

## Variation in Hydrogen Peroxide Sensitivity between Different Strains of *Neisseria gonorrhoeae* Is Dependent on Factors in addition to Catalase Activity

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**Catalase, which catalyzes the reduction of hydrogen peroxide to oxygen and water, is considered the primary defense of *Neisseria gonorrhoeae* against exogenous hydrogen peroxide. Recent reports have demonstrated drastically different sensitivities of the organism to hydrogen peroxide ranging from greater than 80% survival after challenge with 30 mM hydrogen peroxide to less than 0.001% survival after challenge with 10 mM hydrogen peroxide. In this study, we have examined the hydrogen peroxide sensitivities of six clinical gonococcal isolates. The study demonstrates that the variations in gonococcal hydrogen peroxide sensitivities previously reported can be attributed to (i) differences in experimental methods employed or (ii) variation among different gonococcal strains. All of the gonococcal isolates examined generated similar concentrations of catalase, implying that the differences in the H<sub>2</sub>O<sub>2</sub> sensitivity observed may depend on factors in addition to catalase.**

*Neisseria gonorrhoeae* is a gram-negative, human mucosal pathogen. Gonococci can grow in both aerobic and anaerobic atmospheres in vitro and in vivo (4, 5, 16). However, the organism clearly encounters phagocytic cells, including macrophages and neutrophils, during infection. Under aerobic conditions phagocytes utilize molecular oxygen to generate oxidants with antimicrobial activity (7, 10). During the respiratory burst of the phagocyte, a membrane-bound NADPH oxidase reduces molecular oxygen by one electron to superoxide (O<sub>2</sub><sup>-</sup>) (2). Previous studies have shown that *N. gonorrhoeae* is highly resistant to extracellular O<sub>2</sub><sup>-</sup> (1) probably because exogenous O<sub>2</sub><sup>-</sup> cannot traverse bacterial membranes (8). However, within acidic environments (such as neutrophils), O<sub>2</sub><sup>-</sup> spontaneously disproportionates to a stronger oxidant, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). In fact, the neutrophil phagosomal concentration of H<sub>2</sub>O<sub>2</sub> has been estimated to be as high as 100 mM (11). H<sub>2</sub>O<sub>2</sub> can freely diffuse across bacterial membranes and can lead to mutagenic events caused by single-strand nicks in DNA (13), as well as oxidation of critical respiratory chain components (17). Therefore, the oxygen-dependent killing of gonococci by phagocytic cells would be expected to be dependent, in part, on the ability of the organism to respond to increased levels of exogenous H<sub>2</sub>O<sub>2</sub> (18).

Two previous studies have examined the sensitivity of *N. gonorrhoeae* to H<sub>2</sub>O<sub>2</sub>. Using *N. gonorrhoeae* FA1090 cells, Hassett et al. (9) observed less than 0.001% survival after exposure of the bacteria to 10 mM H<sub>2</sub>O<sub>2</sub>. Using bacteria harvested from solidified gonococcal culture (GC) medium, Johnson et al. (14) observed greater than 50% survival of strains 28B1 and F62, which were exposed to 30 mM H<sub>2</sub>O<sub>2</sub>. In

the present study we have attempted to determine the differences between these results.

Gonococcal strain FA1090 was provided by P. F. Sparling, University of North Carolina at Chapel Hill, Chapel Hill. Strain 28B1 is a clinical isolate provided by S. R. Johnson,

