Ceftibuten Resistance and Treatment Failure of *Neisseria gonorrhoeae* Infection[▽]

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Neisseria gonorrhoeae infections have been empirically treated in Hong Kong with a single oral 400-mg dose of ceftibuten since 1997. Following anecdotal reports of the treatment failure of gonorrhea with oral extended-spectrum cephalosporins, the current study was undertaken to determine the antimicrobial susceptibility pattern and molecular characteristics of isolates of *N. gonorrhoeae* among patients with putative treatment failure in a sexually transmitted disease clinic setting. Between October 2006 and August 2007, 44 isolates of *N. gonorrhoeae* were studied from patients identified clinically to have treatment failure with empirical ceftibuten. The ceftibuten MICs for three strains were found to have been 8 mg/liter. These strains were determined by *N. gonorrhoeae* multiantigen sequence typing to belong to sequence type 835 (ST835) or the closely related ST2469. The testing of an additional eight archived ST835 strains revealed similarly elevated ceftibuten MICs. The *penA* gene sequences of these 11 isolates all had the mosaic pattern previously described as pattern X. Of note is that the ceftriaxone susceptibility results of these strains all fell within the susceptible range. It is concluded that ceftibuten resistance may contribute to the empirical treatment failure of gonorrhea caused by strains harboring the mosaic *penA* gene, which confers reduced susceptibility to oral extended-spectrum cephalosporins. Screening for such resistance in the routine clinical laboratory may be undertaken by the disk diffusion test. The continued monitoring of antimicrobial resistance and molecular characteristics of *N. gonorrhoeae* isolates is important to ensure that control and prevention strategies remain effective.

Neisseria gonorrhoeae continues to be an important bacterial agent that causes sexually transmitted infections (3). The recognition of the infection with prompt empirical, but effective, treatment is the mainstay to prevent sequelae and further transmission. Nevertheless, this organism has a propensity for the development of drug resistance under antimicrobial selective pressure and for this resistance to spread rapidly. Thus, continued surveillance is mandatory to ensure that the optimal agent for empirical therapy is employed (12). In Hong Kong, penicillin was the treatment of choice for *N. gonorrhoeae* infections until 1985, when rising resistance resulted in its replacement by ofloxacin, which in turn became obsolete in 1997; it was replaced by ceftibuten in a single oral dose of 400 mg (4). Ceftibuten was chosen in Hong Kong because it was an orally active agent with potent in vitro activity against *N. gonorrhoeae*. Cefixime, one of the standard recommended agents in a similar class (3), was not adopted as the standard treatment, because it was not available in Hong Kong. In terms of the pharmacokinetics of an oral dose of 400 mg of ceftibuten and cefixime, respectively, the bioavailabilities are 80 and 50%, the peak serum levels are 15 mg/liter and 3 to 5 mg/liter, the protein binding level is 65% for both, and the serum half-lives are 2.4 and 3.1 h

(7). These properties suggest that a single dose of either agent administered orally is effective for the treatment of gonorrhea. Concerning other countries in the western Pacific region, cefixime is used in Japan (17), while other countries appear to be using various other oral extended-spectrum cephalosporins in a less systematic manner.

With the increasing use of extended-spectrum cephalosporins in the management of *N. gonorrhoeae* infections, reports of clinical treatment failure and resistance have been published (1, 6, 17), or at least failure and resistance have been implied (13). In particular, specific resistance mechanisms for oral extended-spectrum cephalosporins have been determined to be related to mosaic *penA* gene sequences (11). The possibility that alternative or additional resistance determinants, found alone or in combination, also contribute to the decreased susceptibility to extended-spectrum cephalosporins has been raised (9). Additionally, it appears that the in vitro (as measured by MICs) and the in vivo (as reflected by treatment outcomes) effects of alterations in penicillin-binding protein 2 differ between the oral agents and the injectable ceftriaxone.

