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Trends in Susceptibility of *Neisseria gonorrhoeae* to Ceftriaxone from 1985 through 1991

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The antimicrobial susceptibilities of 16,441 gonococcal isolates from Seattle-King County were determined for ceftriaxone, cefoxitin, penicillin G, and tetracycline. From 1985 to 1989, ceftriaxone, in combination with doxycycline, was increasingly used for treatment of gonorrhea, and by 1989, it was used as therapy for >80% of cases in Seattle-King County. MICs of ceftriaxone correlated significantly ($P < 0.001$) with those of the other beta-lactam antibiotics included in this study. Geometric mean MICs of penicillin G for isolates that did not produce β-lactamase increased from 1985 to 1991. The geometric mean MICs of cefoxitin, ceftriaxone, and tetracycline began to decline in 1987 but increased in 1990 and 1991. The percentage of strains with decreased susceptibility to ceftriaxone (MIC, 0.06 to 0.25 μg/ml) rose from 0.3% in 1985 to 5.3% in 1987 but subsequently declined steadily to 2.6% in 1991, despite increased use of ceftriaxone as routine therapy for gonorrhea. Changes in patterns of antimicrobial susceptibility may be related not only to antimicrobial selection pressures but also to less well understood population shifts among *Neisseria gonorrhoeae* strains within a community.

Neisseria gonorrhoeae has consistently developed resistance to the antimicrobial agents used for the treatment of gonorrhea (23). An excellent example of the effects of antibiotic selection pressure occurred in Korea, where penicillin resistance prompted primary therapy with spectinomycin, only to be followed by development of resistance to this antibiotic (1).

In 1989, the Centers for Disease Control (Atlanta, Ga.) recommended ceftriaxone given as a single intramuscular dose of 250 mg as primary therapy for gonorrhea (3). Although a 125-mg dose of ceftriaxone had proven effective in clinical trials (8, 15), it was not widely recommended because of concern regarding the development of resistance to this antimicrobial agent with the use of the smaller dose (3). However, the Seattle-King County Department of Public Health Sexually Transmitted Diseases (STD) Clinic at Harborview Medical Center employed ceftriaxone in a dose of 125 mg given intramuscularly, followed by doxycycline, 100 mg twice daily by mouth for 7 days, since 1985. By mid-1987, this regimen became the standard therapy for gonorrhea in the STD clinic. By 1989, over 80% of the gonorrhea cases in both public clinics and the private sector in Seattle-King County were treated with ceftriaxone and doxycycline, and this remained the most commonly used therapy for gonorrhea through 1991.

A laboratory surveillance system for gonococcal antimicrobial susceptibility was implemented in Seattle-King County in late 1985, since which time >80% of gonococcal isolates from reported cases have been referred to the Neisseria Reference Laboratory for antimicrobial susceptibility testing. This surveillance system permitted the assessment of the relationship of widespread ceftriaxone therapy for gonorrhea to trends in susceptibility of *N. gonorrhoeae* to ceftriaxone and other antibiotics.

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MATERIALS AND METHODS

Cultures for *N. gonorrhoeae* from various providers within the Seattle area, including the Harborview STD Clinic, were submitted to the Seattle-King County Department of Public Health Laboratory. Isolates were identified presumptively as *N. gonorrhoeae* by growth on selective medium, positive oxidase reaction, and typical Gram stain morphology. The identity of isolates from the rectum, pharynx, and other nongenital sites was confirmed by carbohydrate degradation tests. Isolates presumptively identified as *N. gonorrhoeae* were then forwarded to the Neisseria Reference Laboratory for protein I serotyping, auxotyping, and antimicrobial susceptibility testing. Bacteria were harvested for susceptibility testing from GC agar base medium with defined supplement (IsoVitaleX; BBL Microbiology Systems, Cockeysville, Md.) after 48 h of incubation and suspended in phosphate-buffered saline as previously described (19). An inoculum of 10^5 CFU was spotted onto GC agar base medium (BBL) with defined supplement, containing serial dilutions of ceftriaxone, cefoxitin, penicillin G, or tetracycline.

Chromosomal resistance to penicillin was defined by a penicillin G MIC of ≥2 μg/ml for strains that did not produce β-lactamase (14). Chromosomal tetracycline resistance was defined by a tetracycline MIC of 2.0 to 8.0 μg/ml (14). Resistance to cefoxitin was defined as a MIC of ≥2 μg/ml in accordance with the definition of the Centers for Disease Control (21). Decreased susceptibility to ceftriaxone was defined by a MIC of ≥0.06 μg/ml. Plasmid-mediated resistance to penicillin (PPNG) was determined by detection of β-lactamase with the chromogenic cephalosporin method (Cefinase; BBL). Plasmid-mediated resistance to tetracycline (TRNG) was defined phenotypically by a MIC of ≥16 μg/ml.

