Impact of Quinolone Restriction on Resistance Patterns of *Escherichia coli* Isolated from Urine by Culture in a Community Setting

Bat Sheva Gottesman,¹,² Yehuda Carmeli,²³ Pnina Shitrit,¹,² and Michal Chowers¹,²

¹Infectious Diseases Unit, Meir Medical Center, Kfar Saba, and ²Sackler Medical School, Tel Aviv University, and ³Division of Epidemiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

(See the editorial commentary by Davey and Urquhart, on pages 876–7.)

**Background.** Decreased antimicrobial susceptibility after increased antibiotic use is a known phenomenon. Restoration of susceptibility once antimicrobial use is decreased is not self-evident. Our objective was to evaluate, in a community setting, the impact of quinolone restriction on the antimicrobial resistance of *E. coli* urine isolates.

**Methods.** We conducted a retrospective, quasi-experimental ecological study to assess the proportion of quinolone-susceptible *E. coli* urine isolates in the periods before, during, and after a nationwide restriction on ciprofloxacin use was implemented. We used an interrupted time interval analysis for outcome evaluation.

**Results.** We found a significant decline in quinolone consumption, measured as defined daily doses (DDDs) per month, between the preintervention and intervention periods (point estimate, $-1827.3$ DDDs per month; $95\%$ confidence interval [CI], $-2248.8$ to $-1405.9$ DDDs per month; $P < .001$). This decline resulted in a significant decrease in *E. coli* nonsusceptibility to quinolones, from a mean of 12% in the preintervention period to a mean of 9% in the intervention period (odds ratio, 1.35; $P = .004$). The improved susceptibility pattern reversed immediately when quinolone consumption rose. Moreover, a highly significant inverse relationship was found between the level of quinolone use (regardless of intervention period) and the susceptibility of *E. coli* urine isolates to quinolone (odds ratio, 1.70; 95% CI, 1.26–2.28). During the months of highest quinolone use (8321 DDDs per month), the proportion of nonsusceptibility was 14%, whereas during the months of lowest quinolone use (4027 DDDs per month), the proportion of nonsusceptibility was 9%. An average decrease in resistance of 1.16% was observed for each decrease of 1000 DDDs.

**Conclusion.** Reducing quinolone consumption can lead to an immediate increase in the susceptibility of *E. coli* urine isolates to quinolones.

The association between antimicrobial consumption and bacterial resistance has been known since the first days of antimicrobial use. Soon after the introduction of penicillin, reports of bacteria developing resistance to penicillin appeared [1]. Quinolones were introduced into clinical practice in the 1980s. Their spectrum of activity, good oral absorption, and tolerability has resulted in their extensive use. Resistance of gram-negative bacteria to quinolones is known and is reported concomitantly with their use [2, 3]. The current recommendation for antibiotic stewardship [4] aims at preventing the development of additional resistance. Whether this phenomenon is reversible and reduction in antimicrobial use would result in a parallel reduction in bacterial resistance is still to be determined. The answer to this question may be even more complex, because it could vary with different combinations of bacteria and antibiotic class.

Studies addressing this issue have reported conflicting results [5–8]. Moreover, most of these studies were conducted in a hospital setting, and data regarding this question in the community are lacking. In contrast to a hospital setting, a large-scale restriction of antimicrobial consumption is more difficult to enforce in the community; therefore, the opportunities to assess the impact of antibiotic restriction in this setting are rare.
In Israel, because of a possible anthrax bioterrorism attack in October 2001, there was a need to preserve quinolones for use in postexposure prophylaxis, and a nationwide restriction of ciprofloxacin use was enacted. The aim of the present study was to evaluate, in a defined community, the effect that this intervention had on the antimicrobial resistance of gram-negative bacteria in urine.

