0·125	0·125	0·25	64	64	128	32	32	64	2	1	4	18	18	4
Coumermycin	⩽0·125	⩽0·125	⩽0·125	⩽0·125	⩽0·125	⩽0·125	4	8	8	8	8	16	2	1	2	9	9	9
Doxycycline	0·125	0·125	2	0·25	0·25	4	0·5	0·5	4	0·5	0·5	16	⩽0·125	⩽0·125	0·25	27	27	17
Erythromycin	0·25	0·5	8	0·25	0·25	4	32	64	⩾256	32	32	⩾256	⩽0·125	⩽0·125	1	25	25	17
Fosfomycin in 25 mg/L G-6-P[a]	1	1	2	1	1	2	2	1	2	1	1	0·5	128	128	128	9	9	9
Fusidic acid	⩽0·125	⩽0·125	1	⩽0·125	⩽0·125	1	⩾256	⩾256	⩾256	⩾256	⩾256	⩾256	1	1	4	21	20	9

Gentamicin	⩽0·125	⩽0·125	⩽0 125	0·25	0·25	0·25	0·25	0 5	0·5	1	0·5	0·5	⩽0·125	⩽0·125	⩽0·125	24	24	24
Imipenem	⩽0·125	⩽0·125	⩾256	⩽0·125	⩽0·125	⩾256	0 25	0·5	⩾256	0·25	0·25	⩾256	⩽0·125	⩽0·125	⩾256	32	31	9
Josamycin	2	2	4	2	2	2	—	—	—	—	—	—	0·25	0·25	0 5	20	20	19
Kanamycin	0 5	0·5	1	1	1	1	2	2	4	2	4	2	⩽0·125	⩽0·125	⩽0·125	22	22	22
Latamoxef	8	8	⩾256	8	8	⩾256	⩽0 125	⩽0·125	⩾256	⩽0·125	⩽0 125	⩾256	4	4	⩾256	10	10	9
Meropenem	⩽0·125	⩽0·125	2	⩽0·125	⩽0·125	2	⩽0·125	⩽0 125	0 25	⩽0·125	⩽0 125	0·25	⩽0·125	⩽0·125	1	27	27	16
Methicillin	2	2	8	1	2	32	—	—	—	—	—	—	⩽0·125	⩽0·125	1	26	25	14
Mezlocillin	1	1	2	4	2	8	4	4	8	—	—	—	2	2	4	22	21	17
Mupirocin	0·25	0·25	0·5	0·25	0·25	0 5	128	128	128	64	128	128	⩽0·125	⩽0 125	0·25	23	23	21
Nalidixic acid	64	64	64	64	64	64	2	2	2	2	2	2	2	2	2	14	14	14
Netilmicin	⩽0·125	⩽0·125	⩽0·125	0·25	0·25	0·25	0·5	0·25	0·5	0·25	0·5	0·5	⩽0·125	⩽0 125	⩽0·125	24	24	24
Nitrofurantoin	8	4	64	8	8	32	8	8	32	8	16	32	8	16	34	9	9	9
Norfloxacin	1	1	1	1	1	1	⩽0·125	⩽0·125	⩽0·125	⩽0 125	⩽0 125	⩽0·125	⩽0·125	⩽0 125	⩽0·125	28	27	26