As there are no standard interpretation guidelines for ceftibuten susceptibility testing, results for ceftriaxone have been used as a surrogate for inferring ceftibuten susceptibility in our setting (5). In order to determine the validity of this surrogacy and to monitor for ceftibuten resistance, we studied *N. gonorrhoeae* isolates from putative treatment failure cases with a view to characterizing their antimicrobial susceptibility pattern and molecular resistance mechanisms.

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MATERIALS AND METHODS

Clinical isolates of *N. gonorrhoeae*. Our laboratory processes specimens from patients from government sexually transmitted disease (STD) clinics. Culture for *N. gonorrhoeae* was performed by near-patient inoculation of male urethral or female endocervical specimens onto modified Thayer Martin medium (MTM). Immediately upon inoculation, culture plates were incubated at 36.5°C in a carbon dioxide environment produced by a candle jar. The plates were transported to the laboratory within 48 h. The identification of *N. gonorrhoeae* isolates was undertaken by conventional methods (15). Isolates of *N. gonorrhoeae* were preserved in a commercial bead system at -70°C.

Patients from the government STD clinics with positive microscopy on site for genital *N. gonorrhoeae* infection were given a single 400-mg dose of ceftibuten under direct supervision and requested to attend a follow-up clinic session after 1 week, when a second specimen was obtained for microscopy and for *N. gonorrhoeae* test-of-cure (TOC) culture. Patients whose second TOC specimen was culture positive for *N. gonorrhoeae* were considered to represent possible treatment failure and were given a single 2-g intramuscular dose of spectinomycin, which was effective in virtually all cases. More prolonged courses of treatment were prescribed for those with complicated infections. Specific treatment for *Chlamydia trachomatis* was prescribed only for patients with persistent urethritis after successful treatment for *N. gonorrhoeae* or for those with positive *C. trachomatis* nucleic acid testing results.

Antimicrobial susceptibility testing. A retrospective study was undertaken to identify possible treatment failure cases of gonorrhea between October 2006 and August 2007 from clinical records as defined above. *N. gonorrhoeae* isolates from these case patients were subjected to antimicrobial susceptibility testing for penicillin, ofloxacin, and tetracycline using the disk diffusion method strictly in accordance with guidelines from the CLSI, including test medium requirements and quality control procedures (5). Ceftibuten disk diffusion tests also were performed using disks containing 30 µg of the agent. Beta-lactamase detection was performed using the nitrocefin disk method (Cefinase; bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. The MICs for spectinomycin, ceftriaxone, and cefixime were determined using the Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. This laboratory has been an active member for many years of the World Health Organization Western Pacific Gonococcal Antimicrobial Surveillance Programme (16), with full participation in its external quality assessment program, which includes isolates less susceptible to ceftriaxone. The ceftibuten MIC was determined using the agar dilution method according to the manufacturer's instructions for ceftibuten Adatab (Mast Diagnostics, Bootle, United Kingdom).

Genetic characterization. All strains of *N. gonorrhoeae* were subjected to *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) and the nucleotide sequencing of the *penA* gene according to published protocols (8, 10).

RESULTS

Isolates of *N. gonorrhoeae* from putative treatment failure cases. In 2006, our STD clinics managed approximately 1,600 cases of gonorrhea. In the current study, 45 cases of putative treatment failure following ceftibuten treatment were detected in 11 months from October 2006 to August 2007, during which period our laboratory had positive isolations of *N. gonorrhoeae* from 1,228 patient visits to the STD clinics. Gonococcal isolates were retrieved from all but 1 of these 45 patients identified as possible treatment failures. For another patient, the pretreatment urethral swab specimen was culture negative for *N. gonorrhoeae*, although the direct smear was positive for intracellular gram-negative diplococci; the posttreatment specimen was culture positive. Thus, before and/or after ceftibuten treatment, *N. gonorrhoeae* isolates from 44 patients were available for further study. Intervals between the first and second isolation of *N. gonorrhoeae* ranged from 3 to 25 days. The majority of patients (20) had second isolates grown 7 days after initial treatment, while 11 and 13 patients had strains recultured fewer than and more than 7 days, respectively, following treatment. In three patients, the pre- and posttreatment isolates had different NG-MAST types. For one patient whose

