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In vitro activity of four fluoroquinolones on selected bacteria

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IN VITRO ACTIVITY OF FOUR FLUOROQUINOLONES ON SELECTED BACTERIA

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ABSTRACT

In 1990-1991, in a national surveillance study, and in 1991-1992, in a follow-up study, both by Thornsberry et al. (1993), ciprofloxacin data from various geographical and demographical institutions were collected. Several species of bacteria have shown resistance to ciprofloxacin and norfloxacin, but the degree of resistance to these drugs has not been reported for the Stockton area. To determine the extent of this resistance, Dameron Hospital antibiograms generated from 1990 to 1994 were reviewed and compared. Results of the comparison show that susceptibility among the Gram-negative isolates, with the exception of *Providencia stuartii*, *Acinetobacter lwoffi*, and to a lesser extent *Aeromonas hydrophila*, has changed very little. Consistent with the national surveys, resistance of *Pseudomonas aeruginosa* has not changed appreciably during the five-year period.

Among the Gram-positive isolates that were tested against both ciprofloxacin for a five-year period (1990-1994) and norfloxacin for a three-year period (1992-1994), increased resistance was seen among strains of *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, and *Enterococcus faecalis*, but not among strains of *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, and *S. agalactiae*. To determine whether resistance to one fluoroquinolone occurs also to other fluoroquinolones, several isolates of Gram-positive cocci and *P. aeruginosa* from the

Gram-negative bacilli that showed resistance to either ciprofloxacin, norfloxacin, or both were selected from Dameron Hospital isolates and tested by the disk diffusion technique against ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin. The results indicate that differences do exist among these selected strains. Comparison of the in-vitro effectiveness of the various quinolones confirms that methicillin-resistant staphylococci (*S. aureus*, *S. epidermidis*, and *S. haemolyticus*) exhibit a higher degree of resistance to the four fluoroquinolones compared with the methicillin-susceptible strains of the same species. Resistance of the enterococci (*Enterococcus faecalis* and *E. faecium*) is also high. Generally, when the four fluoroquinolones were compared with each other, ofloxacin seemed to have better in vitro activity.

Resistance to the quinolones consists of two proposed mechanisms: (1) mutation of one or both of the structural genes of the A and B subunits of DNA gyrase and (2) decreased drug accumulation due either to lower uptake by the cell or enhanced efflux out of the cell. These mechanisms of resistance are reviewed.

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INTRODUCTION

> "Fluoroquinolones such as ciprofloxacin, which may be administered orally or intravenously, exhibit a wide spectrum of antibacterial activity. This broad spectrum of activity, combined with ease of administration, excellent absorption, high tissue permeability, and favorable safety profile have made these agents highly desirable in the treatment of a wide variety of infections.
>
> Although the fluoroquinolones have proven to be highly effective antimicrobial agents, some concern has arisen that resistance may develop rapidly among previously susceptible organisms. This concern was supported by a series of published reports demonstrating the development of resistance in *P. aeruginosa, S. aureus* (predominantly methicillin-resistant) and other organisms" (Thornsberry et al., 1993: 4).

Based on two studies, 1990-91 and 1991-92, Thornsberry et al. (1993) reported the results of a national surveillance of bacterial resistance based on data from 162 institutions in 38 states. The susceptibility data for the two years involved over 921,000 isolates representing 56 genera and 176 species or groups of Gram-positive and Gram-negative bacteria. Ciprofloxacin was the only fluoroquinolone reported and its susceptibility data was based on 441,218 isolates.

Using MIC (minimum inhibitory concentration) data and interpretations based on National Committee for Clinical Laboratory Standards (NCCLS) publication M7-A2, Thornsberry et al. (1993) found 86% of the 1991-92 isolates susceptible (S) to ciprofloxacin, 3.1% intermediate (I), and 10.9% resistant (R) compared with 88.2%, 4.6%, and 7.2% respectively for 1990-91.

This led to the conclusion that the occurrence of limited ciprofloxacin resistance in clinically important bacterial species is well established, especially in methicillin-resistant staphylococci and that various mechanisms of resistance have been determined, but the extent of this resistance is less well known. Since the degree and extent of this resistance to ciprofloxacin and other quinolones for the Stockton area is unknown, it was proposed that I conduct a retrospective study beginning with 1990, the year that ciprofloxacin came to be used at Dameron hospital, and extending through 1994. This retrospective study, to determine degree and extent of resistance to ciprofloxacin and other quinolones among bacterial isolates from Dameron Hospital in Stockton, California, became my primary objective.

Thornsberry's data does not include susceptibility results to norfloxacin or other quinolones. Dameron Hospital Microbiology Laboratory has been testing norfloxacin since 1990, and during January and February of 1994, tested ofloxacin and lomefloxacin. During these five years, a number of articles appeared comparing the in vitro activity of fluoroquinlones with one another; among the recent ones are Wadworth and Goa (1991), Walker and Wright (1991), Bongaerts and Hoogkamp-Korstanje (1993), Canton et al. (1993), Marshall et al, (1993), Tanaka et al. (1993), Chang et al. (1994), and Jones et al. (1994). Since mechanisms of action of the quinolones are likely to be the same and that factors responsible for ciprofloxacin resistance are also assumed to be involved in cross-resistance to the other quinolones (Cambau and Gutmann, 1993), I decided to test this assumption by comparing in vitro activities of the four fluoroquinolones on various bacterial species including both

methicillin-resistant and methicillin-susceptible strains. I would like to emphasize at this point that these data and results are based on in vitro studies and the discussion will be limited to in vitro results. Neither pharmacokinetics of the different quinolones nor in vivo advantages of one over another is a component of this study.

HISTORICAL REVIEW

The quinolones are a group of synthetic antimicrobial drugs that are highly active against bacteria but show low toxicity to eukaryotic cells. Selectivity to bacterial cells is due to the unique features of their DNA gyrase, or topoisomerase II, an intracellular enzyme which was originally identified by Gellert et al. (1976) and upon which quinolones are believed to act. Prokaryotic topoisomerase II is required in DNA synthesis, specifically to insert negative supercoils into the covalently closed circular bacterial chromosome, an essential step for the cell to accommodate the very long chromosome (Smith, 1986). In addition to inserting negative supercoils, topoisomerase II provides the information required for such functions as transcription, DNA repair, recombination, and transposition (Wolfson and Hooper, 1985; von Rosentiel and Adam, 1994). In mammalian cells, this function is performed by topoisomerase I (Crumplin, 1986).

This class of chemotherapeutic agents can be defined, according to Wentland (1990), as 1-substituted 1,4-dihydro-4-oxo-3-pyridinecarboxylic acids with an additional ring fused to positions 5 and 6 (Fig. 1). The antimicrobial characteristics of these compounds were discovered in the late 1950s (Wentland, 1990) when researchers at the Sterling Winthrop Research Institute isolated several byproducts of the antimalarial agent chloroquine. One such byproduct, 7-chloro-1-ethyl-1,4-

dihydro-4-oxo-3-quinolinecarboxylic acid (Fig. 2), was found to have sufficient in vitro antibacterial qualities to render it worthy of further study. This compound provided the basis for further analog design and synthesis leading to the introduction of nalidixic acid, 1-ethyl-7-methyl-1,8-napthyridine-4-one-3-carboxylic acid (Fig. 3), the first clinically useful quinolone (Lesher et al., 1962).

Shortly after the introduction of nalidixic acid, it became evident that this drug was of limited therapeutic value because of its weak potency against Gram-positive bacteria, rapid development of resistance to it in vitro and in vivo, irrespective of dosage (Ronald et al., 1966), and its lack of activity against *Pseudomonas* (Neu, 1989). Although its low serum level due to rapid renal clearance limits its use for treatment of systemic bacteremias, it renders it ideal for the treatment of urinary tract infections caused by Gram-negative bacteria.

