ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1997, p. 781–784 Vol. 41, No. 4
0066-4804/97/$04.00+0

Comparative Study of Bactericidal Activities, Postantibiotic Effects, and Effects on Bacterial Virulence of Penicillin G and Six Macrolides against *Streptococcus pneumoniae*

KURT FUURSTED,[1]* JENNY DAHL KNUDSEN,[1] METTE BARENDORFF PETERSEN,[1] RIKKE LYKKE POULSEN,[1] AND DORTE REHM[2]

Department of Research and Development in Microbiology, Division of Microbiology,[1] and Department of Biostatistics,[2] Statens Serum Institut, Copenhagen, Denmark

Received 22 July 1996/Returned for modification 17 October 1996/Accepted 4 February 1997

In this report, we present MIC, bactericidal activity, postantibiotic effect (PAE), and in vivo infectivity data for postantibiotic-phase pneumococci. We compared and evaluated penicillin G and six macrolides, erythromycin, azithromycin, clarithromycin, dirithromycin, roxithromycin, and spiramycin, against 10 strains of pneumococci with various levels of susceptibility to penicillin. All of the agents, except azithromycin, exhibited a bactericidal effect (a ≥3 $\log_{10}$ decrease in the number of CFU per milliliter) after 4 h of exposure to a concentration equal to 10 times the MIC, displaying the following hierarchy: spiramycin = penicillin G = erythromycin = dirithromycin = clarithromycin = roxithromycin > azithromycin. The bactericidal rate of penicillin G was significantly lower for resistant strains (MIC, ≥2 μg/ml), while bactericidal rates of macrolides were unaffected by penicillin susceptibility. A PAE was induced in all of the strains by all of the antibiotics after exposure for 1 h to a concentration equivalent to 10 times the MIC. The mean duration of PAEs varied between 2.3 and 3.9 h, showing the following hierarchy: spiramycin = dirithromycin = clarithromycin = erythromycin = roxithromycin > azithromycin > penicillin G. Virulence studies were performed with immunocompetent mice by intraperitoneal inoculation of virulent, penicillin-susceptible serotype 3 pneumococci which had been pre-exposed to penicillin G or a macrolide for 1 h. A significant decrease in the virulence of postantibiotic-phase pneumococci was induced only by erythromycin, azithromycin, dirithromycin, and spiramycin, displaying 5.9-, 7.1-, 4.2-, and 3.6-fold increases in the 50% lethal dose (LD_{50}) compared to a control suspension, respectively. No significant correlation could be demonstrated between the LD_{50} and the MIC, bactericidal activity, or PAE duration. These results suggest that antimicrobial interaction with host defenses in terms of virulence might be a significant parameter that could influence the drug or drug regimen of choice.

Erythromycin has been widely used for over 30 years as an alternative to penicillin for the treatment of upper and lower respiratory tract infections caused by pneumococci. In recent years, several new macrolides with extended spectra and improved pharmacokinetics have emerged (4, 6). The aim of this study was to evaluate and compare penicillin G with six macrolides (erythromycin, azithromycin, clarithromycin, dirithromycin, roxithromycin, and spiramycin) with respect to MIC, bactericidal activity, and postantibiotic effect (PAE).

PAE studies classically analyze interference with the regrowth of organisms during the postantibiotic growth phase (PA phase). In recent years, however, a broader definition of the PAE has emerged (12, 17, 18, 20, 22) which acknowledges the fact that the "post-activities" of an antibiotic are not only inhibition of regrowth but additional effects that might be of clinical significance. These additional effects include morphological and physiological changes inducing potential alterations of cellular functions and virulence factors (12, 17, 22). Only a few studies have examined the interaction between an antimicrobial agent and host defenses directly in terms of bacterial virulence (8, 10, 19). For this reason, we were also interested in analyzing the interference with the virulence of PA-phase bacteria induced by penicillin G or a macrolide as measured by survival rates after intraperitoneal (i.p.) inoculation of immunocompetent mice with pneumococci.