METHODS

Setting. Clalit Health Services is one of 4 nationally mandated health maintenance organizations (HMOs) that serve 60% of the Israeli population. Our study took place in the Sharon district, an area in the center of Israel that serves a population of ~167,000. Of the population studied, 5.9% were children 0–4 years old, 31.9% were children 5–24 years old, 22.5% were individuals 25–44 years old, 25.0% were individuals 45–64 years old, and 14.8% were elderly individuals (≥65 years old). The age distribution was similar in all study periods (P = .94).

Design. We conducted a retrospective, quasi-experimental ecological study in which 3 periods were defined. The first period was the preintervention period, from January 2000 through October 2001 (22 months).

The second period was the intervention period, from November 2001 through May 2002 (7 months). The beginning of the intervention period was defined as the time when restriction of ciprofloxacin was implemented and preapproval by authorized heads of clinics was required for all ciprofloxacin prescriptions in the community. No specific criteria were implemented. This action took place nationwide.

Restriction was enforced only for ciprofloxacin prescriptions. There were no restrictions on other quinolones for 2 reasons: first, it was assumed that, in case of an anthrax bioterrorism attack, ciprofloxacin would be the drug of choice; second, ciprofloxacin accounted for 90% of quinolone consumption.

The third period was the postintervention period, from June 2002 through July 2004 (26 months). The restriction was never formally waived. Nevertheless, generic ofloxacin was added to the formulary at the end of May 2002, which enabled circumvention of the restriction, and enforcement was not imposed over time. The result was an increase in total quinolone consumption, thus marking the end of intervention period.

Antibiotic consumption. The entire Clalit Health Services system, including the Sharon district, is served by a single computerized pharmacy system. All prescriptions for systemic antibiotics issued from 1 January 2000 through 31 July 2004 were recorded. We focused on oral antibiotics prescribed in the community that have activity against gram-negative organisms. The antibiotics included in the study were quinolones (ciprofloxacin and ofloxacin), β-lactam antibiotics (amoxicillin-clavulanate and cefuroxime), trimethoprim-sulfamethoxazole, and nitrofurantoin. Consumption was calculated and expressed by the computerized pharmacy system as defined daily doses (DDDs) per month.

Antimicrobial susceptibility. The entire Sharon district is served by a single bacteriology laboratory. All urine cultures with positive results performed between 1 January 2000 and 31 July 2004 were recorded. Urine cultures and susceptibility testing were performed using the Walkaway-96 microbiology system (Dade International).

Antimicrobial susceptibility was expressed for each organism as the ratio of the number of isolates that were susceptible to each target antibiotic to the total number of isolates tested per month. Susceptibility to ofloxacin and ciprofloxacin were identical and, therefore, were referred as to quinolone susceptibility.

Statistical analysis. Multiple regression analysis was used to examine the effect that the intervention had on the use of quinolones. A priori in the analysis, the first observation after the beginning of the intervention and the postintervention periods were omitted to allow for the possibility that the effects might not be instantaneous. The model included a linear time trend for each period and monthly effects to control for seasonality.

The independence assumption of the residuals was examined by computing bootstrapped P values for the Durbin-Watson statistics. No significant residual autocorrelations were observed, which justified the independence assumption.

Multiple regression analysis was also used to examine the time trend in the use of cefuroxime and amoxicillin-clavulanate antibiotics. The relationships between the use of quinolones, cefuroxime, amoxicillin-clavulanate, and susceptibility to Escherichia coli were tested by applying logistic regression. No significant serial correlations were observed in all cases, justifying the independence assumption between observations. On the basis of the fitted logistic model, the odds ratio was estimated for the susceptibility comparing high DDD versus low DDD quinolone use. High and low were respectively defined as the average of the 2 largest and smallest quinolone DDD values in the sample.

RESULTS

From 1 January 2000 through 31 July 2004, we analyzed data from 19,570 urine cultures that were positive for E. coli. Of the samples, 95% were from females, 3% were from children <5 years old, and 28% were from elderly persons >75 years old. The number of specimens from which E. coli was isolated increased during the study period, from a mean ± standard deviation (SD) of 313 ± 55.9 before the intervention, to 383 ± 36.7 during the intervention, to 431 ± 54.2 after the intervention.