Isolates were further characterized as to protein I serovar and auxotype by previously described methods (11, 16). Auxotyping and MIC testing were performed on all isolates, as was serotyping except for the period of January 1989 to June 1989, when serotyping was performed on only every fourth specimen.

Rates of ceftriaxone usage for the treatment of gonorrhea were derived from gonorrhea case reports submitted to the Department of Public Health by all types of health care providers.

Data were analyzed with Epi Info, version 5 (Centers for Disease Control). Means were compared by one-way analysis of variance or by Kruskal-Wallis one-way analysis of variance (22). Spearman rank correlation coefficient was used to compare the susceptibilities of isolates to two antimicrobial agents. MICs were standardized to account for year-to-year phenotypic differences by the method described by Rothman (20). For purposes of standardization, the following phenotypes were considered: IA-1 and -2 AHU; other IA phenotypes; IB-1, -3, and -6; other IB serovars; and TRNG regardless of autotype or serovar. Observed geometric mean MICs of ceftriaxone were compared with standard-

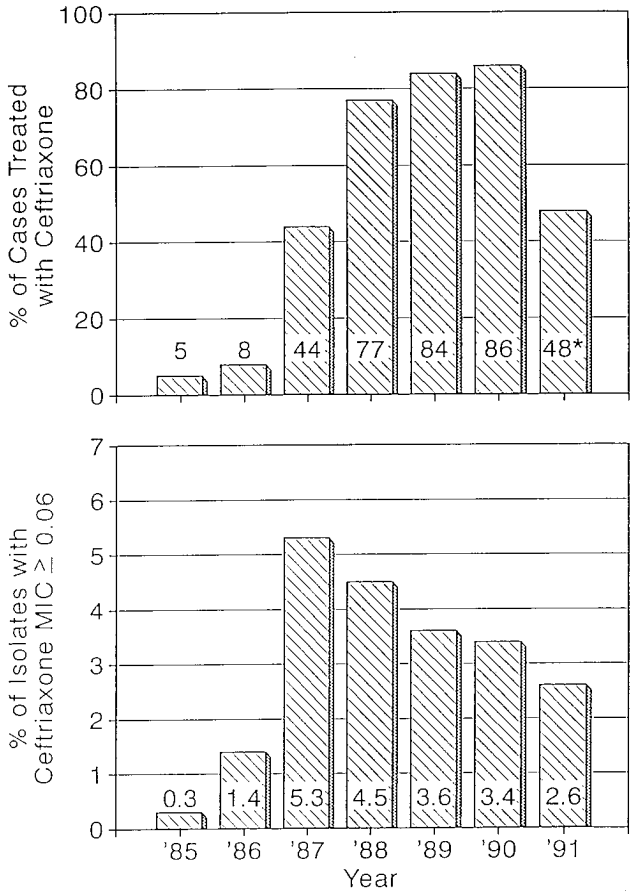

FIG. 1. Percentage of gonorrhea cases treated with ceftriaxone versus percentage of *N. gonorrhoeae* isolates with ceftriaxone MIC of ≥0.06 μg/ml. *, an additional 40% of patients were treated with cefixime.

ized values by comparison of the variances of the observed and standardized values.

RESULTS

During the 7-year study period, isolates from 16,441 gonorrhea cases were received. Isolates were received during only a portion of 1985 and predominantly from the Harborview STD Clinic. Beginning in 1986, isolates from greater than 75% of reported Seattle-King County cases were tested each year. From 39 to 48% of isolates originated from patients evaluated at the Harborview STD Clinic, while the remainder of isolates were forwarded from other clinical providers.

Ceftriaxone use grew steadily until by 1990 over 85% of cases of gonorrhea were treated with this antibiotic (Fig. 1). During 1991, the Harborview STD Clinic changed from injectable ceftriaxone to oral cefixime (400 mg), and one or the other of these therapies was used for 87.6% of gonorrhea treatments in that year.

PPNG isolates peaked in 1987 at 6.0% and subsequently declined to 2.7% in 1991. In contrast, TRNG isolates were rarely present prior to 1990. In that year, the prevalence of TRNG increased dramatically to 6.3% of gonococcal isolates. Geometric mean MICs of ceftriaxone did not differ significantly between PPNG and non-PPNG isolates. However, the geometric mean MIC of ceftriaxone was significantly higher for TRNG than for non-TRNG isolates (0.0091 versus 0.0045 μg/ml, $P < 0.001$).