* Corresponding author. Mailing address: Department of Research and Development in Microbiology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. Phone: (45) 32 68 34 57. Fax: (45) 32 68 38 87.

MATERIALS AND METHODS

Bacteria. One reference strain (*Streptococcus pneumoniae* ATCC 27336) and nine clinical isolates of *S. pneumoniae* obtained from different sources were tested. These strains were selected from a previous study done in our institution (16). Four strains were susceptible to penicillin G with MICs of 0.015 and 0.031 μg/ml (serotypes 3, 6B, 14, and rough); three were intermediately penicillin resistant, with MICs of 0.5 μg/ml (serotypes 4, 6B, and 15B); and three were penicillin resistant, with MICs between 2 and 4 μg/ml (serotypes 14 and 23F). All strains were macrolide susceptible. Strains were stored at −80°C and subcultured on 5% blood agar plates before testing.

Antimicrobial agents, media, and susceptibility testing. The penicillin G used in this study was obtained from Leo Pharmaceuticals, Ballerup, Denmark; erythromycin was obtained from Sigma Chemical Company, St. Louis, Mo.; dirithromycin was obtained from Eli Lilly, Copenhagen, Denmark; and azithromycin, clarithromycin, roxithromycin, and spiramycin were obtained from A/S Rosco, Taastrup, Denmark. Bacteria were grown in serum broth (infusion broth supplemented with 0.05% hemin and 5% serum) (Statens Serum Institut, Copenhagen, Denmark), and viability counts were performed on 5% blood agar plates (Statens Serum Institut). All studies (except the MIC determinations) were done with organisms from logarithmic-phase cultures. This log-phase inoculum was obtained by suspending colonies from an overnight culture directly in serum broth, adjusting the suspension by spectrophotometric measurement to the desired concentration, and then incubating it at 35°C for 1 to 2 h before each experiment. Viable counts were performed immediately to verify the size of the original inoculum. MICs of the six different macrolides were determined by a standard broth macrodilution method (13).

Time-kill determination. Time-kill experiments were performed with all 10 strains of pneumococci with penicillin G and the six different macrolides. The rate of killing was tested in two or three separate experiments with a final antibiotic concentration 10 times the MIC. A drug-free control was included in all experiments. Log-phase cultures with an initial inoculum of approximately 5×10^6 CFU/ml were mixed with drugs and incubated without shaking at 35°C

in air. Killing kinetics were monitored by viable counts made immediately before drug addition and after 0.5, 1, 2, and 4 h. Aliquots were removed, serially diluted 10-fold in saline, and then plated on 5% blood agar plates. Plates were incubated in air at 35°C for 18 to 24 h, and then colonies were counted.

PAE determination. A log-phase pneumococcal inoculum of approximately 5×10^6 CFU/ml was exposed to penicillin G or a macrolide at 10 times the MIC for 1 h. A control culture with no drug added was processed in parallel. All PAE determinations were performed in two separate experiments. Drug activity was removed by 1,000-fold dilution of the bacterial suspension in prewarmed (35°C) fresh medium. To ensure that residual drug activity did not affect the regrowth curve, additional drug controls were run in parallel by inoculating similarly diluted unexposed organisms into fresh media to which a 10^{-3} dilution of the test concentration was added. The residual drug effect was proved not to affect the slope of the regrowth curve (data not shown). The resulting regrowth curve was constructed by doing viability counts at the time of drug removal and at appropriate intervals (1.5 to 2 h) thereafter. Aliquots were removed from all cultures, serially diluted in saline, and plated on 5% blood agar plates. After incubation in air for 20 to 24 h at 35°C, CFU were counted. Duration of PAE was calculated by means of the equation $PAE = T - C$, where T is the time required for the viable count in the test culture to increase 1 $\log_{10}$ above the count immediately after the dilution procedure and C is the time required for the CFU count in an untreated culture to increase by 1 $\log_{10}$ above the count observed after drug removal.