Quinolone use. During the 55-month study period, we observed an overall increase in quinolone use (P = .01). There was, however, a significant difference between the 3 study pe-
Impact of Quinolone Restriction

Figure 1. Quinolone consumption, illustrated as defined daily doses (DDDs) per month. Diamonds indicate values for the preintervention period, squares indicate values for the intervention period, and triangles indicate values for the postintervention period.

periods, with a constant increase in DDDs per month in both the preintervention (39.3 DDDs per month; \( P \leq .012 \)) and postintervention (12.7 DDDs per month; \( P = .006 \)) periods compared with the intervention period, which showed a constant decrease in DDDs per month (−88.5 DDDs per month; \( P = .006 \)) (figure 1).

In the assessment of the effect that the intervention had on quinolone use, after seasonal effects were controlled for there was a highly significant difference in quinolone consumption between the preintervention (mean ± SD, 6996 ± 661 DDDs per month) and intervention (mean ± SD, 5067 ± 755 DDDs per month) periods (point estimate, −1827.3 DDDs per month; 95% confidence interval [CI], −2248.8 to −1405.9 DDDs per month; \( P < .001 \)) and between the intervention and postintervention (mean ± SD, 6895 ± 640 DDDs per month) periods (point estimate, 1294–2131 DDDs per month; \( P < .001 \)). No significant difference in quinolone consumption was found between the pre- and postintervention periods (point estimate, −114.8 DDDs per month; 95% CI, −375.8 to 146.1 DDDs per month; \( P = .38 \)) (figure 1).

Effect of the intervention and quinolone use on E. coli susceptibility. We observed a significant decrease in E. coli nonsusceptibility to quinolones, from a mean of 12% in the preintervention period to a mean of 9% in the intervention period (odds ratio, 1.35; \( P = .014 \)) (figure 2). However, even more pronounced was the significant inverse relationship between the level of quinolone use and susceptibility of E. coli to quinolones (odds ratio, 1.70; 95% CI, 1.26–2.28; \( P = .001 \)) (figure 3). It should be noted that the level of quinolone use and the periods were highly correlated; therefore, only quinolone use was included as an explanatory variable in the models. During the months of highest quinolone use (8321 DDDs per month), the proportion of nonsusceptibility was 14%, whereas during the months of lowest quinolone use (4027 DDDs per month), the proportion of nonsusceptibility was 9%. Thus, the reduction of 50% in quinolone consumption was associated with a 36% reduction in the proportion of nonsusceptibility. An average absolute decrease of 1.16% in nonsusceptibility was observed for each decrease in 1000 DDDs.

\( \beta \)-lactam use. Use of amoxicillin-clavulanate and cefuroxime, which were highly correlated (\( r = 0.84 \)), had a strong seasonal effect. Regression curves for the mean time receiving amoxicillin-clavulanate and cefuroxime (linear trend) and monthly use (to allow for seasonal effects) showed a significant positive relationship (\( P = .002 \)), indicating increased use over time. As expected, the monthly effect showed lower use during the summer months, with the highest values in January. No statistically significant increase was observed in the intervention period (figure 4). No change in the susceptibility of E. coli to amoxicillin-clavulanate and cefuroxime was observed between the preintervention and intervention periods.

DISCUSSION

In the present study, we present the effect, in one district, of a nationwide intervention restricting the use of quinolones. This intervention succeeded in significantly reducing quinolone
consumption in the community by $>40\%$. More interestingly, the intervention was also associated with a statistically significant 25% decrease in *E. coli* nonsusceptibility to quinolones. The improved susceptibility pattern was observed concomitantly with the decrease in quinolone use and was immediately reversed when quinolone consumption rose again. Moreover, a highly significant inverse relationship was found between the level of quinolone use (regardless of time) and the quinolone susceptibility of *E. coli* in urine samples. No unusual compensatory increase in $\beta$-lactam antibiotic use was observed during this time.

