ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2011, p. 1814–1818 Vol. 55, No. 4
0066-4804/11/$12.00 doi:10.1128/AAC.00886-10

Comparative *In Vitro* Activities of the Novel Antibacterial Finafloxacin against Selected Gram-Positive and Gram-Negative Bacteria Tested in Mueller-Hinton Broth and Synthetic Urine▽

Axel Dalhoff,[1]* Will Stubbings,[2] and Sabine Schubert[1]

University Hospital Schleswig Holstein, Campus Kiel, Brunswiker Str. 4, D-24105 Kiel, Germany,[1] and MerLion Pharmaceuticals GmbH, Robert-Rössle Str. 10, D-13125 Berlin, Germany[2]

Received 29 June 2010/Returned for modification 13 August 2010/Accepted 22 December 2010

Kill kinetics and MICs of finafloxacin and ciprofloxacin against 34 strains with defined resistance mechanisms grown in cation-adjusted Mueller-Hinton broth (CAMHB) at pH values of 7.2 and 5.8 and in synthetic urine at pH 5.8 were determined. In general, finafloxacin gained activity at low pH values in CAMHB and remained almost unchanged in artificial urine. Ciprofloxacin MICs increased and bactericidal activity decreased strain dependently in acidic CAMHB and particularly in artificial urine.

Bacteria colonizing or infecting a host grow under hostile conditions, sense changing environmental stress via diverse quorum sensing, and other two-component systems, and respond by up- or downregulating the expression levels of appropriate proteins, which in turn can affect the susceptibility of the cell to antibiotics (2, 11). Examples of environmental stress include, e.g., growth at extreme temperatures, pH, osmotic pressure, and depletion of nutrients, etc.; *Escherichia coli* and *Staphylococcus aureus* causing uncomplicated cystitis or growing in urine or in biofilms on indwelling urethral catheters are such examples (3, 8, 24, 28, 31, 34, 35, 38).

Thus, it is physiologically relevant and clinically important to study the antibacterial activities of agents under test conditions which mimic the infectious focus most closely. Therefore, we used cation-adjusted Mueller-Hinton broth (CAMHB), pH 7.2 and pH 5.8 (Oxoid GmbH, Wesel, Germany), and synthetic urine, pH 5.8, containing 11 solutes each, in concentrations found in a 24-h period in the urine of healthy men (14) for the comparison of the bacteriostatic and -cidal activities of finafloxacin (batch CBC000288) and ciprofloxacin (batch CBC000290).

(Part of the data presented in this paper were shown as a poster at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 2008.)

Thirty-four phenotyped and/or genotyped strains were used for the examination of MICs and kill kinetics, according to CLSI guidelines (6, 7, 19). *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212, as well as moxifloxacin as an external standard, served as controls. Tests were run in duplicate; the higher values were reported in case of deviation (1.2%). Kill kinetics were examined by using finafloxacin and ciprofloxacin at bioequivalent concentrations of 16×, 4×, and 1× the individual MIC values, as measured in the corresponding media. Samples for determination of viable counts were taken at 0, 1, 2, 4, 6, 8, and 24 h. Drug carryover was minimized first by dilution and second by plating the aliquots on cation-enriched agar, thus inactivating the fluoroquinolones. In order to quantify the reduction of viable counts and the speed of kill, the times needed to reduce the inocula by 3-$\log_{10}$ titers and kill rates were calculated as described recently (32). Furthermore, kill rates were normalized to a drug exposure of 1 mg/liter, as the drug concentration/isolate/media and pH associations vary considerably under the different growth conditions studied.

A comparison of MIC values of finafloxacin generated in CAMHB at either pH 7.2 or pH 5.8 reveals that the MICs of finafloxacin decreased in CAMHB at the lower pH by 1 to 3 dilution steps for almost all of the indicator strains tested, except for *E. coli* ATCC 25922 and the *E. coli* wild-type (WT) strain (no change in MICs). The decrease in MICs was independent of both the fluoroquinolone susceptibility and species of strains tested (Table 1).