TABLE 1. Geometric mean MICs (micrograms per milliliter) of penicillin G, tetracycline, cefoxitin, and ceftriaxone for *N. gonorrhoeae* isolates in Seattle-King County, 1985 to 1991

Yr	n^a	Penicillin G[b]	Tetracycline[c]	Cefoxitin	Ceftriaxone
1985	635	0.137 (n = 635)	0.709 (n = 634)	0.412	0.0031
1986	3,323	0.163 (n = 3,290)	0.673 (n = 3,314)	0.454	0.0041
1987	3,171	0.169 (n = 2,982)	0.596 (n = 3,163)	0.452	0.0051
1988	2,687	0.181 (n = 2,570)	0.577 (n = 2,682)	0.429	0.0040
1989	2,704	0.200 (n = 2,597)	0.497 (n = 2,665)	0.424	0.0040
1990	2,053	0.238 (n = 1,986)	0.647 (n = 1,924)	0.501	0.0057
1991	1,868	0.275 (n = 1,818)	0.601 (n = 1,767)	0.549	0.0070

[a] Total number of strains.
[b] Excludes PPNG.
[c] Excludes TRNG.

The geometric mean MICs of penicillin G, tetracycline, cefoxitin, and ceftriaxone are shown by year in Table 1. The geometric mean MICs of penicillin G for non-PPNG isolates rose steadily from 1985 to 1991, from 0.137 to 0.275 μg/ml. MICs of tetracycline (exclusive of TRNG isolates) declined steadily from 1985 to 1989 but then increased from 1989 to 1990. Geometric mean MICs of cefoxitin and ceftriaxone fluctuated only slightly from 1985 to 1989 but began to increase in 1990, with 1991 levels significantly greater than those observed in each of the previous years ($P < 0.001$). No important differences were found between geometric mean MICs of ceftriaxone for Harborview STD isolates (0.0042 μg/ml for all years) and those for the other sectors (0.0045 μg/ml for all years).

MICs of ceftriaxone were highly correlated with those of each of the other three antibiotics ($P < 0.001$) for each comparison (Spearman rank correlation coefficient) (Fig. 2). Of strains with MICs of ceftriaxone ≥ 0.06 μg/ml, 56, 68, and 60% had evidence of chromosomal resistance to penicillin, tetracycline, and cefoxitin, respectively.

Despite a significant increase in the geometric mean MIC of ceftriaxone from 1985 to 1991, the proportion of strains with decreased susceptibility to ceftriaxone (MIC ≥ 0.06 μg/ml) peaked at 5.3% in 1987 and subsequently declined to 2.6% in 1991 ($P < 0.0001$). This occurred despite the increased use of ceftriaxone (Fig. 1).

During the study period, there was no evidence of clinically significant resistance to ceftriaxone. Sporadic instances of per-

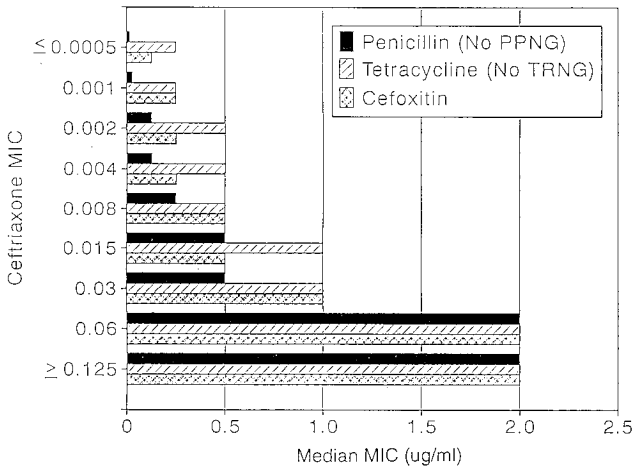

FIG. 2. Correlation of MICs of penicillin G, tetracycline, and cefoxitin with those of ceftriaxone for *N. gonorrhoeae* isolates.

sistent pharyngeal infection unrelated to MICs of ceftriaxone occurred during the study period (9), but persistence of infection with the same strain following treatment with 125 mg of ceftriaxone was well documented in only one instance. This was a pharyngeal infection with pre- and posttreatment isolates susceptible to 0.015 μg of ceftriaxone per ml. Infection subsequently cleared with a dose of 250 mg of ceftriaxone. Throughout the study period, we documented no instances of persistent genital or rectal infection with the same strain of *N. gonorrhoeae*.