Subsequent work on these compounds during the 1970s produced oxolinic acid (Fig. 4) and cinoxacin (Fig. 5), two 4-quinolones with a common skeleton, 4-oxo-1,4-dihydroquinolone (Fig. 6). These, however, had only marginal improvements over nalidixic acid (Norris and Mandell, 1988). Other 4-quinolones produced shortly after were acrosoxacin, pipedemic acid, and piromidic acid. Definite progress was made when it was discovered that the addition of a fluorine atom to 4-oxo-1,4-dihydroquinolone extends its in vitro activity to the Gram-positive bacteria. The substitution of fluorine at position 6 appears also to improve the compound's binding to DNA gyrase and penetration across the bacterial cell wall (Neu, 1989). This 4-quinolone with 6-fluorine became the basic quinolone from which other

fluoroquinolones were derived. Norfloxacin (Fig. 7) was the first to be synthesized through the addition of an ethyl group at position 1, a carboxyl group at position 3, and a piperazinyl group at position 7. As the first fluorquinolone, norfloxacin demonstrated improved Gram-positive and Gram-negative antibacterial activity including that against staphylococci and pseudomonads, respectively. With the development of norfloxacin, the fluoroquinolone era had begun (Wentland, 1990).

Since the synthesis of norfloxacin, many other fluoroquinolones have been synthesized including amifloxacin, difloxacin, flumequine, levofloxacin, sparfloxacin, tosufloxacin, ciprofloxacin, enoxacin, ofloxacin, pefloxacin, temafloxacin (withdrawn from worldwide markets in 1992), fleroxacin, and lomefloxacin (von Rosenstiel and Adam, 1994), all containing a similar skeleton. The quinolones most commonly used in Stockton hospitals at the start of this study were nalidixic acid, cinoxacin, norfloxacin, ciprofloxacin, ofloxacin, and lomefloxacin. The various chemical groups that are added to manufacture ciprofloxacin, ofloxacin and lomefloxacin are shown in figures 8, 9, and 10, respectively.

Fluorine at position 6, as stated earlier, appears to be the most active substituent leading to improved penetration across the bacterial cell wall and >10-fold increase in gyrase inhibition. According to Neu (1989) and Domagala (1994), the carboxyl group and keto group at positions 3 and 4, respectively, mediate the binding of these compounds to DNA gyrase. The proximity of these two groups probably contributes to passage across the bacterial cell wall due to a chelating effect that allows increased entry of the compound. Microbiological and pharmacokinetic

properties are greatly influenced by the substituents at the N-1 or C-7 positions. The cyclopropyl group at the N-1 position, as in ciprofloxacin, appears to be the most potent substitution (Domagala, 1994) against *Enterobacteriaceae* and *Pseudomonas aeruginosa* compared to the addition of an ethyl group as in norfloxacin and lomefloxacin (Neu, 1989). The greatest modification of all positions has been at C-7; excellent in vitro activity is seen with a piperazinyl group, as in norfloxacin and ciprofloxacin, or with a methylpiperazinyl group as in ofloxacin. Both of these groups increase the potency of these compounds towards both Gram-positive and Gram-negative bacteria (Neu, 1989) and greater in vivo efficacy (Domagala, 1994). Alkyl substitutions of the piperazinyl ring at position 7, such as in lomefloxacin, further increase serum half-life and activity against Gram-positive bacteria (Domagala, 1994). Position 8 controls efficiency in vivo with an optimal addition of a fluorine as in lomefloxacin. A ring formed between C-8 and N-1, as in ofloxacin, provides improved Gram-positive and anti-anaerobic activity (Neu, 1989; Domagala, 1994). The substituent at position 5 controls in vitro potency with optimal groups being NH_2, OH, and CH_3, and greatest improvements being in Gram-positive organisms (Domagala, 1994) evident in fluoroquinolones not included in this study. Comprehensive reviews of structure-activity relationships of the fluoroquinolones have been published (Chu and Fernandes, 1989; Domagala, 1994).

MATERIALS AND METHODS

This study is based, in part, on data accumulated between 1990 and 1994 at Dameron Hospital and partly on selected species of bacteria tested by me (in-house testing) during 1993.

Dameron Hospital Antibiogram 1990-1994 The number of isolates encountered in the retrospective study consisted of 10,764 strains belonging to 20 species of Gram-negative bacilli (Table 1) and 6445 belonging to 7 species of Gram-positive cocci (Table 2).

In-House testing The data of 1990-1992 suggested that most resistance to the fluoroquinolones was among the Gram-positive cocci and in *Pseudomonas aeruginosa* among the Gram-negative bacilli. The sample selected, in most cases, consisted of strains that showed resistance to either ciprofloxacin, norfloxacin, or both. These included 208 isolates of Gram-positive cocci: 8 methicillin-susceptible *Staphylococcus aureus* (MSSA), 26 methicillin-resistant *S. aureus* (MRSA), 7 methicillin-susceptible *Staphylococcus epidermidis* (MSSE), 64 methicillin-resistant *S. epidermidis* (MRSE), 27 *Staphylococcus haemolyticus*, 44 *Enterococcus faecalis*, 19 *E. faecium*, 6 *Streptococcus agalactiae*, 7 *S. bovis*; and 21 isolates of *Pseudomonas*

aeruginosa. These specimens, collected during 1993, were tested against 4 fluoroquinolones: ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin.

Antimicrobial drugs The quinolones were obtained from various sources: ciprofloxacin disks (5 ug) were donated by Miles Pharmaceutical Division (West Haven, Conneticut) through the courtesy of their local representative; norfloxacin (10 ug), ofloxacin (5 ug), and lomefloxacin (10 ug) were purchased from Becton Dickenson Microbiology Systems (Cockeysville, Maryland).

Media Media used included Mueller-Hinton antimicrobial susceptibility plates for staphylococci and *P. aeruginosa*; mannitol salt agar (MSA) plates, eosin methylene blue (EMB) plates, tryptic soy agar (TSA) slants, and saline solution were prepared according to the manufacturer's instructions or standard procedures and autoclaved at 250 F, 15 lbs pressure for 20 minutes. For streptococci and enterococci, Mueller-Hinton blood agar plates (BAP) were purchased from PML Microbiologicals (Prepared Media Laboratory, Tualatin, OR).

Stock cultures Isolates were brought from Dameron Hospital on TSA, blood agar, or chocolate agar slants. The slants were subcultured on the following media to ascertain purity: MSA for staphylococci, BAP for streptococci and enterococci, and EMB for *P. aeruginosa*. Following 24-hour incubation, two to three colonies from each plate were selected, transferred to appropriate slants, and

incubated overnight and used as stock culture. Stock cultures were kept in the refrigerator at 2-8 C.

Antimicrobial susceptibility testing Disk diffusion susceptibility testing was performed using the proper Mueller-Hinton plates: clear Mueller-Hinton for staphylococci and *P. aeruginosa* and BAP for streptococci and enterococci. The stock culture of each isolate was removed from the refrigerator and reincubated for 2-4 hours at 35 C. While in their logarithmic growth phase, an inoculum was taken from each slant and introduced into 5.0 ml saline to obtain a turbidity equivalent to 0.5 McFarland Standard (0.05 ml barium chloride in 9.95 ml 1% sulfuric acid) and a cell density of approximately 10^8/ml. The turbidity was estimated visually against the Standard tube and adjusted, if necessary, by either adding inoculum or sterile saline. A sterile swab (Hardwood Products Company) was then dipped into the saline suspension, drained by pressing against the inside of the saline tube above the level of the fluid, and a single streak was made in the center of the appropriate Mueller-Hinton susceptibility plate. The same swab was then used to spread the organisms on the streak line in three dimensions, horizontal, vertical, and diagonal, to ensure uniform inoculation of the entire surface of the plate. Antimicrobial disks of ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin were applied by gently pressing the disks with sterile forceps to ensure adherence to the plates. Plates were incubated at 35 C for 18 to 24 hours. The diameter of the zone of inhibition for each disk was measured using a millimeter ruler and transmitted light. When excessive confluent growth or

contamination was observed, the entire test for that organism was repeated. The observed zones of inhibition were interpreted according to NCCLS document M2-A4 (Appendix) and recorded.

Quality control testing Quality control strains, purchased from American Type Culture Collection, of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), and *Streptococcus bovis* (ATCC 49147) were tested weekly to ascertain proper performance of media and antimicrobial disks.