Virulence experiments. Virulence experiments were done by using a clinical isolate of a serotype 3, fully penicillin-susceptible *S. pneumoniae* strain. To determine the dose-response relationship, immunocompetent outbred female *ssc* CF1 mice weighing 30 ± 2 g were used in a mouse peritonitis model. Inoculation was performed by i.p. injections of log-phase or PA-phase cultures. Suspensions of log-phase bacteria consisting of approximately 5×10^6 CFU/ml were exposed to 10 times the MIC of penicillin or a macrolide for 1 h, and non-drug-exposed cultures were prepared in parallel. After 1 h of incubation without shaking at 35°C in air, the drug effect was removed by washing and centrifugation twice at $3{,}000 \times g$ for 10 min each time at ambient temperature. After the last centrifugation, the pellet was resuspended in fresh, drug-free, prewarmed (35°C) medium. After serial (three- to fivefold) dilution in drug-free medium, 0.5 ml of each dilution was injected i.p. into mice via a 25-gauge syringe (10 mice per dilution for log-phase cultures and 5 mice per dilution for PA-phase cultures). A total of 345 mice was used (120 mice for log-phase cultures and 225 mice for PA-phase cultures). The animals had free access to food and water and were observed daily for 5 days, and the number of survivors of each dilution was recorded.

Statistical analysis. The data were processed as duplicate values by using the following statistical tests for comparison of bactericidal activity and PAE (Prism; GraphPad Software, Inc.): for comparison of groups, two-way analysis of variance; for comparison of paired means, Tukey's multiple-comparison test; for correlation analysis, Spearman's correlation test. A level of $P < 0.05$ was considered significant.

The 50% lethal dose (LD_{50}) was estimated from day 5 survival rates by using the software program SAS 6.11 (SAS Institute Inc.). The relationships between survival rates and bacterial doses were modeled with a logistic regression model under the assumption that the logit transformed survival rates are linearly related to the logarithmically transformed doses (2). A test for linearity was performed by the log-likelihood ratio chi-square test. The same test was used to test for difference in slopes and difference in intercepts compared to the untreated control. A logistic regression model which defines a common slope for drug-treated and untreated controls was used to calculate LD_{50}s. In this model, the LD_{50} was estimated as the antilogarithm of the negative ratio between the intercept and the slope. The relative LD_{50}, defined as the ratio between the LD_{50} of the drug in question and that of the control, was also calculated in the above-described model as the antilogarithm of the horizontal distance between two parallel logistic dose-response curves. The 95% confidence limits for both the LD_{50} and the relative LD_{50} were calculated by using Fieller's theorem (9). Relative LD_{50}s were used for comparative analysis.

RESULTS

MIC determinations. The MICs of the six macrolides tested are shown in Table 1. The in vitro activity of macrolides, in terms of their MICs, showed the following hierarchy: erythromycin = dirithromycin = azithromycin = clarithromycin > spiramycin > roxithromycin. The MICs of the various macrolides were not correlated to the MIC of penicillin G.

Time-kill experiments. The results of time-kill experiments with drug concentrations 10 times the MIC after 1 and 4 h of exposure are shown in Table 2 and Fig. 1. Spiramycin and clarithromycin showed significantly greater killing effects at 1 h than did azithromycin, dirithromycin, and erythromycin. After 4 h of exposure, penicillin G and spiramycin demonstrated the highest killing rates, while azithromycin displayed the poorest killing activity, which was significantly lower than those of the other drugs. After 1 and 4 h of exposure, penicillin G displayed significantly reduced killing of penicillin-resistant strains compared to strains with MICs of 1 μg/ml or lower (Fig. 1). No significant correlation between the MIC of penicillin and the killing rates of the six macrolides could be demonstrated (Fig. 1).

TABLE 1. MICs of six macrolides against 10 strains of pneumococci determined by a standard broth macrodilution technique[a]

Macrolide	MIC_{50}	MIC_{90}	MIC range
Azithromycin	0.031	0.063	0.008–0.063
Clarithromycin	0.031	0.063	0.008–0.125
Dirithromycin	0.031	0.063	0.008–0.063
Erythromycin	0.016	0.031	0.008–0.063
Roxithromycin	0.125	0.125	0.031–0.5
Spiramycin	0.063	0.125	0.016–0.125

[a] MIC_{50} and MIC_{90} represent the MICs for 50 and 90% of the strains tested, respectively. All values are in micrograms per milliliter.