TABLE 1. Susceptibility of 42 posttreatment *Neisseria gonorrhoeae* strains to ceftibuten using a 30-µg ceftibuten disk

MIC (mg/liter)	No. of strains	Disk diffusion zone diam in mm (no.) [range, median]
0.06	2	40 (2)
0.12	1	33 (1)
0.25	12	32 (2), 34 (1), 35 (1), 36 (2), 38 (3), 39 (2), 41 (1) [32–41, 37]
0.5	20	30 (1), 31 (1), 32 (2), 34 (5), 35 (5), 36 (5), 38 (1) [30–38, 35]
1	4	30 (1), 33 (1), 34 (2) [30–34, 33.5]
2	0	
4	0	
8	3	19 (1), 23 (1), 27 (1) [19–27, 23]

isolates were 15 days apart, the NG-MAST types were ST1961 and ST2471 pre- and posttreatment, respectively, while those for another patient with isolates 25 days apart were ST1731 and ST2466, respectively. Since the pre- and posttreatment isolates had very different NG-MAST types, they were considered to be cases of reinfection rather than treatment failure and were not further analyzed in this study. For the remaining patient whose isolates were 4 days apart, the NG-MAST types ST2323 and ST2335 differ only by one nucleotide at the *por* gene. These strains thus were taken into account in the antimicrobial susceptibility study.

Antimicrobial susceptibility pattern of treatment failure isolates. For the treatment failure isolates from the remaining 42 patients, the pre- and posttreatment isolates were indistinguishable on the basis of identical NG-MAST types (except for the ST2324/ST2335 pair described above) and antimicrobial susceptibility patterns. There was no more than a twofold difference in any of the MICs. All 42 strains had intermediate resistance (16 strains) or were resistant (26 strains) to penicillin, with eight of the resistant strains harboring beta-lactamase activity. All strains were resistant to ofloxacin, except for one intermediate strain, as was the case for tetracycline. All isolates were susceptible to spectinomycin, which had MICs of 4, 8, and 16 mg/liter for 3, 31, and 8 strains, respectively. Ceftriaxone susceptibility testing yielded similar susceptible findings by CLSI criteria, with MICs of ≤0.016, 0.032, 0.064, and 0.125 mg/liter for 9, 10, 19, and 4 strains, respectively. MICs for cefixime were ≤0.016, 0.032, 0.064, 0.125, and 0.25 mg/liter for 9, 19, 9, 4, and 1 strain, respectively. Ceftibuten susceptibility results are presented in Table 1. The ceftibuten MICs for three strains were 8 mg/liter; two of these strains belonged to NG-MAST type ST835, while the other was of ST2469. This latter type differs from ST835 in only one nucleotide at the *por* gene, suggesting that these two sequence types have close evolutionary relationships. In order to provide a more complete picture, we retrieved susceptibility results from additional strains of *N. gonorrhoeae* isolated from 40 consecutive patients without treatment failure during October 2006. Ceftibuten disk diffusion zone diameters ranged from 31 to 49 mm (median, 38 mm). The corresponding ceftriaxone MICs ranged from <0.002 to 0.125 mg/liter. None of these isolates belonged to ST835 or ST2469. The *penA* sequences of these isolates are not available.

Strains with reduced susceptibility to ceftibuten. As a result of the above findings, laboratory records for the year 2006 were

TABLE 2. Characteristics of 11 *Neisseria gonorrhoeae* strains for which the ceftibuten MIC was 8 mg/liter^a

Strain no.	NG-MAST ^b	MIC (mg/liter)				Ceftibuten zone diam (mm)
		Spectinomycin	Ceftriaxone	Cefixime	Ceftibuten	
1	835	8	0.064	0.25	8	26
2	835	16	0.064	0.25	8	24
3	835	16	0.064	0.25	8	25
4	835	8	0.064	0.25	8	22
5	835	8	0.064	0.25	8	20
6	835	8	0.064	0.25	8	22
7	835	8	0.064	0.25	8	25
8	835	8	0.125	0.25	8	21
9	835	8	0.064	0.125	8	27
10	835	8	0.064	0.25	8	19
11	2469	8	0.064	0.125	8	23

^a All strains were resistant to penicillin, ofloxacin, and tetracycline, were negative for beta-lactamase, and had *penA* genes harboring the mosaic pattern X.