Finafloxacin MICs were higher in synthetic urine than in acidic CAMHB but identical to those determined in CAMHB at a pH value of 7.2 (exceptions include most Gram-positives, three *E. coli* strains, and *Citrobacter freundii*). *E. coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145, and *Serratia marcescens* ATCC 13880 were more susceptible in synthetic urine than they were in CAMHB, pH 7.2 (Table 1). In contrast, ciprofloxacin generally lost activity in acidic CAMHB and particularly did so in artificial urine (Table 1).

An extraordinary increase in the MICs of both finafloxacin and ciprofloxacin at the acidic pH in synthetic urine (from 0.5 and 0.06 to 64 and 128 μg/ml, respectively) was recorded for the extended-spectrum β-lactamase (ESBL)-producing clinical isolate *E. coli* CIA TEM 7. The reasons for this phenomenon are unknown, as this isolate has not yet been characterized genetically.

* Corresponding author. Mailing address: University Hospital Schleswig Holstein, Campus Kiel, Institute for Infection Medicine, Brunswiker Str. 4, D-24105 Kiel, Germany. Phone: 49-202-2655236. Fax: 49-202-2655297. E-mail: adalhoff@t-online.de.

▽ Published ahead of print on 18 January 2011.

TABLE 1. Antibacterial activities of finafloxacin and ciprofloxacin

Strain (resistance mechanism)[b]	MIC (μg/ml)					
	Finafloxacin			Ciprofloxacin		
	CAMHB		Synthetic urine, pH 5.8	CAMHB		Synthetic urine, pH 5.8
	pH 7.2	pH 5.8		pH 7.2	pH 5.8	
S. aureus						
ATCC 29213 (MSSA)[a]	0.25	0.125	0.25	0.5	2	8
ATCC 12600 (MSSA)	0.25	0.015	0.5	0.5	1	8
133 (WT)	0.25/0.125	0.015	0.25	0.06	0.5	8
clone 16 (*grlA*[*S80P*])	0.25	0.03	2	1	2	64
105-11 (*grlA*[*S80F*] *gyrA*[*S84A*])	1	0.5	4	4	16	128
104-13 (*grlA*[*S80F*] *gyrA*[*S84L*])	2	1	8	16	32	>128
103-17 (*grlA*[*S80F*] *grlA*[*E84V*] *gyrA*[*S84L*])	8	4	32	64	128	>128
Staphylococcus saprophyticus ATCC 15305[a]	0.25	0.015	0.5	0.5	0.5	8
Streptococcus agalactiae ATCC 13813	0.25	0.125	1	0.5	0.5	8
E. faecalis						
ATCC 19433[a]	1	0.5	2	1	4	8
ATCC 29212	1	0.25	1	1	2	8
E. coli						
ATCC 25922[a]	0.03	0.015	0.06	0.03	0.06	0.5
ATCC 11775	0.25	0.015	0.125	1	0.06	2
WT	0.015	0.015	0.06	0.015	0.015	2
WT-2 (*gyrA*[*D87Y*])[a]	1	0.06/0.125	1	0.125	2	16
WT-3-M4 (*gyrA*[*S83L*] *gyrA*[*D87G*] *parC*[*S80I*])	128	16	128	32	>128	>128
WT-3-1-M4 (*gyrA*[*S83L*] *gyrA*[*D87G*] *parC*[*S80R*])	32	4	32	2	32	>128
WT-3-M21 (*gyrA*[*S83L*] *gyrA*[*D87G*], lacks *parC*, *marR* deletion at 80 bp)	64	8	64	8	64	>128
WT-4-M2-1 (lacks *gyrA*, *parC*[*S80I*])[a]	0.25	0.015	0.25	0.03	0.03	2
MI (*gyrA*[*S83L*])	4	0.5	8/4	0.5/0.25	4	64
MI-4 (*gyrA*[*S83L*] *parC*[*S80I*])[a]	4	1	4	0.5	8/4	128
MII (*gyrA*[*S83L*], lacks *parC*, *marR* deletion at 175 bp)	16	2	16	2	32	>128
MIII (*gyrA*[*S83L*] *gyrA*[*D87G*], *parC* [*S80I*], *marR* deletion at 74 bp)	>128	64	>128	128	>128	>128
ESBL-producing CIA TEM-7	0.5	0.015/0.03	64	0.06	4	>128
ESBL-producing 85 (Ur 4731/06)	64	8	64	64	64	>128
K. pneumoniae						
ATCC 13883	0.6	0.015	0.25	0.03	1	2
ESBL-producing ATCC 700603	4	1	4	0.25	4	32
ESBL-producing 20 SHV-27 TEM	32/16	4	16	4	64	>128
ESBL-producing *Klebsiella oxytoca* 23 SHV-12 TEM-1	128	16	128	>128	>128	>128
C. freundii ATCC 8090	0.015	≤0.0075	0.06	≤0.0075	≤0.0075	≤0.0075
Proteus mirabilis ATCC 9240[a]	0.5	0.25/0.125	0.25/0.5	0.015	0.06	2
Enterobacter cloacae ATCC 13047	0.25	0.03	0.25	0.015	0.015	2
S. marcescens ATCC 13880	2	0.5/0.25	0.06/0.125	0.03	0.5	2
P. aeruginosa ATCC 10145[a]	4	1	1	0.06	0.5	4