Although it is well accepted that resistance rates will increase
with increasing antibiotic use, conflicting answers exist as to the question of whether susceptibility patterns will be restored once antimicrobial use is decreased, which has evolutionary importance. For example, in many parts of the world chloramphenicol has been rarely used during the last 3 decades, yet resistance rates remain very high [9]. The majority of studies examining the reversal of antibiotic resistance have been performed in the hospital setting. Extrapolation from the hospital setting to the community is difficult, because the hospital contains a dynamic population in which rapid changes in resistance may be observed that reflect the discharge rate of colonized patients. Moreover, resistance in hospitalized patients may reflect the emergence of new resistance patterns or the spread of existing resistance to new patients. As a result, studies that were conducted in hospitals and that demonstrated efficacy included infection-control measures, which might have contributed to the decrease in resistance [7].

Few studies have examined the association between antimicrobial use and bacterial resistance in the community, and only 2 of them demonstrated restoration of susceptibility to the antimicrobial after halting its use. Both studies evaluated the susceptibility of gram-positive bacteria. In the first study, following a report [10] of increased resistance to macrolides by Streptococcus pyogenes in Finland, a nationwide recommendation for a reduction in macrolide use was implemented. This resulted in a reduction in macrolide prescriptions from 2.5–3 per 1000 population per month during 1986–1990 to only 1.4–1.6 during 1992–1994. The rate of S. pyogenes resistance to macrolides, which was 13.2% in 1990, peaked at 19% in 1993 and dropped to 8.6% in 1996. Of note, Seppala et al [11] described a concurrent increase in Streptococcus pneumoniae resistance to macrolides despite the decrease in macrolide use.

The second example of reversal of antibiotic resistance in the community was published by Kristinsson et al [12], who described a major campaign directed at the public and physicians in Iceland that resulted in a decline in the consumption of antimicrobials. The incidence of penicillin-resistant pneumococcal infections, after peaking at nearly 20% in 1993, declined to 16.9% in 1994. Carrier status of penicillin-resistant pneumococci in healthy children attending day care centers dropped as well, from 20% in 1992 to 15% in 1995.

The only study addressing the restoration of Enterobacteriaceae susceptibility in the community failed to demonstrate such a phenomenon. In 1995, a national restriction on sulfonamide use in the United Kingdom caused a dramatic decrease in the number of prescriptions, from 320,000 in 1991 to 7000 in 1999. The frequency of E. coli resistance to sulfamethoxazole, however, was not reduced; it was reported to be 39.7% in 1991 and 46% in 1999 [13].

Lipstich, in his review “The Rise and Fall of Antimicrobial Resistance” [14], states that what determines whether reversal of existing antimicrobial resistance will occur in response to a reduction in antimicrobial use is the fitness cost (ie, if drug resistance imposes a cost on the transmissibility of a certain strain, then the resistant strain will be replaced by a susceptible one once antimicrobial selection pressure is removed). If there
is no fitness cost, then there is no force favoring the reversal of resistance, even when antimicrobial exposure is halted. Thus, one possible explanation for our observation is that the bacteria pay a high fitness cost for quinolone resistance, although this might not be the case for resistance to sulfonamides. Indeed, 2 studies exploring the effect of mutations in *E. coli* that confer resistance to quinolones found that such mutations resulted in a marked reduction in the growth rate and in biological fitness (up to a 98% reduction) [15–17]. This effect was not observed in *E. coli* with rifampicin-resistance mutations [18]. Another explanation for the disparity in reversal of resistance between quinolones and sulfonamides is that a sulfonamide-resistant gene is often closely linked to other resistance determinants on mobile genetic elements, which helps to maintain sulfonamide resistance in the face of various selection pressures, whereas quinolone resistance is most often the result of chromosomal mutations.