PAE experiments. The data from the PAE experiments are shown in Table 1 and Fig. 2. All of the antibiotics had a PAE on all of the strains tested. The mean duration of the PAE of penicillin G was shorter than that of all of the macrolides. Azithromycin had a significantly lower mean PAE than spiramycin, dirithromycin, clarithromycin, and erythromycin. The duration of the PAE induced by spiramycin was significantly shorter in penicillin-resistant strains than in penicillin-susceptible strains (Fig. 2). For the other macrolides, a similar trend between PAE duration and penicillin susceptibility was noticed but the differences were not statistically significant. The PAE induced by penicillin G was unaffected by penicillin susceptibility (Fig. 2).

Pathogenicity experiments. Virulence in mice was determined by using a clinical serotype 3 penicillin-susceptible pneumococcus. The data obtained by logistic regression on slopes, intercepts, LD_{50}s, and relative LD_{50}s are shown in Table 3. Analysis of the different logistic transformed curves revealed similar slopes for all of the drugs compared with the control but significantly different intercepts for erythromycin, azithromycin, dirithromycin, and spiramycin compared with the untreated control. Pretreatment with penicillin G, clarithromycin, or roxithromycin had no statistically significant effect on virulence based on the calculated LD_{50}s. Erythromycin, azithromycin, spiramycin, and dirithromycin, on the other hand, produced significant decreases in virulence with 3.6- to 7.1-fold higher LD_{50}s than the control (Table 2). A correlation between

TABLE 2. Bactericidal activities and PAEs of penicillin and six macrolides against 10 strains of pneumococci[a]

Drug	Log_{10} reduction in CFU at:		PAE duration (h)
	1 h	4 h	
Penicillin G	1.02 (0.73–1.31)	3.35 (3.04–3.65)	2.33 (2.12–2.45)
Erythromycin	0.82 (0.61–1.03)	3.21 (3.01–3.40)	3.50 (3.20–3.78)
Azithromycin	0.50 (0.35–0.65)	2.61 (2.44–2.77)	2.83 (2.56–3.10)
Clarithromycin	1.40 (1.04–1.76)	3.04 (2.89–3.20)	3.60 (3.31–3.90)
Dirithromycin	0.76 (0.60–0.92)	3.15 (2.98–3.32)	3.64 (3.38–3.90)
Roxithromycin	1.09 (0.80–1.38)	3.01 (2.78–3.24)	3.13 (2.91–3.35)
Spiramycin	1.55 (1.15–1.95)	3.41 (3.22–3.59)	3.88 (3.69–4.08)

[a] All values are means and 95% confidence intervals.