[a] Time-kill experiments were performed with these strains.
[b] MSSA, methicillin-susceptible *S. aureus*.

TABLE 2. Kill rates and times needed for reduction of viable counts by 3-$\log_{10}$ titers calculated for the log-linear phases of time-kill curves

Strain	Kill rate (h^{-1})/time (h) needed for 3-$\log_{10}$ kill											
	Finafloxacin						Ciprofloxacin					
	CAMHB, pH 7.2			Synthetic urine, pH 5.8			CAMHB, pH 7.2			Synthetic urine, pH 5.8		
	1× MIC	4× MIC	16× MIC	1× MIC	4× MIC	16× MIC	1× MIC	4× MIC	16× MIC	1× MIC	4× MIC	16× MIC
S. aureus ATCC 29213	1.2/5.6	1.9/3.7	1.7/3.9	0.5/12.8	1.6/4.3	1.4/4.9	1.1/6.3	1.5/4.6	1.4/4.9	1.1/6.3	0.9/7.7	1.0/6.9
S. saprophyticus ATCC 15305	0.2/27.6	1.2/5.7	1.5/4.6	0.4/17.2	1.2/5.7	1.3/5.3						
E. coli ATCC 25922	1.1/6.3	3.2/2.1	6.3/1.1	1.3/5.3	2.3/3.0	5.6/1.2	1.9/3.6	1.9/3.6	1.8/3.8	1.1/6.3	2.9/2.4	6.6/1.0
E. coli WT-2	1.0/6.9	1.2/5.7	1.8/3.8	0.06/>24	1.4/4.9	1.9/3.6	0.7/9.8	1.9/3.6	2.4/2.8	0.3/23	2.5/2.7	3.1/2.2
E. coli WT-4-M2-1	1.5/4.6	2.9/2.4	4.5/1.5	2.0/3.4	3.1/2.2	3.1/2.2						
E. coli MI-4	1.8/3.8	2.3/3.0	2.4/2.8	1.7/4.0	2.7/2.6	2.8/2.5						
P. mirabilis ATCC 9240	0.6/11.5	3.6/1.9	6.2/1.1	1.5/4.6	2.8/2.5	5.0/1.4						
E. cloacae ATCC 13047	0.8/8.6	1.6/4.3	2.3/3.0	1.0/6.9	2.0/3.4	2.2/3.1						
E. faecalis ATCC 29212	0.9/7.7	1.4/4.9	1.5/4.6	0.2/>24	1.2/5.7	1.3/5.3	1.0/6.9	1.3/5.3	1.2/5.7	0.6/11.5	1.2/5.7	1.4/4.9
P. aeruginosa ATCC 10145	1.9/3.6	2.6/2.6	3.3/2.1	1.3/5.3	1.8/3.8	2.2/3.1						

The bactericidal activities of bioequivalent concentrations (i.e., multiples of the MICs, as determined in the two media) of finafloxacin and ciprofloxacin were compared with each other in CAMHB, pH 7.2, and in synthetic urine, pH 5.8. The absolute drug concentrations used under these conditions for finafloxacin were almost the same, as the MICs differed in the two media by one titration step, if at all (except for *P. aeruginosa*), whereas the ciprofloxacin concentrations were twice as high (*E. coli* ATCC 25922) to up to 128 times higher (*E. coli* WT-2) in synthetic urine than in CAMHB, pH 7.2.