RESULTS

These results are based on information accumulated between 1990 and 1994 from Dameron Hospital and on data obtained from in-house testing. It should also be noted that the data from Dameron Hospital is based on MIC results whereas in-house testing utilizes disk diffusion methods. Between 1990 and 1994, 10 different types of MIC panels for Gram-negative bacilli and three different types of MIC panels for Gram-positive cocci have been manufactured by Microscan (Microscan; Sacramento, California) and used at Dameron Hospital. Three Gram-negative MIC panels were used for isolates from the urinary tract and seven for isolates from other body sites. Certain panels contained all four quinolone types while others contained only ciprofloxacin and norfloxacin. The results of the retrospective study are summarized in tables 1 and 2 and those of in-house testing in tables 3-12.

Retrospective study

Table 1 shows results of the activities of the 4 fluoroquinolones on 20 species of Gram-negative bacteria, 16 Enterobacteriaceae and 4 non-Enterobacteriaceae. A total of 10,764 isolates were tested for susceptibility to ciprofloxacin, 5246 isolates to norfloxacin, 328 to ofloxacin, and 204 to lomefloxacin (only 1994 data available for ofloxacin and lomefloxacin).

Ciprofloxacin susceptibility Among the 20 Gram-negative species, 9 Enterobacteriaceae (*Escherichia coli, Shigella sonnei, Klebsiella pneumoniae, Citrobacter diversus, C. amalonaticus, Proteus mirabilis, P. vulgaris, Morganella morganii,* and *Salmonella* spp.) showed 100% susceptibility to ciprofloxacin; for 6 species, susceptibilities varied from 99.7% to 95.4% (*Klebsiella oxytoca*, 99.7%; *Enterobacter cloacae*, 99.6%; *E. aerogenes*, 99.4%; *Citrobacter freundi*, 99.3%; *Providencia rettgeri*, 96.2%; *Serratia marcescens*, 95.4%); and *Providencia stuartii*, 85.4%. Among the 4 non-Enterobacteriaceae, *Aeromonas hydrophilia* and *Pseudomonas aeruginosa* showed susceptibilities of 95.9%, *Acinetobacter lwoffi* 92.9%, and *Xanthamonas maltophilia* 75.6% for this quinolone. Among the Enterobacteriaceae, only *P. stuartii* showed susceptibility of less than 90% (85.4%) to ciprofloxacin, and *X. maltophilia* was least susceptible among the non-Enterobacteriaceae. A consistent downward trend was evident among *A. hydrophila* strains.

Norfloxacin susceptibility Among the 16 species of Enterobacteriaceae, 8 (*S. sonnei, K. oxytoca, E. cloacae, C. diversus, C. amalonaticus, P. vulgaris, M. morganii,* and *Salmonella* spp.) displayed 100% susceptibility to norfloxacin; 8 showed susceptibilities between 99.6% and 60% (*E. coli*, 99.6%; *K. pneumoniae*, 99.1%; *P. mirabilis*, 99.1%; *E. aerogenes*, 98.3%; *C. freundii*, 94.3%; *P. rettgeri*, 84.6%; *S. marcescens*, 78.4%; and *P. stuartii*, 60%). Among the non-

Enterobacteriaceae, the 4 species showed the following susceptibilities to norfloxacin: *A. hydrophilia*, 100%; *P. aeruginosa*, 90%; *A. lwoffi*, 88.2%; and *X. maltophilia*, 0%. Again the least susceptible among the Enterobacteriaceae was *P. stuartii* (60%); among the non-Enterobacteriaceae, all four strains of *X. maltophilia* showed no susceptibility.

Ofloxacin susceptibility Among the 15 species of Gram-negative Enterobacteriaceae, 12 showed 100% susceptibility during the one year tested (*S. sonnei, K. pneumonia, K. oxytoca, E. cloacae, E. aerogenes, S. marcescens, C. diversus, C. amalonaticus, P. mirabilis, P. vulgaris, M. morganii,* and *Salmonella* spp.). *Escherichia coli* isolates showed 99% susceptibility, *C. freundii* 94%, and *P. stuartii* 50%. Only two species of non-Enterobacteriaceae were tested: *A. lwoffi* displayed 90% susceptibility and *P. aeruginosa* 81%. Among the Enterobacteriaceae, *P. stuartii* again showed least susceptibility (50%).

Lomefloxacin susceptibility Among the 12 species of Enterobacteriaceae, 8 showed 100% susceptibility during the one year tested (*K. pneumoniae, K. oxytoca, E. cloacae, E. aerogenes, C. diversus, P. mirabilis, P. vulgaris,* and *M. morganii*); the remaining 4 displayed the following susceptibilities: *E. coli*, 98%; *C. freundii*, 83%; and *S. marcescens* and *P. stuartii*, 0%. Among the non-Enterobacteriaceae, *P. aeruginosa* showed susceptibility of 76% while *A. lwoffi* displayed 67%. *P. stuartii*

was also the least susceptible among the Enterobacteriaceae; however, it should be emphasized that only one strain was tested.

Among the Gram-negative bacilli, 13 of 20 species (65%) show greater than 99% susceptibility to ciprofloxacin, 12 of 20 (60%) to norfloxacin, 13 of 17 (76.5%) to ofloxacin, and 8 of 14 (57.1%) to lomefloxacin. The Enterobacteriaceae showed a high level of susceptibility to all quinolones, while the non-Enterobacteriaceae showed relatively lower susceptibility levels. For the Gram-negative bacteria tested, *P. stuartii* and *X. maltophilia* displayed non-susceptibility rates to ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin that exceeded 10%; *P. aeruginosa* displayed both ofloxacin and lomefloxacin non-susceptibility rates of 19% and 24% respectively, and *P. rettgeri* and *S. marcescens* showed norfloxacin non-susceptibility rates of 15.4% and 21.6% repectively.

When the 4 quinolones are compared to one another, certain trends are evident. Ciprofloxacin and norfloxacin seemed to be equally effective on most species with the exception of *S. marscens*, *P. rettgeri*, and *P. stuartii*; 95.4% of *S. marscens*, 96.2% of *P. rettgeri*, and 85.4% of *P. stuartii* were susceptible to ciprofloxacin compared with 78.4%, 84.6%, and 60%, respectively, to norfloxacin. These observations suggest that ciprofloxacin is more effective in vitro than norfloxacin against certain species of Gram-negative bacilli. The number of isolates of these 3 species tested against ofloxacin and lomefloxacin was too small (*P. rettgeri* was not tested) to allow for comparison. When the activities of the 4 quinolones were compared with species having 10 or more isolates, table 1 shows that ciprofloxacin,

norfloxacin, ofloxacin, and lomefloxacin are equally effective against *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, C. *freundii*, and *P. mirabilis*. Comparing the in vitro efficacy of the quinolones against *P. aeruginosa*, the data suggest that ciprofloxacin is the most effective (95.9%) compared with norfloxacin (90%), ofloxacin (81%), and lomefloxacin (76%).

Table 2 shows the activities of two quinolones, ciprofloxacin over a five year period and norfloxacin over a three year period and their effect, on 7 species of Gram-positive cocci. A total of 6445 organisms were tested against ciprofloxacin and 1525 against norfloxacin.

Ciprofloxacin susceptibility Among the staphylococci tested, *S. saprophyticus* isolates were 100% susceptible. *S. aureus* showed 85.6% susceptibility compared to 70.8% and 56.1% for *S. epidermidis* and *S. haemolyticus*, respectively. Among the streptococci, *S. pyogenes* showed 99.3% susceptibility and *S. agalactiae* 98.9%. *E. faecalis* showed 89.3% susceptibility. A susceptibility trend is evident for *S. epidermidis* decreasing every year from 1990 to 1994 with an average of 70.8% susceptibility; *S. haemolyticus* showed a similar trend from 1990 to 1994 and an average of 56.1% susceptibility. *S. haemolyticus* was the least susceptible overall.

Norfloxacin susceptibility Among the staphylococci tested, *S. saprophyticus* was 100% susceptible, *S. aureus* 77.1%, *S. epidermidis* 61.3%, and *S. haemolyticus* 59.4%. For the streptococci, *S. pyogenes* showed 100% suceptibility and

S. agalactiae 83.9%. *E. faecalis* displayed 77.9% susceptibility. Again, *S. haemolyticus* showed the least susceptibility.

Methicillin-resistant staphylococci are known to exhibit resistance to many antibiotics (hence also the designation multiply-resistant staphylococci), including the fluoroquinolones, compared with methicillin-susceptible ones. The data in table 2 does not, however, distinguish between the two groups.