Novobiocin	0·25	0·5	0·5	0·25	0·5	0·5	128	128	128	64	64	128	1	1	2	17	17	15
Ofloxacin	0·25	0·25	0·25	0·5	0·5	0·5	⩽0·125	⩽0·125	⩽0·125	⩽0 125	⩽0 125	⩽0·125	⩽0·125	⩽0 125	⩽0·125	28	28	27
Oxacillin	0·25	0·25	0·5	0·25	0·25	0·5	—	—	—	—	—	—	⩽0·125	⩽0·125	0·25	24	24	21
Penicillin G	⩽0·125	⩽0·125	0·25	2	2	16	—	—	—	—	—	—	4	8	16	32	32	26
Piperacillin	1	1	16	2	2	32	2	2	64	—	—	—	0 5	1	16	21	20	11
Piperacillin + tazobactam 8:1	0·5	1	16	1	1	16	2	2	32	4	8	32	0 5	0·5	2	21	20	11
Polymyxin B	32	32	128	32	32	64	⩽0 125	⩽0·125	0·25	⩽0·125	⩽0·125	0·5	4	4	4	9	9	9
Rifampicin	⩽0·125	⩽0·125	⩽0·125	⩽0·125	⩽0·125	⩽0·125	4	8	16	4	4	8	⩽0·125	⩽0·125	⩽0·125	23	23	21
Sulbactam	128	128	⩾256	—	—	—	64	32	64	32	32	64	64	32	64	9	9	9
Tazobactam	64	64	128	64	64	128	—	—	—	128	128	⩾256	16	16	64	9	9	9
Teicoplanin	1	0·5	1	0·25	0·25	2	—	—	—	—	—	—	⩽0 125	⩽0·125	0·5	15	15	11
Temocillin	⩾256	⩾256	⩾256	—	—	—	16	32	⩾256	4	8	⩾256	—	—	—	—	—	—
Tetracycline	0·5	0·5	32	0·25	0·25	32	0·5	0 5	32	0 5	0 5	64	⩽0·125	⩽0·125	32	25	25	9
Ticarcillin	1	1	0·5	4	4	8	8	8	128	⩾256	256	⩾256	0 25	0·25	2	23	23	23
Ticarcillin in 4 mg/L clavulanic acid	0·5	0·5	0·5	0·5	1	1	4	8	128	8	16	⩾256	⩽0·125	⩽0·125	⩽0 125	23	23	23
Tobramycin	0·125	0·125	0 125	0·25	0·25	0·25	0·25	1	1	0 5	0 5	0·5	⩽0·125	⩽0·125	⩽0 125	25	25	24
Trimethoprim	1	1	1	2	2	2	1	1	1	1	1	1	⩽0·125	⩽0 125	⩽0·125	29	29	29
Co-trimoxazole	0·06/ 1 2	0·12/ 2·38	0·12/ 2 38	0·5/ 9·5	0·5/ 9·5	0·5/ 9·5	0 12/ 2·38	0·12/ 2 38	0·12/ 2·38	0·5/ 9·5	0·5/ 9 5	0·5/ 9 5	⩽0·03/ 0·6	0·06/ 1·2	⩽0·03/ 0 6	29	29	29
Vancomycinm	1	1	2	0·5	0 5	1	—	—	—	—	—	—	0·25	0·25	0·25	17	17	14