In general, the bactericidal activities of both of the fluoroquinolones are comparable under these prevailing conditions, as evidenced by the times needed to reduce the inocula of the test strains by 3-$\log_{10}$ titers (Table 2).

The kill rates calculated for finafloxacin against the two staphylococci tested are almost concentration independent in both media (Table 2). In contrast, the kill rates for finafloxacin against all *E. coli* and the remaining reference strains tested were concentration dependent in both media (Table 2). Similar kill rates were calculated for most of the strains (except *E. coli* ATCC 25922) exposed to ciprofloxacin (Table 2).

These data demonstrate that the absolute kill rates for both of the fluoroquinolones are almost comparable. However, the absolute finafloxacin or ciprofloxacin concentrations to which the strains studied in this series of experiments were exposed differed by up to 32-fold, so the values were concentration normalized in order to enable a direct comparison of the two agents. A comparison of the normalized kill rates reveals that the values of finafloxacin are higher, i.e., more rapid killing occurs, than those of ciprofloxacin for *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, lower for *E. coli* WT-2, and comparable for *E. faecalis* ATCC 29212 when the strains were grown in CAMHB at pH 7.2. The normalized kill rates of finafloxacin are up to 47 times higher than those of ciprofloxacin for all four strains grown in synthetic urine at pH 5.8 (Fig. 1).

The data generated in this series of experiments confirm, in agreement with previous findings (reviewed in references 10 and 36), that the MICs and bactericidal activity of ciprofloxacin are reduced in acidic media and in synthetic urine. Higher urinary concentrations of magnesium, which are commonly in the range of 8 to 10 mM, account in large part for this reduction in activity. Supplementation of standard media with magnesium at levels present in human urine results in 2- to 16-fold increases in the MICs of any commercially available fluoroquinolone for various bacterial species. The reduced potencies of the fluoroquinolones in urine can also be ascribed in part to the diminished activities of many fluoroquinolones at low pH levels prevailing in these media. Decreased bacteriostatic or bactericidal activities in acidified media could be demonstrated for every commercially available fluoroquinolone. Finafloxacin, however, is almost not affected by growth in urine and is even activated by growth in an acidic standard medium.

Other drug classes like aminoglycosides, macrolides, and tetracyclines and trimethoprim are also affected by these phenomena (1, 15, 20, 21, 26, 27, 29, 33, 37) and are characterized by an increased propensity for resistance development (9). In general, acidity—provided the molecules are not hydrolyzed—has little effect on the bacteriostatic activity of β-lactams (4, 18, 30), but their bactericidal activity is significantly diminished to an almost bacteriostatic effect (13). Furthermore, the β-lactamase inhibitory activities of tazobactam and sulbactam, but not clavulanate, are variably reduced at low pH values, with 50% inhibitory concentrations being up to 300-fold higher at pH 6.5 than at pH 8.0 (18).

These examples demonstrate that the bacteriostatic and/or bactericidal activities of many agents used in the treatment of bacterial infections are impaired at the low pH values and/or high osmolarity which prevail at many infectious sites (5, 9, 12, 16, 17, 22, 23, 25).