Ofloxacin and lomefloxacin were not tested. Overall, 83.9% of the Gram-positive isolates were susceptible to ciprofloxacin and 75.6% to norfloxacin.

In-house testing

The second component of this study was to compare the results of the 4 quinolones with one another. In-house testing was conducted in 1993 using the disk diffusion method on a group of Gram-positive cocci belonging to 7 species in 9 groups: [(methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), methicillin-susceptible *S. epidermidis* (MSSE), methicillin-resistant *S. epidermidis* (MRSE), *S. haemolyticus*, *E. faecalis*, *E. faecium*, *S. agalactiae*, and *S. bovis*)] and *P. aeruginosa*. These species were selected because the 1992 antibiogram had shown that resistance to ciprofloxacin and norfloxacin was common among these Gram-positive cocci; *P. aeruginosa* was also selected to compare with Thornsberry et al. (1993) who had concluded that resistance by this organism to ciprofloxacin is on the increase.

There were 208 Gram-positive and 21 *P. aeruginosa* isolates tested against each of the 4 quinolones (ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin). Tables 3 through 13 summarize these results.

Methicillin-Susceptible *Staphylococcus aureus* (MSSA) Table 3 shows the results of the 4 quinolones tested on 8 isolates; one isolate (12.5%) was completely resistant to all quinolones (pattern 1); another (12.5%) was susceptible to all 4 quinolones (pattern 2). Patterns 3 through 7 show a mixture of reactions of S, I, and R. Highest susceptibility was seen to ofloxacin with 7 strains (87.5%) susceptible followed by ciprofloxacin (62.5%), norfloxacin (25%) and lomefloxacin (25%).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Table 4 shows only 2 patterns for the 26 isolates, and 25 isolates (96%) exhibited complete resistance to all 4 quinolones (pattern 1), with only 1 strain (4%) showing suceptibility to all quinolones (pattern 2).

Methicillin-susceptible *Staphylococcus epidermidis* (MSSE) Table 5 shows a predominance of complete resistance to all 4 quinolones (pattern 1) in 5 of the 7 isolates (71.4%). Of the other 2 strains, one (14.3%) showed complete susceptibility (pattern 2), and one (14.3%) intermediate susceptibility to 3 quinolones and resistance to lomefloxacin. Thus, 71.4% of the isolates showed susceptibility to ciprofloxacin, norfloxacin, and ofloxacin; 85.7% of the isolates were resistant to lomefloxacin.

Methicillin-Resistant *Staphylococcus epidermidis* (MRSE) Table 6 displays the results of testing of 64 strains and shows 7 different patterns of activity. A predominance of resistance to all four quinolones was exhibited in 49 strains (76.6%) compared with 2 (3.1%) that were completely susceptible (pattern 2). Patterns 3 through 7 showed a mixture of reaction of S, I, and R to the fluoroquinolones. Overall, 96.9% of the isolates were resistant to lomefloxacin, 92.2% to norfloxacin, 79.7% to ofloxacin, and 78.1% to ciprofloxacin.

Staphylococcus haemolyticus Table 7 shows the results of 27 isolates tested; all 27 isolates displayed complete resistance to all four quinolones.

Enterococcus faecalis Table 8 shows the results of testing of 44 strains. Twenty-seven isolates (61.4%) exhibited resistance to all 4 quinolones (pattern 1) and 2 (4.5%) show complete susceptibility (pattern 2). For 7 strains, pattern 3 showed intermediate susceptibility to 3 quinolones but resistance to lomefloxacin. Overall, 42 isolates (95.5%) were resistant to lomefloxacin, 70.5% to norfloxacin, 68.2% to ciprofloxacin, and 63.6% to ofloxacin.

Enterococcus faecium Table 9 shows results of the 19 strains tested. Thirteen isolates (68.4%) were resistant to all 4 quinolones (pattern 1), 5 strains (26.3%) showed resistance to ciprofloxacin and lomefloxacin and intermediate

susceptibility to norfloxacin and ofloxacin (pattern 2), and one strain (5.3%) had intermediate susceptibility to ciprofloxacin, norfloxacin, and ofloxacin but was resistant to lomefloxacin. No isolates exhibited susceptibility to any of the 4 quinolones, and all 19 (100%) were resistant to lomefloxacin.

Streptococcus agalactiae Table 10 shows the results of testing of 6 isolates. Five different patterns of S, I, and R were evident with no strong trends for any strain except that there was 100% resistance to lomefloxacin; 66.7% of the isolates showed susceptibility to ofloxacin.

Streptococcus bovis Table 11 shows results of testing on 7 strains and 5 different patterns. No strong trends were apparent; one isolate (14.3%) was resistant to all 4 quinolones (pattern 1). Of the 7 isolates, 85.7% showed resistance to lomefloxacin and highest susceptibility was to ofloxacin (42.9%).

Pseudomonas aeruginosa Table 12 shows the results of testing of 21 isolates; resistance to all 4 quinolones (pattern 1) was exhibited by 11 isolates (52.4%), and one (4.8%) showed susceptibility to all 4 quinolones. The rest of the isolates (38.1%) displayed a mixture of patterns (patterns 3-8). Overall, 95.2% were resistant to lomefloxacin, followed by 90.5% to ofloxacin, 61.9% to norfloxacin, and 57.1% to ciprofloxacin.

Ciprofloxacin susceptibility Table 13 shows that 62.5% of MSSA isolates were susceptible compared with 3.8% of MRSA. MSSE showed a susceptibility of 14.3% compared with 3.1% for MRSE. The other isolates showed susceptibilities between 0% and 30%: *S. haemolyticus*, 0%; *E. faecalis*, 4.5%; *E. faecium*, 0%; *S. agalactiae*, 16.7%; *S. bovis*, 28.6%; and *P. aeruginosa*, 14.3%.

Norfloxacin susceptibility Table 13 shows that 25% of MSSA isolates were susceptible compared with 3.8% for MRSA. MSSE showed 14.3% susceptible compared to 3.1% for MRSE. The other isolates showed susceptibilities between 0% and 20%: *S. haemolyticus*, 0%; *E. faecalis*, 4.5%; *E. faecium*, 0%; *S. agalactiae*, 0%; *S. bovis*, 14.3%; and *P. aeruginosa*, 19%.

Ofloxacin susceptibility Table 13 shows that 87.5% of MSSA isolates were susceptible compared with 3.8% for MRSA; *S. epidermidis* isolates were 14.3% susceptible compared with 6.3% for MRSE. The other species showed susceptibilities between 0% and 66.7%: *S. haemolyticus*, 0%; *E. faecalis*, 11.4%; *E. faecium*, 0%; *S. agalactiae*, 66.7%; *S. bovis*, 57.1%; and *P. aeruginosa*, 4.8%.

Lomefloxacin susceptibility Table 13 shows that 25% of MSSA isolates were susceptible compared with 3.8% for MRSA. MSSE showed 14.3% susceptibility compared to 3.1% for MRSE. The remaining species showed

susceptibilities between 0% and 4.8%: *S. haemolyticus*, 0%; *E. faecalis*, 4.5%; *E. faecium*, 0%; *S. agalactiae*, 0%; *S. bovis*, 0%; and *P. aeruginosa*, 4.8%.

DISCUSSION

The results of this study are compared with those of Thornsberry et al. (1993) (national survey) for the same species (Tables 14 and 15). Overall, 99.7% of the Enterobacteriaceae showed susceptibility to ciprofloxacin compared with 97.4% in the national survey. The non-Enterobacteriaceae represent a heterogeneous group: *Pseudomonas aeruginosa* and *Xanthomonas maltophilia* are members of the order Pseudomonadales; *Acinetobacter lwoffi* and *Aeromonas hydrophila* are neither related to each other nor to the Pseudomonadales. The data in Table 14 show that there are some differences between the Dameron data and the national data with respect to *A. lwoffi* (92.9% compared with 86.4%), *P. aeruginosa* (95.9% compared with 86.4%), and *X. maltophilia* (75.6% compared with 33.6%). Table 14 also indicates similar patterns and trends of susceptibility among Dameron isolates and those of the national data.

