NOTES

Effect of Elevated Atmospheric Pressure on Antibiotic Susceptibility of *Staphylococcus aureus* and *Streptococcus pyogenes*

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Received for publication 10 April 1972

Staphylococcus aureus showed decreased susceptibility to penicillin, vancomycin, sodium cephalothin, and tetracycline, but increased susceptibility to sodium colistimethate, at a pressure of 68 atm in helium or helium-oxygen gas. Susceptibility of *Streptococcus pyogenes* was unchanged by pressurization.

It was previously shown that the susceptibility of *Staphylococcus aureus* to penicillin is reduced by a pressure of 68 atm [1,000 psi (1)]. Because of the obvious implications of this finding to

treatment of personnel during prolonged deep sea dives, these studies were extended to include other antibiotics and another human pathogen, *Streptococcus pyogenes*.

TABLE 1. Effect of elevated atmospheric pressure on antibiotic susceptibility of bacteria under a normal partial pressure of oxygen

Antibiotic ^a	Pressure (atm)	Minimal inhibitory concn ^b			
		<i>Staphylococcus aureus</i>		<i>Streptococcus pyogenes</i>	
		ATCC 13301	Clinical isolate	ATCC 11435	Clinical isolate
Penicillin	68	19 ^c	9.4 ^c	0.010	0.009
	1	15	6	0.011	0.009
Vancomycin	68	1.7 ± 0.02 ^d	11.2 ± 0.20 ^e	0.61	4.9
	1	1.6 ± 0.01	10.6 ± 0.21	0.62	4.7
Sodium cephalothin	68	0.54 ± 0.02 ^d	0.44 ± 0.01 ^e	0.10	0.068
	1	0.30 ± 0.03	0.32 ± 0.01	0.11	0.069
Tetracycline	68	72 ± 1.3 ^d	0.28 ± 0.004 ^e	0.32	0.038
	1	65 ± 1.0	0.26 ± 0.005	0.32	0.038
Erythromycin	68	3.7	0.22	0.033	0.071
	1	3.6	0.21	0.032	0.076
Sodium colistimethate	68	195 ± 9 ^d	159 ± 6 ^e	25	22
	1	237 ± 9	201 ± 5	25	21
Polymyxin	68	453	325	63	59
	1	453	326	71	61

^a Antibiotics were purchased from the following sources: Penicillin (potassium penicillin G), Pfizer Co., Inc., New York, N.Y.; vancomycin and Keflin (sodium cephalothin), Eli Lilly & Co., Indianapolis, Ind.; tetracycline, J. B. Roerig and Co., New York, N.Y.; erythromycin, Abbott Laboratories, Chicago, Ill.; Colymycin (sodium colistimethate), Warner-Chilcott Laboratories, Morris Plains, N.J.; polymyxin (B sulfate), Burroughs Wellcome and Co., Inc., Tuckahoe, N.Y.

^b Expressed as units (penicillin and polymyxin) or micrograms (other antibiotics) per milliliter of broth. Values are means of at least 12 replicate tests.

^c From previous publication (1).

^d Mean ± standard error. Probability of random distribution between pressurized and control groups (P) < 0.05 > 0.001.

^e P < 0.001.

TABLE 2. *Effect of elevated atmospheric pressure on antibiotic susceptibility of S. aureus under anaerobic conditions*^a

Antibiotic	Pressure (atm)	Minimal inhibitory concn ^b
Penicillin.....	68	16 ± 0.25
	1	13 ± 0.62
Vancomycin.....	68	1.8 ± 0.02
	1	1.6 ± 0.03
Sodium cephalothin.....	68	0.42 ± 0.05
	1	0.22 ± 0.02
Tetracycline.....	68	85 ± 0.5
	1	77 ± 1.7
Sodium colistimethate...	68	138 ± 2
	1	165 ± 5

^a The clinical isolate was used to test penicillin; strain ATCC 13301, for the other antibiotics.

^b Units (penicillin) or micrograms (other antibiotics) per milliliter of broth medium. Values are means ± standard errors of four replicate tests.

S. aureus ATCC 13301 and *S. pyogenes* ATCC 11435 were obtained from the American Type Culture Collection. *S. aureus*, coagulase- and mannitol-positive, phage type 3B/3C/55/71, used in previous experiments (1), and *S. pyogenes*, type A, beta-hemolytic, were clinical isolates.

The antibiotic susceptibility tests were done in brain heart infusion broth (BHI) as previously described (1). Similarly, a description of the pressurization equipment used was presented earlier (1, 2). The cultures were pressurized to 68 atm in a gas mixture of helium and 0.3% oxygen, which has a partial pressure of oxygen equivalent to air at 1 atm. Control cultures were maintained in room air in the same types of cylinders.

As is apparent from Table 1, pressurization of both strains of *S. aureus* resulted in decreased susceptibility to vancomycin, cephalothin, and tetracycline. Although some of the observed differences were very small, they were highly reproducible and were reinforced by the observation that, in cultures containing somewhat less than minimal inhibitory concentrations, turbidity was higher in the pressurized groups. Of particular interest is the observation that elevated pressure caused the same effect on both strains, despite great differences in susceptibility to vancomycin and tetracycline.

Elevated pressure affected the susceptibility of *S. aureus* to sodium colistimethate as well, but in this case antibiotic susceptibility was increased. No effect was noted with erythromycin or polymyxin.

The possibility that the above-described differences in antibiotic susceptibility of *S. aureus* were due to variation in oxygen tension rather than elevated pressure was also tested (Table 2). Thioglycolate medium was substituted for BHI, and the gas phase at 68 and 1 atm was 100% helium instead of oxygen-containing mixtures. Table 2 shows that the antibiotic susceptibilities of *S. aureus* under such anaerobic conditions were entirely comparable to those obtained at the two pressures under normoxic conditions.

As shown in Table 1, the antibiotic susceptibility of *S. pyogenes* was also examined in parallel with that of *S. aureus*. Pressurization in this case produced no demonstrable alterations in antibiotic susceptibility.

Since the antibiotics studied in these experiments vary greatly in mode of action, a single mechanism cannot explain the effect of elevated pressure on susceptibility of *S. aureus*. Since *S. aureus* and *S. pyogenes* differ markedly in response to pressurization, it is plausible to assume that the effect was on surface structures. In fact, an earlier paper showed that elevated pressure affected the β -galactoside permeases of *Escherichia coli* (2). It can, therefore, be concluded that pressurization may influence a number of reactions which take place at the cell surface or are involved in transport. From a practical point of view, these results reinforce previous evidence (1) that under conditions of deep sea diving antibiotic treatment may not have exactly the same effect as at sea level.

I am indebted to Emilio Weiss for invaluable advice and to James E. Perry for excellent technical assistance.

This work was supported by the Bureau of Medicine and Surgery, Department of the Navy, Project MF12.524.014-9006 AC9K.

LITERATURE CITED

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