The *in vitro* activity of finafloxacin, however, is not negatively affected by these conditions, thus indicating that finafloxacin may be effective in the treatment of infections within acidic

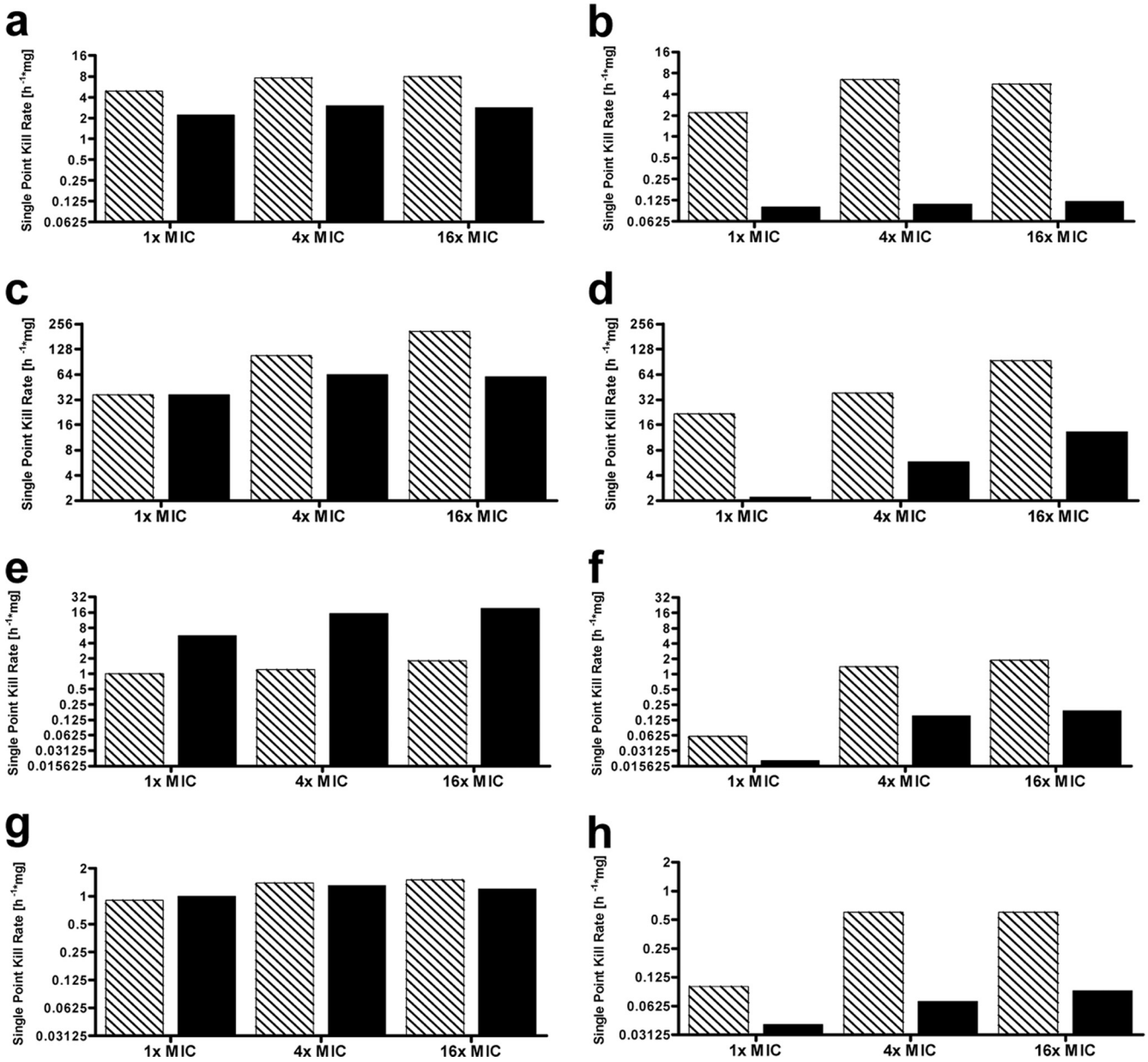

FIG. 1. Relative single-point kill rates normalized to a drug concentration of 1 mg/liter. Relative single-point kill rates of finafloxacin (hatched bars) and ciprofloxacin (filled bars) in CAMHB, pH 7.2 (left), and synthetic urine, pH 5.8 (right), against *S. aureus* ATCC 29213 (a and b), *E. coli* ATCC 25922 (c and d), *E. coli* WT-2 (e and f), and *E. faecalis* ATCC 29212 (g and h).

foci. Controlled clinical studies to address this hypothesis are warranted.