^b NG-MAST, *N. gonorrhoeae* multi-antigen sequence typing.

reviewed for the presence of additional strains of ST835. A total of eight patients were found to harbor these strains, of whom six had putative treatment failure, insofar as posttreatment isolates were detected in TOC cultures 3 to 7 days after the initial isolation of the bacterium. For the remaining two patients, one defaulted on follow-up and the other had a negative urethral swab culture 7 days after standard ceftibuten treatment. No further strains of ST2469 were found. All additional ST835 strains were subjected to the antimicrobial susceptibility and characterization tests described above. As the strains from each patient had virtually identical antimicrobial susceptibility patterns and the ceftibuten MICs were 8 mg/liter, the details of only one strain from each of these eight patients are presented in Table 2, together with those from the three patients in the current study for whom the ceftibuten MIC level was 8 mg/liter. The disk diffusion zone sizes obtained with 30- μ g ceftibuten disks for these isolates ranged between 19 and 27 mm, whereas those with the same ceftriaxone MIC (0.064 or 0.125 mg/liter) but for which ceftibuten treatment was successful (among 14 of the 40 patients described above) had zone sizes ranging from 33 to 38 mm.

Sequencing of the *penA* gene. The sequencing of the *penA* gene revealed that all 11 isolates shown in Table 2 had the mosaic pattern previously described as pattern X (11), while none of the remaining treatment failure isolates exhibited this pattern.

Correlation of ceftibuten susceptibility results with treatment status and genotypic data. In patients with ceftibuten treatment failure, the ceftibuten MICs for all strains of ST835/ST2469 and harboring the *penA* gene were 8 mg/liter, with disk diffusion zone diameters of ≤ 27 mm. In contrast, the ceftibuten MICs of *penA*-negative strains of other sequence types were ≤ 1 mg/liter, with corresponding disk diffusion zone diameters of ≥ 30 mm. Similarly, patients successfully treated with ceftibuten had isolates with disk diffusion zone diameters of ≥ 31 mm.

DISCUSSION

With the propensity for *N. gonorrhoeae* strains to develop antimicrobial resistance under the pressure of drug treatment, it is important to monitor its resistance rates, especially to the

agent employed for its empirical treatment. In Hong Kong, oral ceftibuten has been used in this role since 1997. As standard antimicrobial susceptibility testing guidelines do not have interpretation criteria for the disk diffusion test results or MICs for ceftibuten, results from ceftriaxone have been used as surrogates. This study indicated the possibility of ceftibuten treatment failure while the isolates remained susceptible to ceftriaxone by laboratory testing criteria. It is sometimes difficult to distinguish between true treatment failure and reinfection from the same partner with the original pathogen (12). Nevertheless, the data obtained in this study represent a clinical treatment failure rate of empirical ceftibuten of around 3.7% (45/1,228), which is still within the 5% figure generally considered an acceptable resistance level for an empirical agent (12).

Among the study patients designated as having putative treatment failure, the majority (39 out of 42) harbored isolates of *N. gonorrhoeae* for which the ceftibuten MICs were ≤ 1 mg/liter, while the MIC levels for the three remaining patients' isolates were 8 mg/liter. With a 400-mg oral dose of ceftibuten, the peak serum level is 15 mg/liter (7). Taking the drug with food decreases the rate and extent of absorption (2). Furthermore, the agent is 65% protein bound after absorption, such that the effective serum level would approximate 9 mg/liter at best. It is conceivable that the patients harboring isolates with low MIC levels had clinical treatment failure due to pharmacokinetic factors such as poor oral absorption. Alternatively, they may have had reinfection from the same source. In our setting, as patients are directly observed to take the single-treatment dose in the clinic setting, there should not be an issue with noncompliance.