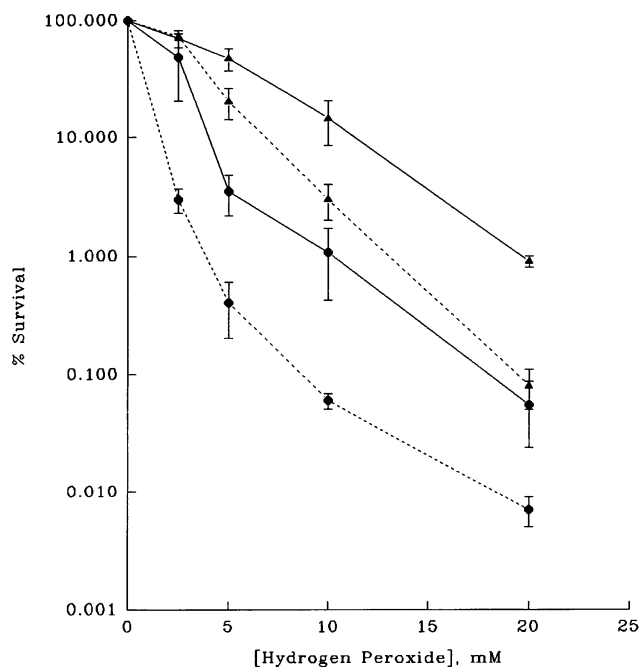


FIG. 1. Percent survival of gonococci after challenge with various concentrations of H<sub>2</sub>O<sub>2</sub>. Each result is the mean and one standard deviation of three separate experiments. The circles indicate the survival of strain FA1090, and the triangles indicate the survival of strain 28B1. Bacteria were grown either in Trypticase soy broth without agitation (—) or in GC broth (---) with agitation prior to H<sub>2</sub>O<sub>2</sub> challenge.

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TABLE 1. Hydrogen peroxide sensitivities and catalase activities of various gonococcal strains<sup>a</sup>

Strain	% Survival after H <sub>2</sub> O <sub>2</sub> challenge				Catalase activity (U/mg)	[Iron] (fg/CFU)
	2.5 mM	5 mM	10 mM	20 mM		
FA1090	3 ± 0.7	0.4 ± 0.2	0.06 ± 0.009	0.007 ± 0.002	1,290 ± 45	0.027
28B1	73 ± 3	20 ± 6	3 ± 1	0.08 ± 0.03	1,203 ± 27	0.17
L4401	63 ± 10	26 ± 6	4 ± 1	0.6 ± 0.2	1,144 ± 67	0.25
L8800	40 ± 5	3 ± 1	0.3 ± 0.009	0.006 ± 0.003	1,169 ± 42	1.19
L2370	64 ± 11	8 ± 1	0.4 ± 0.06	0.04 ± 0.01	1,178 ± 86	ND
L6200	58 ± 5	15 ± 3	1 ± 0.4	0.05 ± 0.002	1,213 ± 40	ND
L4933	66 ± 2	30 ± 1	3 ± 2	0.7 ± 0.2	1,248 ± 63	ND

<sup>a</sup> Bacteria were grown according to the method of Hassett et al. (9). Values are the means and standard deviations of five separate experiments. Catalase activity was determined spectrophotometrically at 240 nm according to the method of Beers and Sizer (3) by using 17.6 mM H<sub>2</sub>O<sub>2</sub>. One unit of activity is that which decomposes 1 μM H<sub>2</sub>O<sub>2</sub> per min at room temperature. ND, not determined.

Centers for Disease Control and Prevention, Atlanta, Ga. Strains L2370, L4401, L6200, and L8800 are clinical isolates from the Wilson County Sexually Transmitted Disease Clinic (Wilson, N.C.). The methods employed by Hassett et al. (9) and Johnson et al. (14) differed in the type of growth media used to propagate the bacteria and the specific phase of growth achieved by the organisms prior to challenge with H<sub>2</sub>O<sub>2</sub>. Hassett et al. (9) grew the bacteria aerobically in GC broth until mid-logarithmic phase and, after diluting the bacteria into fresh, prewarmed GC broth, exposed the gonococci to various concentrations of H<sub>2</sub>O<sub>2</sub> for 15 min at 37°C. After exposure, the bacteria were diluted in GC salts containing catalase and spread on GC agar plates for enumeration of viable CFU. Johnson et al. (14) grew the bacteria in a liquid layer of Trypticase soy broth over a solid medium of GC agar. In both methods, the concentration of bacteria was adjusted to approximately 1 × 10<sup>7</sup> to 5 × 10<sup>7</sup> CFU/ml prior to exposure to H<sub>2</sub>O<sub>2</sub>.