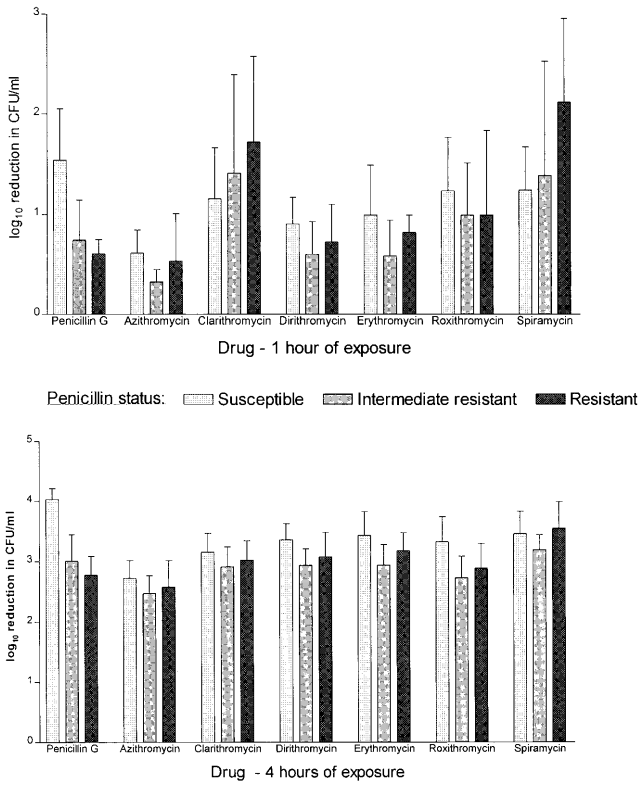

FIG. 1. Bactericidal activities of penicillin G and six macrolides against strains of pneumococci in relation to penicillin susceptibility (four susceptible, three intermediately resistant, and three resistant strains). The bars indicate mean values with 95% confidence limits.

a decrease in virulence and various pharmacodynamic parameters was examined. However, none of the in vitro pharmacodynamic parameters tested (MIC, bactericidal activity, and PAE) significantly correlated with the LD_{50}.

DISCUSSION

The increasing emergence of penicillin-resistant strains of pneumococci is already a therapeutic problem in many areas around the world (1). A macrolide could be an alternative to penicillin, especially for patients allergic to penicillin.

As shown in this study, the MICs of macrolides for the 10 strains of pneumococci tested were comparable to those of

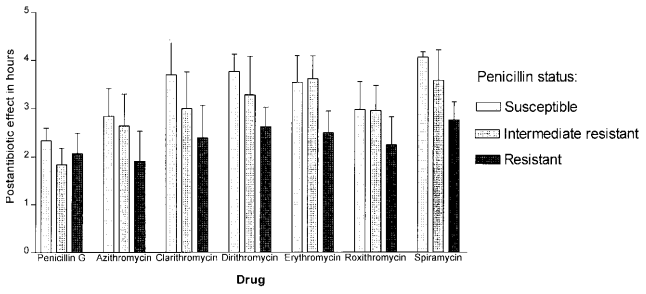

FIG. 2. Duration of the PAEs of penicillin G and six macrolides against strains of pneumococci with respect to penicillin susceptibility (four susceptible, three intermediately resistant, and three resistant strains). The bars indicate mean values with 95% confidence limits.

TABLE 3. Data calculated by logistic regression analysis of survival rates of a serotype 3 *S. pneumoniae* strain exposed to penicillin G or a macrolide at 10 times the MIC for 1 h

Drug	Slope (SE)	Intercept (SE)	LD_{50} (95% CI[a])	Relative LD_{50} (95% CI)
None (control)	2.9 (0.5)	−7.2 (1.4)	323 (216–487)	
Penicillin G	3.0 (0.9)	−7.6 (2.0)	315 (158–664)	1.0 (0.4–2.3)
Erythromycin	4.6 (1.5)	−14.0[b] (4.7)	1,916 (993–3,872)	5.9[b] (2.7–13.3)
Azithromycin	3.2 (0.6)	−10.7[b] (1.6)	2,287 (886–5,964)	7.1[b] (2.5–20.0)
Clarithromycin	3.2 (0.6)	−8.9 (1.6)	596 (228–1,537)	1.8 (0.7–5.2)
Dirithromycin	3.4 (1.4)	−10.6[b] (4.4)	1,360 (527–3,510)	4.2[b] (1.5–11.8)
Roxithromycin	2.2 (0.7)	−5.4 (1.9)	316 (154–639)	1.0 (0.4–2.21)
Spiramycin	0.8 (0.7)	−2.1[b] (2.6)	1,174 (435–3,085)	3.6[b] (1.2–10.3)

[a] CI, confidence interval.
[b] Significantly different from control at 5% test level.

other reports, with roxithromycin the least potent macrolide (3, 16).

All of the drugs tested exhibited a bactericidal effect (a ≥ 3 $\log_{10}$ decrease in the number of CFU per milliliter) on pneumococci, except azithromycin. Penicillin G and spiramycin demonstrated the highest killing rates, which were significantly better than that of azithromycin. An unexpected observation was the association between penicillin susceptibility and the bactericidal activity of penicillin G. However, this finding is based on a small number of strains in each susceptibility group, and with the lack of supporting data from the literature, we can draw no conclusions about a relationship between bactericidal activity and susceptibility to penicillin.

