Emergence of decreased susceptibility to extended-spectrum cephalosporins in Neisseria gonorrhoeae in India

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ABSTRACT

Background. In the past, Neisseria gonorrhoeae has developed resistance to antimicrobial agents used for its treatment. Consequently, extended-spectrum cephalosporins form the mainstay of treatment for gonorrhoea.

Methods. Samples from 88 patients attending the sexually transmitted diseases clinics from December 2009 to January 2011 in two referral hospitals in New Delhi were studied. Antimicrobial susceptibility testing was done using the disc diffusion method as per the calibrated dichotomous sensitivity technique against the following antibiotics: penicillin (0.5 i.u.), tetracycline (10 µg), nalidixic acid (30 µg), ciprofloxacin (1 µg), spectinomycin (100 µg), ceftriaxone (0.5 µg) and cefpodoxime (10 µg) (Oxoid, UK). Azithromycin (15 µg) (Oxoid, UK) was tested as per the guidelines of the Clinical and Laboratory Standards Institute. Minimum inhibitory concentrations were determined using the Etest for penicillin, tetracycline, ciprofloxacin, ceftriaxone, spectinomycin and these are not used in many parts of the world. Even the more recently available macrolides now have limited utility, leaving extended-spectrum cephalosporins (ESCs, both oral and injectable) and spectinomycin (when and where it is available) as the mainstay of treatment.

Results. Eighteen isolates of Neisseria gonorrhoeae were obtained. Three of these had decreased susceptibility to ceftriaxone and cefpodoxime by the disc diffusion method. The minimum inhibitory concentrations of ceftriaxone for two isolates were 0.064 µg/ml and for one isolate it was 0.125 µg/ml.

Conclusion. Higher minimum inhibitory concentrations to extended-spectrum cephalosporins is of concern as it has been shown to precede treatment failure. This may warrant its use in increased/multiple dosages alone or possibly in combination (dual therapy), thereby complicating effective disease control. Our report is in accordance with earlier reports from different parts of the world. Therefore, a continuous surveillance of antimicrobial resistance is crucial to tailor treatment schedules for Neisseria gonorrhoeae in a particular geographical region.

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INTRODUCTION

The prevention and control of gonorrhoea is an important public health concern due to the high burden of disease, the recent increase in reported rates of infection and the reproductive and economic consequences of infection. In the absence of a vaccine, the control of gonorrhoea is achieved through effective antimicrobial therapy to eradicate the infection, reduce transmission and also prevent complications. The unrestricted access to antimicrobials, inappropriate selection, overuse of antibiotics and suboptimal quality of antibiotics, as well as genetic mutations within the organism have contributed to the development of antibiotic resistance in Neisseria gonorrhoeae. The organism has been reported to develop resistance to multiple classes of antimicrobials including penicillins, tetracyclines and quinolones; and these are not used in many parts of the world. Even the more recently available macrolides now have limited utility, leaving extended-spectrum cephalosporins (ESCs, both oral and injectable) and spectinomycin (when and where it is available) as the mainstay of treatment.

Though there is no report of treatment failure with ESCs from India, there are reports of treatment failure due to oral cephalosporins (cefixime and ceftriben) in genital tract gonorrhoea from Japan, Hong Kong, Norway, United Kingdom and Austria. Also, pharyngeal gonococcal isolate with a high-level of resistance to ceftriaxone has been reported (the last remaining option for empirical first-line treatment) from Japan. Furthermore, elevated minimum inhibitory concentrations (MICs) to cephalosporins have been reported from Japan, Australia, China, Hong Kong, USA, various parts of Europe and India. A worldwide continuous surveillance of gonococcal antimicrobial resistance and treatment failures is crucial to minimize the spread of ESC-resistant gonococcal strains to ensure that gonorrhoea remains a treatable infection. Hence, we determined the antimicrobial susceptibility pattern of Neisseria gonorrhoeae isolates in our population.