The results of susceptibility testing of the Enterobacteriaceae to ciprofloxacin indicate that 15 of 16 species (93.8%) showed susceptibility greater than 90% (95.4%-100%), *Providencia stuartii* exhibiting susceptibility of 85.4%. When the Dameron Hospital data are compared with the national data for the same species, the national data show 13 of 16 (81.3%) with a susceptibility over 90% (93.1%-99.6%). Differences are seen in *Serratia marcescens* 95.4% compared with 88.2% and

P. rettgeri 96.2% compared with 88.8%. Both data indicate that *P. stuartii* is less susceptible to ciprofloxacin than other Enterobacteriaceae, Dameron Hospital reporting susceptibility of 85.4% compared to national survey of 69.7%. In every instance, the susceptibility rates were higher for each of the species for Dameron isolates compared with the national survey.

Dameron Hospital data show the other three quinolones, norfloxacin, ofloxacin, and lomefloxacin, to be equally effective in vitro for most Enterobacteriaceae; exceptions to this are seen in *S. marcescens* and *P. stuartii* which show reduced susceptibility to norfloxacin and lomefloxacin, and *P. stuartii* exhibiting reduced susceptibility to ofloxacin.

Overall, with a few exceptions, the Enterobacteriaceae remain highly susceptible to ciprofloxacin in vitro. This conclusion is supported by Walker and Wright (1991) and Jones et al. (1994). King and Phillips (1986) showed that the N-1 cyclopropyl group gives ciprofloxacin its most potent in vitro activity against Enterobacteriaceae. The same conclusion was reached by Chu and Fernandes (1989) with respect to activity against *P. aeruginosa*. Thornsberry et al. (1993), as in this study, found a large decrease in susceptibility for *P. stuartii*, but indicated that the majority of resistant strains came from five of 162 institutions, of which Dameron Hospital could have been one; they also suggested that quinolone resistance tends to be localized within endemic areas rather than being spread out geographically.

Table 14 also compares the percent susceptibility of the four non-Enterobacteriaceae species from Dameron Hospital with the national survey. Again

the percent susceptibility reported for the Dameron isolates is higher than that reported in the national survey. Thornsberry et al. (1993) suggested that increased resistance to ciprofloxacin has appeared among a few strains of *P. aeruginosa* but that, in general, most strains are still susceptible. The data from Dameron Hospital for the five years (Table 1) support this trend with similar results for norfloxacin. Factors that may explain differences between the two data (95.9% for Dameron compared to 86.4%) include greater consistency in reading of susceptibilities at a single hospital (Dameron Hospital) compared with the national results derived from 162 hospitals, and also extent of the usage of ciprofloxacin in various regions of the country. Overall, the Dameron data show that lomefloxacin and ofloxacin are not as effective as norfloxacin and ciprofloxacin against *P. aeruginosa*.

The in-house testing, based, as indicated earlier, on a biased sample in which 20 of the 21 strains were specifically selected because of their resistance to one or more quinolones, suggests that lomefloxacin shows least in vitro efficacy against *P. aeruginosa*. This conclusion is supported by Wadworth and Goa (1991). Other reports indicate that ciprofloxacin is the most potent of the quinolones, as shown by King and Phillips (1986), Walker and Wright (1991), Wadworth and Goa (1991), von Rosenstiel and Adam (1994). Thornsberry et al. (1993) conclude that ciprofloxacin-resistant strains exist, yet most are still susceptible without a marked change in susceptibility; Dameron Hospital results support this conclusion.

Among the non-Enterobacteriaceae, Dameron Hospital results show that norfloxacin is relatively less effective than the other quinolones against *X. maltophilia*,

and ofloxacin is least effective against *A. lwoffi*. Thornsberry et al. (1993) found a 10% decrease in susceptibility in *A. lwoffi* but also stated that the change could be due to a change in the taxonomic nomenclature for this genus. They also found a large majority of *X. maltophilia* isolates to be resistant to ciprofloxacin in contrast with Dameron isolates that are susceptible.

Comparing the results of the Dameron study with the national survey data for the Gram-positive isolates, Table 15 suggests that, with the exception of *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, and *S. agalactiae*, generally the other Gram-positive cocci (*Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, and *Enterococcus faecalis*) are less susceptible to ciprofloxacin compared with most of the Enterobacteriaceae. The susceptibilities of these four species of Gram-positive cocci range from 56.1% to 89.3% compared with 52.3% to 79.1% from the national study. The differences are especially significant in the percent susceptibility of *E. faecalis* isolates from Dameron Hospital which show susceptibility of 89.3% compared with 58.3% in the national study. This higher susceptibility of Dameron isolates is the result of improper reporting by the Microbiology Department (F. M. Nahhas, personal communication). In late 1994, the microbiologists at Dameron accidentally discovered that 18-24 hour incubation of the Gram-positive panels is not long enough and, thus, does not give an accurate reading for ciprofloxacin and norfloxacin. When these panels were incubated for an additional 24 hours, in about 30% of the cases, resistance was found at 48 hours which was not evident at 24 hours. The hospital has already corrected this problem by not reporting

the results of ciprofloxacin and norfloxacin at 24 hours. It is of interest to note that such a problem was not encountered with the Gram-negative panel.

Among the staphylococci, Dameron data show that *S. saprophyticus* is 100% susceptible to ciprofloxacin and norfloxacin (not tested in-house). Compared with *S. saprophyticus*, other staphylococci showed relatively lower susceptibility rates than the other species tested, with *S. aureus* being highest and *S. haemolyticus* being the lowest. Both *S. epidermidis* and *S. haemolyticus* show trends of decreasing susceptibility to ciprofloxacin and norfloxacin over the five- and three-year period, respectively, with unexplainable results for *S. aureus*. In-house data show that all four quinolones were 100% resistant to *S. haemolyticus*. Fuchs (1991) reported that the staphylococci that show resistance to ciprofloxacin are the methicillin-resistant *S. haemolyticus*; this study confirs that conclusion. In-house data also show that, among the MSSE and MRSE, the majority of strains were resistant to all four quinolones. Also, in-house results show that ofloxacin was most effective against MSSA followed by ciprofloxacin; all four quinolones showed equally poor susceptibility results for MRSA. Thornsberry et al. (1993) report that lower susceptibility to ciprofloxacin is associated with methicillin-resistant strains of staphylococci, especially MRSA and MRSE. Furthermore, most methicillin-susceptible staphylococci remain susceptible to ciprofloxacin while most methicillin-resistant staphylococci are clearly resistant and, thus, follow a definite pattern. Interestingly enough, however, mechanisms of resistance for the two drugs are very different: methicillin-resistance is due to

production of penicillin-binding proteins whereas ciprofloxacin resistance is due to alterations in DNA gyrase.

Among the enterococci, Dameron data show that *E. faecalis* is relatively less susceptible to ciprofloxacin and norfloxacin than streptococci. Over the five- and three-year period, decreasing susceptibility trends can be observed for ciprofloxacin and norfloxacin. In-house data shows that there is relatively high resistance among enterococci to all four quinolones, *E. faecium* showing no susceptibility to any of the four quinolones. Both *E. faecalis* and *E. faecium* show highest resistance to lomefloxacin, and *E. faecalis* shows highest susceptibility to ofloxacin. Thornsberry et al. (1993) found that isolates of enterococci were evenly distributed between ciprofloxacin-susceptible and ciprofloxacin-resistant strains; yet, Dameron data show higher overall susceptibility to ciprofloxacin due to reasons previously mentioned. In contrast, in-house data show very little susceptibility. Schaberg et al. (1992) report that resistance to ciprofloxacin appeared in enterococci over a five-year period (1985-1990) in one hospital in spite of specific restricted access to quinolone use. Walker and Wright (1991) report that the genus *Enterococcus* is among those least susceptible to most quinolones; Piddock et al. (1994) report that *E. faecium* tends to be more resistant to antimicrobial agents than *E. faecalis*.

Among the streptococci, Dameron data show ciprofloxacin and norfloxacin to be relatively highly effective against *S. pyogenes* and *S. agalactiae*. In-house data show that lomefloxacin was least effective and ofloxacin most effective on *S. agalactiae* and *S. bovis*. Piddock (1994) reported that the epidemiology of

quinolone resistance has not been documented and high resistance not reported for streptococci as they have been for (ciprofloxacin-resistant) staphylococci.