[a] G-6-P = glucose-6-phosphate.
[b] —, data not presented as strains were highly resistant.
RT, No treatment; 56 = 56°C for 30 min; 121 = 121°C for 15 min.

Antimicrobials

Reference substances of the antimicrobials listed in Table I were gifts of the respective manufacturers; stock solutions were prepared according to the manufacturers' specifications to a concentration of 2560 mg/L (co-trimoxazole contained 320 mg/L trimethoprim and 6080 mg/L sulphamethoxazole), sterilized by membrane filtration (0·22 μm; Nalge Sybron Corp., Rochester, NY), dispensed as 1·5 mL aliquots, and stored at −65°C.

Heat-exposure of antimicrobials

The stock solution of each antimicrobial drug was diluted five-fold in MHB (512 mg/L) except for co-trimoxazole which was 64/1216 mg/L (trimethoprim/sulphamethoxazole). This solution was divided into three aliquots. The first aliquot was held at room temperature, the second aliquot was exposed to 56°C for 30 min in a waterbath, and the third aliquot was autoclaved at 121°C for 15 min.

Antimicrobial susceptibility tests

A previously described microtiter method (Traub, Spohr & Bauer, 1986), using MHB and bacterial inoculum adjusted to $1{\cdot}5 \times 10^5$ cfu in 0·1 mL, was used to determine the MICs of each treated or untreated solution (RT, 56°C and 121°C) against the five test strains. The concentrations tested ranged from 128 to 0·125 mg/L (co-trimoxazole 32/608–0.06/1·2 mg/L (trimethoprim/sulphamethoxazole)). The microtiter plates were incubated at 35°C overnight. In addition the treated and untreated solutions were further diluted two-fold, and tested for residual activity against *B. subtilis* strain ATCC 6633 using a simple agar diffusion procedure (Traub & Leonhard, 1992); sterile disks (9 mm diameter) (Schleicher & Schüll, Dassel, Germany) were charged with 10 μL of the respective antibiotic solution and pressed gently against the surface of *B. subtilis*-inoculated MHA plates (14 cm diameter) which were incubated at 35°C overnight. The diameters of resultant inhibition zones were recorded in mm (9 mm = No zone of activity).

Results and discussion

Among the 62 individual antimicrobials or combinations tested, the majority of β-lactam antibiotics were extensively or totally inactivated by autoclaving (Table I). Surprisingly, however, azlocillin, aztreonam, mezlocillin, and oxacillin were remarkably heat-stable. All aminoglycosides, all quinolones, chloramphenicol, clindamycin, coumermycin, fosfomycin, josamycin, mupirocin, novobiocin, co-trimoxazole, trimethoprim, and vancomycin proved heat-stable. Several β-lactam antibiotics, fusidic acid, nitrofurantoin, polymyxin B, rifampicin, and teicoplanin were partially heat-labile, in that antibacterial activity was reduced four- to eight-fold. Erythromycin, doxycycline, and tetracycline were markedly heat-labile. Table II summarizes these findings and groups the antimicrobials on the basis of their heat stability.

To the best of our knowledge, this is the first comprehensive examination of a large number of antimicrobials with regard to heat-lability. The data obtained might simplify the development and preparation of antibiotic-containing selection media, in that

Table II. Heat-stability of 62 antimicrobials or combinations, after autoclaving at 121°C, 15 min

Heat-stable (MICs raised ≤two-fold) ($n = 25$)		Partially heat-stable (MICs raised four- to eight-fold) ($n = 11$)	Heat-labile (MICs raised ≥16-fold) ($n = 26$)	
Amikacin	Sulbactam	Amoxycillin	Amoxycillin + clavulanic acid	Piperacillin
Azlocillin	Tobramycin	Ampicillin	Cefamandole	Piperacillin + tazobactam
Aztreonam	Trimethoprim	Ampicillin + sulbactam	Cefazolin	Temocillin
Clarithromycin	Trimethoprim +	Carbenicillin	Cephalothin	Tetracycline
Chloramphenicol	Sulphamethoxazole	Fusidic Acid	Cefixime	Ticarcillin
Ciprofloxacin	Vancomycin	Nitrofurantoin	Cefotaxime	Timentin
Clindamycin		Penicillin G	Cefotiam	
Coumermycin		Polymixin B	Cefoxitin	
Fosfomycin		Rifampicin	Cefpirome	
Gentamicin		Tazobactam	Ceftazidime	
Josamycin		Teicoplanin	Ceftizoxime	
Kanamycin			Ceftriaxone	
Mezlocillin			Cefuroxime	
Mupirocin			Clavulanic acid	
Nalidixic acid			Doxycycline	
Netilmicin			Erythromycin	
Norfloxacin			Imipenem	
Novobiocin			Latamoxef	
Ofloxacin			Meropenem	
Oxacillin			Methicillin	

candidate heat-stable antibiotics may be added to rehydrated media before autoclaving. An additional noteworthy finding was that exposure to 56°C for 30 min failed to raise the MICs of any of the agents tested by more than two-fold (the only exception was tobramycin assayed against *E. coli* ATCC 25922). The disc diffusion test results on the whole agreed with the MIC results. However, since MIC tests are relatively insensitive to small changes in concentration heat treatment at 56°C for 15 min could significantly reduce concentrations measured by a more sensitive technique such as immunoassay, HPLC or bioassay. These data indicate that the majority of the tested antimicrobials would not be destroyed by autoclaving prior to disposal, a potentially ecologically significant finding, although since only antimicrobial activity was measured it cannot be ruled out that degradation or conversion to other microbiologically active products did not take place.

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