As expected, a PAE was demonstrated by all of the agents in this study, which is consistent with earlier reports on pneumococci (7, 14, 15). Penicillin G induced a significantly shorter PAE than all of the macrolides (5, 11). Spiramycin was consistently the most efficient PAE inducer, but a significant difference was demonstrated only in comparison with azithromycin. In a similar study, erythromycin induced a longer PAE than roxithromycin with the same strain as in the present study (11). In another study, roxithromycin produced a longer PAE (and postantibiotic sub-MIC effect) in a single strain than did azithromycin and clarithromycin (15). We observed a tendency for all macrolides to generate a reduced PAE in penicillin-resistant strains compared with strains with a penicillin MIC of <0.1 μg/ml. However, this difference was only significant with spiramycin.

It is increasingly being recognized that the concept of a PAE is not only inhibition of regrowth but additional effects, such as morphological and physiological changes (12, 17, 22), which might be of clinical significance. Thus, it was of interest to examine the interaction of pre-exposed (PA phase) pneumococci on the host immune defense.

By comparing the survival rates of an unexposed control culture and PA phase cultures exposed to penicillin or a macrolide, it was demonstrated that pneumococci exposed to azithromycin, spiramycin, dirithromycin, and erythromycin produced a significant decrease in virulence, suggesting that these drugs affect certain bacterial components that are associated with bacterial virulence. Penicillin G, clarithromycin, and roxithromycin, on the other hand, had no effect on virulence. Virulence is a complex phenomenon that results from the interaction between a bacterium and its host and in pneumococci is related primarily to the capsular polysaccharide, which has an antiphagocytic function (21). As macrolides interfere with protein biosynthesis, the bacterial effects of this drug class are likely to be manifested in both altered cell surface structures and inter-

ference with production of toxins. These changes could modify the interaction between the drug-damaged bacteria and the host immune system and provide an explanation of the decrease in virulence of briefly exposed pneumococci to a macrolide. However, it is not clear why not all macrolides have an effect on bacterial virulence. Note that these experiments were done on only a single strain and there may exist considerable variation between different strains of pneumococci.

Penicillin G could not be demonstrated to affect bacterial pathogenicity, consistent with the original work by Eagle et al. in 1950 (8). In contrast, it was noticed that the LD_{50}s for penicillin-damaged group A and B streptococci were 100- to 1,000-fold higher than those for untreated organisms (8). Moreover, in that study, penicillin-damaged group A and B streptococci had increased susceptibility to the bactericidal effect of the host immune system, which was correlated with reduced virulence for mice. It should be clear that a PAE is not the only postexposure event that should be evaluated. An antibiotic inducing sublethal damage to bacteria might produce increased susceptibility to host defenses, which might contribute to recovery from infections, at least in an immunocompetent host. However, it should be evident that the single most important parameter for the antimicrobial effect of an antibiotic must be its bactericidal activity rather than the unpredictable elements of a PAE (or postantibiotic sub-MIC effect) or reduction of virulence. Yet, these latter effects on an intact host may contribute significantly to success with intermittent drug regimens.

The data presented in this study reveal that spiramycin had significantly greater bactericidal activity and a longer PAE than azithromycin for 10 strains of pneumococci. However, this distinction was abolished when the PAE on bacterial virulence was evaluated and compared.

It is concluded that drug interaction with host defenses might be a significant parameter which could influence the drug or drug regimen of choice. However, the ultimate significance of this decrease in bacterial virulence or of any other postexposure activity besides strictly antimicrobial activity can be determined only in human trials.