The development of resistance to old and new antimicrobials is of great concern in clinical medicine today (Davies, 1994). When a new antibiotic is introduced, many species seem to adapt and develop various degrees of resistance to that drug; thus, the development of resistance to the quinolones is not surprising (Thornsberry et al., 1993).

Quinolones bind and inhibit DNA gyrase inducing cleavage of the bacterial DNA backbone thus producing a bactericidal effect (Walker and Wright, 1991); yet, even with the extensive research, the precise interaction between DNA gyrase and quinolones is incompletely understood (Maxwell, 1992). Prokaryotic DNA gyrase is composed of 4 subunits, 2 A monomers and 2 B monomers. Gellert et al. (1977) discovered that the A subunits function to insert nicks into each strand of DNA, while the B subunits contain sites involved in ATP hydrolysis providing energy for the supercoiling reaction of the nicked DNA. Following the supercoiling, the A subunits lock the supercoils into the chromosome. The specific action of nalidixic acid, the first quinolone studied, on chromosomal replication was found to cause an abnormal accumulation of DNA single-stranded precursors to form, each representing a domain of the chromosome (Crumplin & Smith, 1976). Gellert et al. (1977) proposed that the 4-quinolones prevent the sealing of the staggered nicks in the DNA usually resealed by the A subunits of DNA gyrase. Smith (1986) submitted additional evidence that shows that mutations in the B subunits accord a change in bacterial sensitivity to the 4-

quinolones; thus, both A and B subunits are affected when 4-quinolones attack the DNA gyrase.

Two general mechanisms of resistance to the quinolones have been proposed: (1) the alteration of DNA gyrase and (2) decreased drug accumulation due either to lower uptake or enhanced efflux. Mutation in the structural genes of the A and B subunits, gyrA and gyrB, has been found to be the cause of alteration of DNA gyrase since Gellert et al. (1977) identified the first quinolone-resistance gene in *E. coli*, the product of nalA, a component of DNA gyrase. Biochemical analysis has shown that when purified A and B subunits from susceptible and resistant strains were compared for quinolone resistance, the A subunit from a quinolone-resistant strain combined with the B subunit of a susceptible strain forming a quinolone-resistant DNA gyrase (Gootz & Martin, 1991). Yoshida et al. (1988, 1990) and Wadworth and Goa (1991) found through genetic analysis that a single point mutation causing a single amino acid change in the gyrA gene in *E. coli*, the quinolone-resistance determining region, was found to be responsible for the modification of subunit A leading to decreased affinity of DNA gyrase to the quinolones. Twenty-one gyrA mutants of *E. coli* have been genetically studied and 11 different mutations described both in vivo and in vitro. Similar findings were reported for *S. aureus*; it is not yet established if the same mutations have the same effect on other bacterial species (Cambau and Gutmann, 1993). Complementation experiments with the gyrA gene confirmed that quinolone-resistant strains of different Gram-positive (Nakanishi, et al., 1991) and Gram-negative species (Power et al., 1992) exhibit mutations in the gyrA genes.

Mutation in the gyrB gene can also confer quinolone resistance evidenced by differences in lower resistance patterns from that of the gyrA gene (Cambau and Gutmann, 1993). Yoshida et al. (1991) tested quinolone resistant gyrB mutants of *E. coli* Kl16 and found two types of mutants. Type 1 mutants were resistant to all quinolones and type 2 resistant to acidic quinolones but hypersusceptible to the amphoteric quinolones (norfloxacin, ciprofloxacin, and ofloxacin).

Decreased cell wall permeability and increased quinolone efflux have also been found to cause resistance (Cambau and Gutmann, 1993). Quinolone-resistant mutants often show decreased quinolone uptake. For *E. coli*, most mutations are associated with a decrease in the amount of the OmpF porin, an outer membrane protein that forms water-filled channels through which certain small hydrophilic antimicrobial agents penetrate the outer membrane. In the mutants studied (nfxB, cfxB, norB, nfxC), a gene encodes for an antisense mRNA interfering with translation of OmpF mRNA, decreasing synthesis of OmpF (Hooper et al., 1992), and thus decreasing cell wall permeability. In *P. aeruginosa*, differences in outer membrane proteins OprF (Piddock et al., 1991) and other proteins have been associated with decreased quinolone accumulation within mutants of this species.

Quinolone efflux, another possible cause of quinolone resistance, is a process by which quinolones are exported into or out of the bacterial cell and is the result of an energy-dependent inner membrane process. Resistance conferred by increased efflux is lower than resistance caused by the gyrA mutation but is still present often enough for sufficient resistance to occur during therapy (Cambau and Gutmann, 1993).

Fluoroquinolones have become widely used in clinical practice, and the increase in resistance toward them among methicillin-resistant staphylococci calls for justified concern in their use for long-term efficacy according to Cruciani and Bassetti (1994).

My study indicates that resistance among the Gram-positive cocci in the Stockton community is not restricted to MRSA but also involves other staphylococci (*S. epidermidis* and *S. haemolyticus*), streptococci (*S. agalactiae* and *S. bovis*), and enterococci (*E. faecalis* and *E. faecium*). In contrast, resistance to *P. aeruginosa* has remained relatively unchanged.

CONCLUSION

The retrospective study of the Gram-negative isolates showed that susceptibility of Enterobacteriaceae to ciprofloxacin and norfloxacin had changed very little over the five-year period, except for *Providencia stuartii* which showed a decreased susceptibility trend. Susceptibility of non-Enterobacteriaceae to ciprofloxacin and norfloxacin varied for different species tested and was relatively less than that of Enterobacteriaceae. *Acinetobacter lwoffi* showed decreased susceptibility, specifically for 1994 data, but this could be due to rearrangement of species in the genus *Acinetobacter*. *Aeromonas hydrophila* showed a decreasing susceptibility trend during 1993 and 1994, *Pseudomonas aeruginosa* remained relatively consistently susceptible, and *Xanthomonas maltophilia* consistently showed lowest susceptibility. The few isolates of *Providencia stuartii* showed low susceptibility to ofloxacin and lomefloxacin.

The retrospective study of the Gram-positive isolates showed that *Staphylococcus saprophyticus* was 100% susceptible to both ciprofloxacin and norfloxacin, while *Staphylococcus haemolyticus* showed relatively poor susceptibility against both fluoroquinolones. *Staphylococcus epidermidis* and *Enterococcus faecalis* showed decreasing susceptibility trends against ciprofloxacin and norfloxacin over the years tested. *Streptococcus pyogenes* and *Streptococcus agalactiae* remained

relatively consistently susceptible to ciprofloxacin and norfloxacin. Generally, ciprofloxacin was relatively more effective against Gram-positive isolates than norfloxacin.

The in-house study (a biased sample of Gram-positive and *P. aeruginosa* isolates) showed that methicillin-resistant staphylococci had lower susceptibility to all four fluoroquinolones tested than did methicillin-susceptible staphylococci. Also, these four fluoroquinolones were relatively poorly effective against *S. haemolyticus* and the enterococci. Ofloxacin was most effective and lomefloxacin least effective against Gram-positive cocci compared with ciprofloxacin and norfloxacin. Ciprofloxacin was most effective against *P. aeruginosa*.

In general, the results of this study are in agreement with those of the national survey.

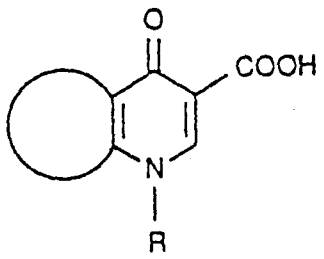

Figure 1 Definition of a quinolone
1-substituted-1,4-dihydro-4-oxo-3-pyridinecarboxylic acid with additional ring fused to positions 5 and 6 representing the position of variation in the quinolones.