This work was supported by a research grant from MerLion Pharmaceuticals GmbH, Berlin, Germany.

This publication is dedicated to Harald Labischinski, who died on 24 August 2010. His work was devoted to the discovery and development of new antibacterials. Finafloxacin was the last project on which he worked.

REFERENCES

1. **Aagaard, J., P. O. Madsen, P. Rhodes, and T. Gasser.** 1991. MICs of ciprofloxacin and trimethoprim for *Escherichia coli*: influence of pH, inoculum size and various body fluids. Infection **19**(Suppl. 3)**:**167–169.
2. **Atkinson, S., and P. Williams.** 2009. Quorum sensing and social networking in the microbial world. J. R. Soc. Interface **6:**959–978.
3. **Beenken, K. E., et al.** 2004. Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol. **186:**4665–4684.
4. **Bodey, G. P., V. Rodriguez, and S. Weaver.** 1976. Pirbenicillin, a new semisynthetic penicillin with broad-spectrum activity. Antimicrob. Agents Chemother. **9:**668–674.
5. **Borrill, Z., C. Starkey, J. Vesttbo, and D. Singh.** 2005. Reproducibility of exhaled breath condensate pH in chronic obstructive pulmonary disease. Eur. Respir. J. **25:**269–274.
6. **Clinical and Laboratory Standards Institute/NCCLS.** 1999. Methods for determining bactericidal activity of antimicrobial agents, vol. 19, p. 18. Approved guideline M26-A. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Clinical and Laboratory Standards Institute/NCCLS.** 2001. Performance standards for antimicrobial susceptibility testing, vol. 21, p. 1. 11th informa-