As shown in Fig. 1, the method employed to grow the bacteria had a significant effect on the H<sub>2</sub>O<sub>2</sub> sensitivities of the two gonococcal strains. Strain FA1090 was more sensitive to H<sub>2</sub>O<sub>2</sub> than 28B1 with either method. However, both strains FA1090 and 28B1 were more sensitive after aerobic growth in GC broth. A likely explanation for this difference is in the metabolic activity of the bacterium at the time of H<sub>2</sub>O<sub>2</sub> challenge. Imlay and Linn (12) have shown that metabolically inactive (starved) *Escherichia coli* organisms are more resistant to H<sub>2</sub>O<sub>2</sub> than are equivalent numbers of mid-logarithmic-phase organisms. A similar decrease in H<sub>2</sub>O<sub>2</sub> sensitivity has been observed with stationary-phase *Pseudomonas syringae* (15). It has been suggested that stationary-phase bacteria possess fewer of the cellular equivalents required to reduce H<sub>2</sub>O<sub>2</sub> to HO·, a reaction which leads to DNA strand breakage (13).

Experiments were conducted in order to determine the peroxide sensitivities of clinical isolates of *N. gonorrhoeae*. As shown in Table 1, there was little difference in the H<sub>2</sub>O<sub>2</sub> sensitivity of gonococcal strains at low concentrations of H<sub>2</sub>O<sub>2</sub> (2.5 and 5.0 mM), with the exception of FA1090. It should be noted that strain FA1090 was isolated from a patient with disseminated gonococcal infection, whereas the other strains tested were urethral isolates.

We observed significant variation in the sensitivity of gonococcal strains after challenge with 20 mM H<sub>2</sub>O<sub>2</sub> (Table 1). These differences were surprising since all test strains possessed similar catalase specific activities (Table 1). Johnson et al. (14) demonstrated that catalase-negative gonococci are significantly more sensitive to H<sub>2</sub>O<sub>2</sub> than isogenic catalase-positive organisms. Likewise, we (18) have previously shown that catalase-negative gonococci demonstrate remarkably

greater sensitivity to exogenous H<sub>2</sub>O<sub>2</sub> and stimulated human neutrophils than catalase-positive strains. However, the present results make it clear that catalase is only one of several factors determining gonococcal sensitivity to H<sub>2</sub>O<sub>2</sub>.

We have previously shown that the iron concentration of *N. gonorrhoeae* affects susceptibility to redox stress (6). The intracellular iron concentrations of several strains of *N. gonorrhoeae* were measured to see if there was a correlation with H<sub>2</sub>O<sub>2</sub> sensitivity. Bacteria were grown to mid-logarithmic phase in 50 ml of GC broth with agitation, and the concentration of bacteria was determined. Cells were harvested by centrifugation, and the resulting cell pellet was washed once with Hanks' balanced salt solution, solubilized in 10 ml of a 10% sodium dodecyl sulfate solution (pH 1.0), and boiled for 10 min in an acid-washed flask. The total iron concentration in each sample was determined by standard neutron activation analysis (14 h of irradiation at 1.5 × 10<sup>13</sup> n/cm<sup>2</sup>-s; Nuclear Energy Services, Nuclear Engineering Department at N.C. State University). As shown in Table 1, there is no correlation between H<sub>2</sub>O<sub>2</sub> sensitivity and intracellular iron concentration.

The intracellular localization of catalase could affect the sensitivity to H<sub>2</sub>O<sub>2</sub>, as previously observed with *E. coli* (12). However, we have shown catalase to be limited to the cytoplasm in *N. gonorrhoeae* (18). Other factors which could affect the sensitivity of *N. gonorrhoeae* to exogenous H<sub>2</sub>O<sub>2</sub> include intracellular glutathione (calculated to be 17 mM in gonococci [1]) and the concentration of endogenous reactive oxygen species and reducing equivalents. In conclusion, this study demonstrates that the sensitivity of gonococci to H<sub>2</sub>O<sub>2</sub> is highly dependent upon the experimental conditions. In particular, differences in the growth of the gonococci prior to challenge with H<sub>2</sub>O<sub>2</sub> can markedly affect its survival.

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