REFERENCES

1. **Appelbaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. **15:**77–83.
2. **Armilage, P., and G. Berry.** 1987. Statistical methods in medical research. Blackwell Scientific Publications, Oxford, England.
3. **Bauernfeind, A.** 1993. In-vitro activity of dirithromycin in comparison with other new and established macrolides. J. Antimicrob. Chemother. **31:**39–49.
4. **Brogden, R. N., and D. H. Peters.** 1994. Dirithromycin—a review of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. Drugs **48:**599–616.
5. **Bundtzen, R. W., A. U. Gerber, D. L. Cohn, and W. A. Craig.** 1981. Postantibiotic suppression of bacterial growth. Rev. Infect. Dis. **3:**28–37.
6. **Chabbert, Y. A.** 1988. Early studies on in-vitro and experimental activity of spiramycin: a review. J. Antimicrob. Chemother. **22**(Suppl. B)**:**1–11.
7. **Craig, W. A., and S. Gudmundsson.** 1991. The postantibiotic effect, p. 403–431. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins, Co., Baltimore, Md.
8. **Eagle, H., R. Fleischman, and A. D. Musselman.** 1950. The bactericidal action of penicillin in vivo: the participation of the host, and the slow recovery of the surviving organisms. Ann. Intern. Med. **33:**544–571.
9. **Finney, D. J.** 1978. Statistical methods in biological assay. Macmillan, New York, N.Y.
10. **Holloway, P. M., R. A. Bucknall, and G. W. Denton.** 1986. The effects of sub-lethal concentrations of chlorhexidine on bacterial pathogenicity. J. Hosp. Infect. **8:**39–46.
11. **Kuenzi, B., C. Segessenmann, and A. U. Gerber.** 1987. Postantibiotic effect of roxithromycin, erythromycin, and clindamycin against selected gram-positive bacteria and Haemophilus influenzae. J. Antimicrob. Chemother. **20**(Suppl. B)**:**39–46.
12. **Majcherczyk, P. A.** 1996. The issue of the true postantibiotic effect. J. Antimicrob. Chemother. **37:**188–189.
13. **National Committee for Clinical Laboratory Standards.** 19●●. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—third edition. Approved standard. NCCLS document M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Odenholt-Tornqvist, I., E. Löwdin, and O. Cars.** 1991. Pharmacodynamic effects of subinhibitory concentrations of β-lactam antibiotics in vitro. Antimicrob. Agents Chemother. **35:**1834–1839.
15. **Odenholt-Tornqvist, I., E. Löwdin, and O. Cars.** 1995. Postantibiotic effects and postantibiotic sub-MIC effects of roxithromycin, clarithromycin, and azithromycin on respiratory tract pathogens. Antimicrob. Agents Chemother. **39:**221–226.
16. **Poulsen, R. L., J. D. Knudsen, M. B. Petersen, K. Fuursted, F. Espersen, and N. Frimodt-Møller.** 1996. In vitro activity of six macrolides, clindamycin and tetracycline on Streptococcus pneumoniae with different penicillin susceptibilities. APMIS **104:**227–233.
17. **Pruul, H., and P. J. McDonald.** 1988. Damage to bacteria by antibiotics in vitro and its relevance to antimicrobial chemotherapy: a historical perspective. J. Antimicrob. Chemother. **21:**695–698.
18. **Ramadan, M. A., A. F. Tawfik, A. M. Shibl, and C. G. Gemmell.** 1995. Post-antibiotic effect of azithromycin and erythromycin on streptococcal susceptibility to phagocytosis. J. Med. Microbiol. **42:**362–366.
19. **Rotter, M. L., A. M. Hirschl, and W. Koller.** 1988. Effect of chlorhexidine-containing detergent, non-medicated soap or isopropanol and the influence of neutralizer on bacterial pathogenicity. J. Hosp. Infect. **11:**220–225.
20. **Shibl, A. M., J. C. Pechere, and M. A. Ramadan.** 1995. Postantibiotic effect and host-bacteria interactions. J. Antimicrob. Chemother. **36:**885–887.
21. **Watson, D. A., D. M. Musher, and J. Verhoef.** 1995. Pneumococcal virulence factors and host immune responses to them. Eur. J. Clin. Microbiol. **14:**479–490.
22. **Winstanley, T. G.** 1990. Penicillin-induced postantibiotic effects on streptococci in vitro and in vivo. J. Antimicrob. Chemother. **26:**165–168.