However, with regard to the isolates with reduced susceptibility to ceftibuten (a MIC level of 8 mg/liter), frank treatment failure is expected when taking into consideration the achievable serum levels even under optimum conditions. The finding that all strains except one belonged to ST835 underlines the significance of public health measures needed to prevent the spread of this strain. The single strain of ST2469 was closely related to ST835, and isolates of both ST835 and ST2469 harbored *penA* genes with the mosaic pattern X. Studies have previously demonstrated that such strains have reduced susceptibility to oral extended-spectrum cephalosporins (8), but

that this change had lesser effects on ceftriaxone MICs (11). Our findings also revealed that such strains remained susceptible to ceftriaxone in vitro by CLSI criteria, yielding MICs of 0.064 mg/liter, and up to 0.125 mg/liter for one strain (Table 2). Cefixime susceptibility testing results also all fell within the susceptible category according to CLSI guidelines, although the MICs of most of our study strains (9 out of 11) were at the upper limit of this category (0.25 mg/liter). Interestingly and importantly, disk diffusion tests with 30- μ g ceftibuten disks yielded dichotomous findings when strains for which the ceftibuten MICs were ≤ 1 mg/liter had zone sizes of at least 30 mm, while strains for which the ceftibuten MICs were 8 mg/liter all had zone sizes under 30 mm. In addition, patients successfully treated empirically with ceftibuten all had strains with zone sizes of 31 mm or more. Taking into account all of the above data together with the known pharmacokinetics of ceftibuten, our findings can be adapted to yield tentative MIC and disk diffusion breakpoints for ceftibuten susceptibility testing for *N. gonorrhoeae*. A MIC of ≥ 8 mg/liter and a disk diffusion zone size of ≤ 27 mm would suggest resistance, whereas respective values of ≤ 1 mg/liter and ≥ 30 mm likely would indicate susceptibility. As the number of strains in the current study is relatively small, continued testing and the characterization of isolates with correlation with clinical and epidemiological data will enable a better definition of various susceptibility categories, including the criteria for an intermediate category.

In practical terms, our findings suggest that in a routine clinical laboratory setting where ceftibuten MIC testing is not a feasible option, the disk diffusion test using 30- μ g ceftibuten disks will give an indication of likely ceftibuten resistance due to the presence of the mosaic *penA* gene. The phenomenon then could be confirmed by further definitive susceptibility testing and/or characterization for the presence of the mosaic X genotype (14) undertaken on the selected isolates. However, the use of existing ceftriaxone breakpoints as a surrogate for detecting reduced susceptibility to ceftibuten seems to be problematic, in that there would be strains regarded both as ceftibuten susceptible and resistant (based on clinical outcomes and ceftibuten disk testing) among gonococci with a ceftriaxone-susceptible MIC. Takahata et al. (11) and Lindberg et al. (9) have suggested that the mosaic X change in *penA*, when associated with additional alterations in other genes, including *ponA*, *mtrR*, and *porB*, had greater and disproportionate effects on the MICs of oral extended-spectrum cephalosporins such as ceftibuten and cefixime than on ceftriaxone. This clearly shows the importance of studies such as this one on gonococci from documented treatment failure and the need for the further clarification and correlation of the clinical and in vitro effects of different combinations of changes in relevant genes.

It is important that these isolates with reduced susceptibility to ceftibuten remain susceptible to the injectable agent spectinomycin, such that it continues to be useful as a second-line agent of treatment.

In conclusion, the reduction of the susceptibility of *N. gonorrhoeae* strains to oral extended-spectrum cephalosporins, currently one of the empirical therapeutic agents of choice, is likely to emerge and spread. Routine clinical laboratories need to be vigilant for the detection of such strains, such that strate-

gies for control and prevention could be reviewed and effective algorithms implemented in a timely manner. While these findings apply directly to ceftibuten, they strongly suggest that further consideration should be given to developing similar and reliable criteria for other oral cephalosporins used or proposed for use for the treatment of gonorrhea. These include cefixime, cefdinir, and cefpodoxime.

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