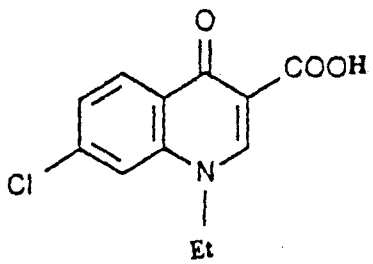

Figure 2 Byproduct of chloroquine
7-chloro-1-ethyl-1,4-dihydro-4-oxo-3-quinoline carboxylic acid

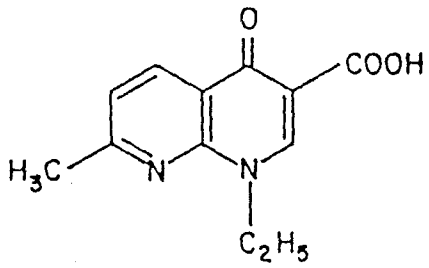

Figure 3 Nalidixic Acid
1-ethyl-7-methyl-1,8-napthyridine-4-one-3-carboxylic acid

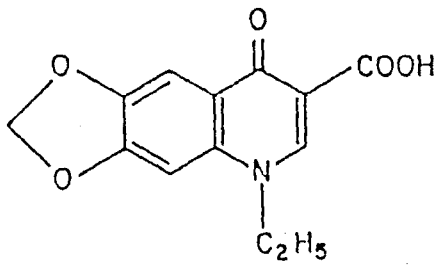

Figure 4 Oxolinic Acid

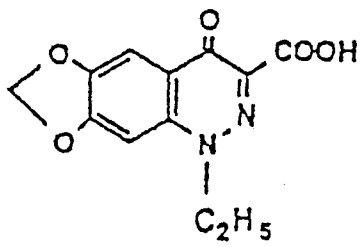

Figure 5 Cinoxacin

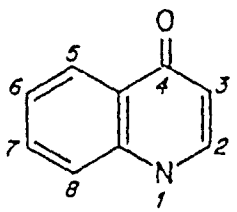

Figure 6 Common skeleton of the quinolone
4-oxo-1,4-dihydroquinolone

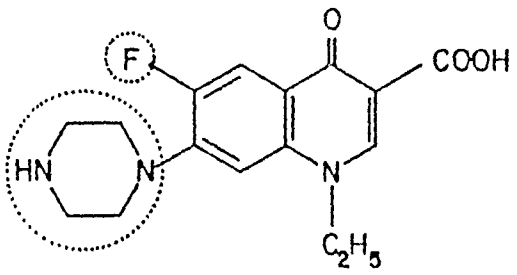

Figure 7 Norfloxacin
The first fluoroquinolone: addition of fluorine extends activity to Gram-positive bacteria and addition of piperazinyl group appears to improve Gram-positive activity.

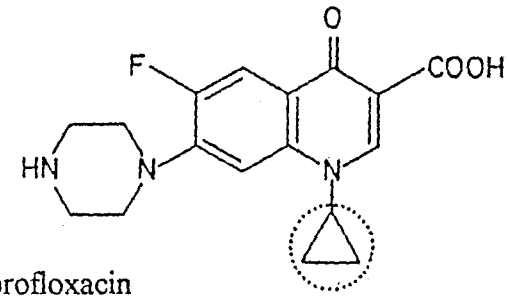

Figure 8 Ciprofloxacin
Addition of the cyclopropyl group appears to improve activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa*.

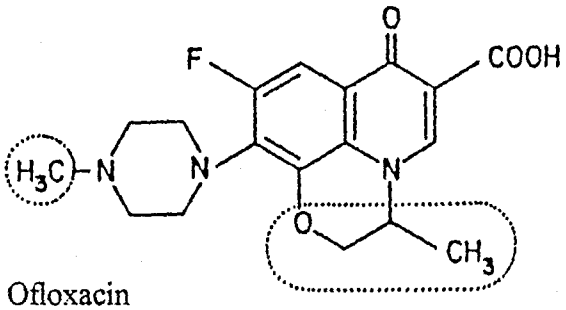

Figure 9 Ofloxacin
The ring surrounded by the oblong circle appears to increase Gram-positive and anti-anaerobic activity and the methyl group appears to increase Gram-positive activity.

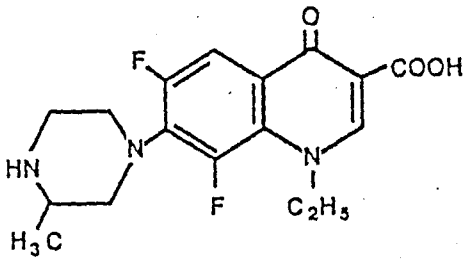

Figure 10 Lomefloxacin
Addition of fluorine at position 8 increases Gram-positive activity.

Bold = % susceptibility Light = total isolates tested		*Escherichia coli*	*Shigella sonnei*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Serratia marcescens*	*Citrobacter freundii*	*Citrobacter diversus*	*Citrobacter amalonaticus*	*Proteus mirabilis*	*Proteus vulgaris*	*Morganella morganii*	*Providencia rettgeri*	*Providencia stuardii*	*Salmonella species*	*Acinetobacter lwoffi*	*Aeromonas hydrophila*	*Pseudomonos aeruginosa*	*Xanthomonas maltophilia*
Ciprofloxacin	1990	**100**	**100**	**100**	**100**	**100**	**97**	**98**	**99**	**100**	**100**	**100**	**100**	**100**	**100**	**81**	**100**	**94**	**100**	**97**	
		1109	12	259	62	141	31	50	86	13	10	209	15	59	8	11	30	18	11	338	
	1991	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**99**	**100**	**100**	**100**	**100**	**100**	**100**	**82**	**100**	**97**	**100**	**98**	**74**
		935	23	264	70	103	38	50	94	16	18	181	4	54	6	11	24	32	9	346	23
	1992	**100**	**100**	**100**	**100**	**98**	**100**	**93**	**99**	**100**	**100**	**100**	**100**	**100**	**67**	**100**	**100**	**100**	**100**	**95**	
		809	31	233	46	121	33	61	84	8	19	124	8	50	3	7	18	25	7	338	
	1993	**100**	**100**	**100**	**100**	**100**	**100**	**84**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**83**	**100**	**100**	**94**	**96**	
		821	15	236	48	111	34	38	72	19	9	165	9	50	3	6	25	13	16	350	
	1994	**100**	**100**	**100**	**98**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**67**	**100**	**82**	**87**	**93**	**77**
		815	38	187	64	95	30	42	73	31	12	116	8	38	6	6	11	38	6	288	22
	Ave.	**100**	**100**	**100**	**99.7**	**99.6**	**99.4**	**95.4**	**99.3**	**100**	**100**	**100**	**100**	**100**	**96.2**	**86.4**	**100**	**92.9**	**96.9**	**95.9**	**75.6**
	Total	4489	119	1179	290	571	166	241	409	87	68	795	44	251	26	41	108	126	49	1660	45
Norfloxacin	1990	**100**		**99**	**100**	**100**	**92**	**76**	**93**	**100**	**100**	**100**	**100**	**100**	**100**	**100**				**93**	
		816		142	17	40	12	4	29	8	2	129	8	12	4	3				122	
	1991	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**97**	**100**	**100**	**100**	**100**	**100**	**100**	**40**	**100**	**100**		**96**	**0**
		618	2	127	21	26	20	4	29	4	4	97	2	13	1	5	1	1		99	1
	1992	**99**		**98**	**100**	**100**	**100**	**50**	**83**	**100**	**100**	**100**	**100**	**100**	**50**			**100**		**89**	
		533		98	12	38	9	6	18	3	5	69	6	16	2			2		75	
	1993	**99**		**100**	**100**	**100**	**100**	**63**	**100**	**100**	**100**	**98**	**100**	**100**	**50**	**50**		**100**	**100**	**88**	
		557		106	19	30	6	8	16	6	3	91	4	4	2	2		4	1	93	
	1994	**100**	**100**	**98**	**100**	**100**	**100**	**93**	**97**	**100**	**100**	**97**	**100**	**100**	**100**	**60**		**80**		**82**	**0**
		560	1	89	27	25	12	15	31	11	4	74	6	12	4	5		10		90	3
	Ave.	**99.6**	**100**	**99.1**	**100**	**100**	**98.3**	**78.4**	**94.3**	**100**	**100**	**99.1**	**100**	**100**	**84.6**	**60**	**100**	**88.2**	**100**	**90**	**0**
	Total	3084	3	562	96	159	59	37	123	32	18	460	26	57	13	15	1	17	1	479	4
Ofloxacin		**99**	**100**	**100**	**100**	**100**	**100**	**100**	**94**	**100**	**100**	**100**	**100**	**100**		**50**	**100**	**90**		**81**	
(Only 1994 available)	1994	120	4	33	10	16	4	3	16	3	3	30	1	7		2	2	10		64	
Lomefloxacin		**98**		**100**	**100**	**100**	**100**	**0**	**83**	**100**		**100**	**100**	**100**		**0**		**67**		**76**	
(Only 1994 available)	1994	120		15	5	7	2	1	6	1		19	1	6		1		3		17	