In the present study, a decrease in *E. coli* resistance to quinolones was observed concomitantly with a decrease in quinolone use. The only 2 studies that have described restoration of microbial susceptibility in the community reported such a change after a lag of a few years. It took ~3 years of macrolide restriction to achieve a 50% decline in *S. pyogenes* resistance [11] and 4 years of decreased antibiotic use to achieve a 25% decline in penicillin resistance in pneumococci [12]. In contrast to the findings of these studies, the immediate relationship we observed between consumption and resistance emphasizes the high fitness cost of quinolone resistance in *E. coli*.

Such a rapid, concomitant correlation between susceptibility restoration and antimicrobial consumption has been reported in the hospital setting after restriction of ceftazidime use [7, 8, 19] when combined with infection-control measures. Another example of this phenomenon was reported with the use of quinolones as prophylaxis in patients with cancer. Reuter et al [20] reported a rapid increase in the incidence of gram-negative quinolone-susceptible strains after discontinuation of quinolone prophylaxis for a period of only 3 weeks. In contrast to our community-based study, that study was conducted in a hospital setting within a specific subgroup. Throughout the 4.5 years of our study, we observed a gradual increase in quinolone consumption except during the intervention period. This is in agreement with worldwide trends [21]. Consumption of β-lactam increased as well, with no change during the intervention period. This is in contrast to other studies, in which restriction of one antibiotic caused an increase in the consumption of another [7, 8, 22, 23]. We speculate that the lack of effect of quinolone restriction on β-lactam use is related to the relatively small burden of quinolone use compared with β-lactam use in the community. Even if a 1:1 replacement had occurred, it would have led to an increase of <8% in β-lactam use, which would be difficult to detect.

The main limitations of our study are its retrospective design and lack of a control group. Because the restriction of quinolones was enforced in all 4 HMOs throughout the country, we could compare our results only to historical controls. To ensure that the changes seen during the intervention period were not random or unrelated, we used an interrupted time-series analysis. This design provides a robust method of measuring the effect of an intervention when randomization or identification of a control group are impractical. Multiple data points are collected before and after the intervention. The intervention effect is measured against the preintervention trend, which serves as a control period for the intervention period [24]. The number of time points before and after intervention confirm our results and validate the relationship between the intervention and the outcome. Moreover, not only did we see improvement in susceptibility with quinolone restriction, but, in the absence of intense and continuous restriction, we observed an increase in quinolone use back to the baseline level with a corresponding reversal of the susceptibility pattern.

Another possible limitation might be sampling bias (ie, restriction of ciprofloxacin use may have affected submission of urine cultures). Unfortunately, we do not have data on the total number of urine cultures sent, and thus we cannot analyze whether this is true. Because the susceptibility of *E. coli* to quinolones was similar to that to β-lactam (cefuroxime and amoxicillin-clavulanate) during the study period, we assume that a change in sampling routine did not occur during the intervention period.

To conclude, we report that a short-term countrywide intervention of quinolone restriction resulted in an immediate increase in *E. coli* susceptibility to this class of antibiotics. This is the first report of such a rapid association between antibiotic use and resistance in the community. As has been demonstrated, the outcome of an intervention in antibiotic use is dependent on the setting, the antibiotic class, and the bacterium. More studies in community settings are needed to help policymakers address the alarming increase in antibiotic resistance in communities worldwide.

**Acknowledgments**

Statistical analysis was done by Ayala Cohen and Etti Doveh of the Faculty of Industrial Engineering and Management, Statistics Laboratory, Haifa, Israel.

**Potential conflicts of interest.** During the last 3 years, Y.C. personally, his laboratory, and/or studies that he has conducted have received grants, honoraria, travel support, consulting fees, and other forms of financial support from the following companies: Basilea Pharmaceutical, Bioline Therapeutics, Cempra Pharmaceuticals, InterCell Pharmaceuticals, IPSAT, Johnson & Johnson Pharmaceuticals, Merck & Co, Neopharm, Pfizer Pharmaceuticals, Teva Medical, Wyeth Pharmaceuticals, and XTL Pharmaceuticals. All other authors: no conflicts.
References