The overall geometric mean MIC of ceftriaxone for strains belonging to the protein IA serogroup was significantly lower than that for the IB strains (0.0021 versus 0.0053, $P < 0.0001$). The prevalence of protein IA strains increased from 13.0 to 22.9% of all isolates from 1985 until 1988 ($P < 0.0001$). However, by 1991, the prevalence of IA strains had declined to 7.4%. AHU/IA-1 and -2 strains, which were extremely susceptible to ceftriaxone (MIC, 0.0007 μg/ml), declined in frequency from 6.9 to 0.5% of all isolates from 1985 to 1991. These declines in highly susceptible strains paralleled the increase in the geometric mean MICs of ceftriaxone.

The influence of phenotypic variation by year on the geometric mean MIC for ceftriaxone was assessed by comparing observed values with values standardized to the phenotypic composition of the strain population by using the phenotypes previously mentioned. Standardization reduced variance by 68%, suggesting that two-thirds of observed variability over time could be explained by standardizing for phenotypic composition.

DISCUSSION

Ceftriaxone is highly effective for the treatment of gonorrhea. The levels in serum achieved by single-dose therapy with 125 to 250 mg are many times higher than the in vitro MICs of ceftriaxone (15). No high-level resistance to ceftriaxone has been documented in vitro to date (21). Likewise, clinical failures with genital or rectal infection are rare and not correlated with decreased in vitro susceptibility. However, since the Centers for Disease Control recommended ceftriaxone for primary therapy for gonorrhea (3, 4), ceftriaxone has been widely used, and there have been concerns about the development of clinically significant resistance due to antibiotic selection pressure.

Clinically significant chromosomal resistance to levels of penicillin G or tetracycline, attainable with single doses of penicillin G or with maximum dosages of tetracycline, has been recognized in the gonococcus since the early 1980s (6, 18), and the genetic loci involved in resistance to these antibiotics are closely related (5, 7, 12). Decreased susceptibility to the expanded-spectrum cephalosporins such as cefoxitin and cefuroxime has also developed (12, 18). In the present study, chromosomally mediated resistance to penicillin G and tetracycline and decreased susceptibility to cefoxitin were highly correlated with increased MICs of ceftriaxone. Decreased susceptibility of *N. gonorrhoeae* to ceftriaxone appears due to chromosomally mediated alterations in outer membrane permeability and altered affinity of penicillin-binding proteins for ceftriaxone, as for penicillin G (2, 5, 12).

The geometric mean MIC of penicillin G for non-β-lactamase-producing strains of *N. gonorrhoeae* has continued to increase in Seattle-King County since our laboratory began continuously monitoring gonococcal susceptibility in 1985. The geometric mean MIC of tetracycline for non-TRNG strains of *N. gonorrhoeae* began to decline in 1986 but sharply increased during 1990 and 1991. The geometric mean MICs of cefoxitin and ceftriaxone began to decline in 1988 but also increased substantially in 1990 and 1991. In contrast, the percentage of strains with MICs of ceftriaxone of ≥0.06 μg/ml declined from 1987 to 1991. This apparent discrepancy can be explained in part by the fact that the very susceptible phenotypes (protein IA strains) have been declining rapidly in Seattle. The fact that fewer strains have ceftriaxone MICs of ≥0.06 μg/ml provides preliminary evidence that widespread use of 125-mg ceftriaxone therapy for gonorrhea in the community has not promoted chromosomally mediated decreased susceptibility to ceftriaxone. The use of doxycycline with ceftriaxone in the majority of cases may have discouraged the development of resistant strains.

The reason for the recent decline in the number of strains with ceftriaxone MICs of ≥0.06 μg/ml in Seattle is unclear. Previous work by others has suggested an association between protein I serovar and chromosomally mediated resistance to antibiotics (18, 24). The pattern of serovar-auxotypes found in a community is not stable from year to year (17, 21), and clonal outbreaks of particular strains may occur (10). Continued shifting of serovars may be due to factors other than pressure from use of a particular antimicrobial agent (17) and yet may contribute to shifts in susceptibility to that antimicrobial agent. As we have shown by standardizing phenotypic composition, although including only five phenotypes, the variance was reduced by 68%, suggesting that two-thirds of observed variability can be explained by shifts among these phenotypes. The remainder may be accounted for by more subtle shifts that could not be identified through our standardization method. Alternatively, antimicrobial selection pressure may play a role.

The present data are reassuring concerning the development of chromosomal resistance of *N. gonorrhoeae* to ceftriaxone. The gonococcus may instead develop resistance to broad-spectrum cephalosporins via plasmid-mediated mechanisms. Mutations in the TEM gene have already resulted in resistance to broad-spectrum cephalosporins among some enteric bacteria (13), and the appearance of such mutated TEM genes may be anticipated in gonococci in the future.

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