Table 1 Gram-negative surveillance isolates from Dameron Hospital: Susceptibility to four quinolones

Bold = % susceptibility Light = total isolates tested		*Stapylococcus aureus*	*Stapylococcus epidermidis*	*Staphylococcus haemolyticus*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Streptococcus pyogenes*	*Streptococcus agalactiae*
Ciprofloxacin	1990	**85**	**81**	**61**	**100**	**92**	**100**	**99**
		553	198	111	14	457	38	167
	1991	**65**	**77**	**48**	**100**	**93**	**100**	**100**
		150	48	65	9	309	7	57
	1992	**85**	**74**	**57**	**100**	**89**	**100**	**100**
		549	195	116	6	416	37	106
	1993	**88**	**67**	**58**	**100**	**87**	**100**	**100**
		574	301	98	4	404	28	76
	1994	**90**	**63**	**51**	**100**	**86**	**97**	**97**
		542	224	70	8	338	33	137
	Ave.	**85.6**	**70.8**	**56.1**	**100**	**89.3**	**99.3**	**98.9**
	Total	2368	966	460	41	1924	143	543
Norfloxacin (1990-91 not available)	1992	**71**	**69**	**59**	**100**	**79**	**100**	**78**
		85	39	63	6	312	3	59
	1993	**82**	**62**	**68**	**100**	**84**	**100**	**90**
		68	61	38	3	222	5	59
	1994	**80**	**56**	**48**	**100**	**71**	**100**	**84**
		83	63	27	7	239	2	81
	Ave.	**77.1**	**61.3**	**59.4**	**100**	**77.9**	**100**	**83.9**
	Total	236	163	128	16	773	10	199

Table 2 Gram-positive surveillance isolates from Dameron Hospital: Susceptibility to ciprofloxacin and norfloxacin

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	1
2	S	S	S	S	1
3	S	I	S	I	2
4	I	I	S	I	1
5	I	R	S	I	1
6	S	S	S	I	1
7	S	I	S	S	1

Table 3 Methicillin-Susceptible *Staphylococcus aureus*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	25
2	S	S	S	S	1

Table 4 Methicillin-Resistant *Staphylococcus aureus*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	5
2	S	S	S	S	1
3	I	I	I	R	1

Table 5 Methicillin-Susceptible *Staphylococcus epidermidis*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	49
2	S	S	S	S	2
3	I	R	I	R	7
4	I	R	R	R	2
5	I	I	S	R	2
6	R	R	I	R	1
7	I	I	I	R	1

Table 6 Methicillin-Resistant *Staphylococcus epidermidis*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	27

Table 7 *Staphylococcus haemolyticus*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	27
2	S	S	S	S	2
3	I	I	I	R	7
4	R	R	I	R	2
5	I	I	S	R	2
6	I	R	S	R	1
7	R	I	I	R	1
8	I	R	I	R	1
9	I	I	R	R	1

Table 8 *Enterococcus faecalis*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	13
2	R	I	I	R	5
3	I	I	I	R	1

Table 9 *Enterococcus faecium*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	I	R	1
2	S	I	S	R	1
3	I	I	S	R	2
4	I	R	I	R	1
5	I	R	S	R	1

Table 10 *Streptococcus agalactiae*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	1
2	S	S	S	S	1
3	R	R	I	R	2
4	I	R	S	R	2
5	S	I	S	R	1

Table 11 *Streptococcus bovis*

Pattern #	Ciprofloxacin	Norfloxacin	Ofloxacin	Lomefloxacin	Total
1	R	R	R	R	11
2	S	S	S	S	1
3	I	I	R	R	3
4	I	R	R	R	2
5	R	I	R	R	1
6	S	S	I	R	1
7	I	S	R	R	1
8	S	S	R	R	1

Table 12 *Pseudomonas aeruginosa*

Bold = % susceptibility Light = total isolates tested	MSSA	MRSA	MSSE	MRSE	*Staphylococcus haemolyticus*	*Enterococcus faecalis*	*Enterococcus faecium*	*Streptococcus agalactiae*	*Streptococcus bovis*	*Pseudomonas aeruginosa*
Ciprofloxacin	**62.5**	**3.8**	**14.3**	**3.1**	**0**	**4.5**	**0**	**16.7**	**28.6**	**14.3**
1993	8	26	7	64	27	44	19	6	7	21
Norfloxacin	**25**	**3.8**	**14.3**	**3.1**	**0**	**4.5**	**0**	**0**	**14.3**	**19**
1993	8	26	7	64	27	44	19	6	7	21
Ofloxacin	**87.5**	**3.8**	**14.3**	**6.3**	**0**	**11.4**	**0**	**66.7**	**57.1**	**4.8**
1993	8	26	7	64	27	44	19	6	7	21
Lomefloxacin	**25**	**3.8**	**14.3**	**3.1**	**0**	**4.5**	**0**	**0**	**0**	**4.8**
1993	8	26	7	64	27	44	19	6	7	21

MSSA=Methicillin-susceptible *Staphylococcus aureus*

MRSA=Methicillin-resistant *Staphylococcus aureus*

MSSE=Methicillin-susceptible *Staphylococcus epidermidis*

MRSE=Methicillin-resistant *Staphylococcus epidermidis*

Table 13 Quinolone susceptibility for in-house isolates

Bold = % susceptibility Light = total isolates tested	*Escherichia coli*	*Shigella spp.*	*Klebsiella pneumonia*	*Klebsiella oxytoca*	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Serratia marcescens*	*Citrobacter freundii*	*Citrobacter diversus*	*Citrobacter amalonaticus*	*Proteus mirabilis*	*Proteus vulgaris*	*Morganella morganii*	*Providencia rettgeri*	*Providencia stuartii*	*Salmonella species*	*Acinetobacter lwoffi*	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*	*Xanthomonas maltophilia*
Dameron Hospital	**100**	**100**	**100**	**99.7**	**99.6**	**99.4**	**95.4**	**99.3**	**100**	**100**	**100**	**100**	**100**	**96.2**	**85.4**	**100**	**92.9**	**95.9**	**95.9**	**75.6**
	4489	119	1179	290	571	166	241	409	87	68	795	44	251	26	41	108	126	49	1660	45
Overall %	<----------99.7---------->																			
National Survey	**99.4**	**96.9**	**95.7**	**97.5**	**97**	**95.3**	**88.2**	**94.8**	**93.1**	**99.5**	**97.5**	**99.3**	**97.7**	**88.8**	**69.7**	**99.6**	**86.4**	**93.2**	**86.4**	**33.6**
	99040	520	27850	5314	13336	7262	6503	5104	3005	421	18962	1226	3337	742	1896	1516	911	250	46798	2726
Overall %	<----------97.4---------->																			

Table 14 Gram-negative surveillance isolates: Percent susceptibility to ciprofloxacin of Dameron isolates compared to national survey

Bold = % susceptibility Light = total isolates tested	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus haemolyticus*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Streptococcus pyogenes*	*Streptococcus agalactiae*
Dameron Hospital	**85.6**	**70.8**	**56.1**	**100**	**89.3**	**99.3**	**98.9**
	2368	966	460	41	1924	143	543
National Survey	**79.1**	**70.5**	**52.3**	**93.6**	**58.3**	**91.1**	**85.1**
	67586	18400	2560	1163	12682	426	4693

Table 15 Gram-positive surveillance isolates: Percent susceptibility to ciprofloxacin of Dameron isolates compared to national survey

APPENDIX

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS
DOCUMENT M2-A4

	Susceptible	Intermediate	Resistant
Ciprofloxacin (5 ug)	≤15	16-18	≥19
Norfloxacin (10 ug)	≤12	13-16	≥17
Ofloxacin (5 ug)	≤12	13-15	≥16
Lomefloxacin (10 ug)	≤18	19-21	≥22

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