tional supplement. M100-S11. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Cotter, P. D., and C. Hill.** 2003. Surviving the acid test: response of Gram-positive bacteria to low pH. Microbiol. Mol. Biol. Rev. **67:**429–453.
9. **Dalhoff, A., S. Schubert, and U. Ullmann.** 2005. Effect of pH on the *in vitro* activity of and propensity for emergence of resistance to fluoroquinolones, macrolides, and a ketolide. Infection **33**(Suppl. 2)**:**36–43.
10. **Eliopoulos, G. M., and C. T. Eliopoulos.** 1993. Activity *in vitro* of the quinolones. *In* D. C. Hooper and J. S. Wolfson (ed.), Quinolone antibacterial agents, 2nd ed. ASM Press, Washington, DC.
11. **Geske, G. D., J. O'Neill, and H. E. Blackwell.** 2008. Expanding dialogues: from natural autoinducers to non-natural analogues that modulate quorum sensing in Gram-negative bacteria. Chem. Soc. Rev. **37:**1432–1447.
12. **Gitlin, N.** 1990. Ascitic fluid acidosis in bacterial peritonitis: a poor prognostic sign? Hepatology **11:**896–898.
13. **Goodell, E. W., R. Lopez, and A. Tomasz.** 1976. Suppression of lytic effect of beta-lactams on *Escherichia coli* and other bacteria. Proc. Natl. Acad. Sci. U. S. A. **73:**3293–3297.
14. **Griffith, D. P., D. M. Musher, and C. Itin.** 1976. The primary cause of infection-induced urinary stones. Invest. Urol. **13:**346–350.
15. **Gudmundsson, A., H. Erlendsdottir, M. Gottfredsson, and S. Gudmundsson.** 1991. Impact of pH and cationic supplementation on *in vitro* postantibiotic effect. Antimicrob. Agents Chemother. **35:**2617–2624.
16. **Hunt, J. F., et al.** 2000. Endogenous airway acidification. Implications for asthma pathophysiology. Am. J. Respir. Crit. Care Med. **161:**694–699.
17. **Kalantzi, L., et al.** 2006. Characterisation of the human upper gastrointestinal content under conditions simulating bioavailability/bioequivalence studies. Pharm. Res. **23:**165–176.
18. **Livermore, D. M., and J. E. Corkill.** 1992. Effects of CO_2 and pH on inhibition of TEM-1 and other β-lactamases by penicillanic acid solutions. Antimicrob. Agents Chemother. **36:**1870–1876.
19. **Lorian, V. (ed.).** 1996. Antibiotics in laboratory medicine, 4th ed. Williams & Wilkins Co., Baltimore, MD.
20. **Miller, T., and S. Phillips.** 1983. Effect of physiological manipulation on the chemotherapy of experimentally induced renal infection. Antimicrob. Agents Chemother. **23:**422–428.
21. **Minuth, J. N., D. M. Musher, and S. B. Thorsteinsson.** 1976. Inhibition of the antibacterial activity of gentamicin by urine. J. Infect. Dis. **133:**14–21.
22. **Montuschi, P.** 2007. Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. Ther. Adv. Respir. Dis. **1:**5–23.
23. **Ojoo, J. C., S. A. Mulrennan, J. A. Kastelik, A. H. Morice, and A. E. Redington.** 2005. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Thorax **60:**22–26.
24. **O'Neill, E. O., et al.** 2008. Novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J. Bacteriol. **190:**3835–3850.
25. **Pandit, N. K.** 2007. Introduction to the pharmaceutical sciences. Chapter 9: drug absorption. Lippincott Williams and Wilkins, Philadelphia, PA.
26. **Papapetropoulou, M., J. Papavassiliou, and N. J. Legakis.** 1983. Effect of the pH and osmolarity of urine on the antibacterial activity of gentamicin. J. Antimicrob. Chemother. **12:**571–575.
27. **Peddie, B. A., and S. T. Chambers.** 1993. Effects of betaines and urine on the antibacterial activity of aminoglycosides. J. Antimicrob. Chemother. **31:**481–488.
28. **Resch, A., R. Rosenstein, C. Nerz, and F. Götz.** 2005. Differential gene expression profiling *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. Appl. Environ. Microbiol. **71:**2663–2676.
29. **Sabath, L. D.** 1978. Six factors that increase the activity of antibiotics in vivo. Infection **6**(Suppl. 1)**:**67–71.
30. **Sanders, C. C.** 1983. Alzocillin: a new broad spectrum penicillin. J. Antimicrob. Chemother. **11**(Suppl. B)**:**21–31.
31. **Sato, S., and H. Kobayashi.** 2003. Bacterial responses to alkaline stress. Sci. Prog. **66:**271–282.
32. **Schaper, K. J., S. Schubert, and A. Dalhoff.** 2005. Kinetics and quantification of anti bacterial effects of beta-lactams, macrolides, and quinolones against gram-positive and gram-negative RTI pathogens. Infection **33**(Suppl. 2)**:** 3–14.
33. **Schlessinger, D.** 1988. Failure of aminoglycoside antibiotics to kill anaerobic, low-pH, and resistant cultures. Clin. Microbiol. Rev. **1:**54–59.
34. **Schumann, W.** 2007. Bacterial stress sensors. *In* S. K. Calderwood (ed.), Protein reviews: cell stress proteins, vol. 7. Springer Science and Business Media LLC, New York, NY.
35. **Stickler, D. J., N. S. Morris, J. C. Robert, R. J. C. McLean, and C. Fuqua.** 1998. Biofilms on indwelling urethral catheters produce quorum-sensing molecules in situ and in vitro. Appl. Environ. Microbiol. **64:**3486–3490.
36. **Thauvin-Eliopoulos, C., and G. M. Eliopoulos.** 2003. Activity in vitro of the quinolones. *In* D. C. Hooper and J. S. Wolfson (ed.), Quinolone antibacterial agents, 3rd ed. ASM Press, Washington, DC.
37. **Wisell, K. T., G. Kahlmeter, and C. G. Giskes.** 2008. Trimethoprim and enterococci in urinary tract infections: new perspectives on an old issue. J. Antimicrob. Chemother. **62:**35–40.
38. **Yarwood, J. M., D. J. Bartels, E. M. Volper, and E. P. Greenberg.** 2004. Quorum sensing in *Staphylococcus aureus* biofilms. J. Bacteriol. **186:**1838–1850.