METHODS

Between December 2009 and January 2011, 47 women with cervicitis and 41 men with urethritis in the age group 15–50 years presenting as outpatients to the Dermatology departments of the All India Institute of Medical Sciences (AIIMS) and Ram Manohar Lohia Hospital, New Delhi were included in the study. Urethral swabs were collected from men and endocervical swabs from women by a physician as per the standard protocol. Culture was done on standard culture media; modified Thayer Martin (GC agar base plus VCNT (A) inhibitors) (Himedia, India) and chocolate agar (Columbia agar base [BD BBL, USA] plus sheep blood) at the bedside. The suspected isolates from culture were presumptively identified by Gram-stain, oxidase test and superoxol test, and confirmed by rapid carbohydrate utilization test (RCUT) as per the standard protocol. The confirmed colonies were used to determine the antimicrobial susceptibility pattern of the isolates and were also tested for beta-lactamase production.

Antimicrobial susceptibility testing was done using the disc diffusion method as per the Calibrated Dichotomous Sensitivity (CDS) technique against the following antibiotics: penicillin (0.5 i.u.), tetracycline (10 µg), nalidixic acid (30 µg), ciprofloxacin (1 µg), spectinomycin (100 µg), ceftriaxone (0.5 µg) and cefpodoxime (10 µg) (Oxoid, UK). It uses a uniform zone size to define susceptible strains with the results being interpreted in terms of annular radius (mm). For tetracycline, those strains with MIC ≥16 µg/ml as determined by the disc diffusion method were described as plasmid-mediated tetracycline-resistant N. gonorrhoeae (TRNG). The chromosomally-mediated resistance to tetracycline has to be detected by the MIC method. For ceftriaxone, strains with annular radius of 5–9 mm and MIC of
0.03–0.25 μg/ml and for cefpodoxime, strains with annular radius of 7–12 mm were defined as decreased susceptible.\textsuperscript{14} Cefpodoxime has an antimicrobial spectrum similar to that of cefixime, the oral ESC recommended and available for use in India; hence it is used for detection of decreased susceptibility to oral ESCs.\textsuperscript{15} Azithromycin (15 μg) (Oxoid, UK) was tested as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI).\textsuperscript{16} According to the CLSI method, discs containing known amounts of antimicrobial agent are used to establish the zone of inhibition around the disc for the tested strains, which is then interpreted as susceptible, intermediate or resistant based on the diameter of the zone of inhibition. The WHO 2008 \textit{N. gonorrhoeae} reference strains (F, G, K–P), provided by the WHO Gonococcal Antimicrobial Susceptibility Programme (GASP) South East Asia Region (SEAR), Regional Reference Laboratory, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India were used as control strains.

The MIC for all isolates was determined by using Etest strips of penicillin, tetracycline, ciprofloxacin, ceftriaxone, spectinomycin and azithromycin as per the manufacturer’s instructions (Biomerieux, France). The MIC of 0.032–0.25 μg/ml was used as the cut-off for decreased susceptibility to ceftriaxone as per the CDS method. The study was approved by the Ethics Committee of AIIMS, New Delhi, India.

RESULTS
A total of 18 isolates of \textit{N. gonorrhoeae} were obtained (17 from men and 1 from a woman). The strains were consecutive and non-repetitive (one strain per patient). The alarming finding in the current context is that three isolates showed decreased susceptibility to ceftriaxone and cefpodoxime by the disc diffusion method (Table I). All the three isolates were from men. Two had an MIC of 0.064 μg/ml and one had an MIC of 0.125 μg/ml. All the three isolates were sensitive to spectinomycin and azithromycin but resistant to penicillin and ciprofloxacin and less sensitive to tetracycline (Table II). Results by both the methods, i.e. disc diffusion and MIC by Etest, were confirmed by the WHO GASP SEAR, Regional Reference Laboratory, New Delhi. There was no treatment failure. None of the strains were multidrug-resistant or extensively drug-resistant \textit{N. gonorrhoeae} as per the revised guidelines.\textsuperscript{17} All the isolates in our study were sensitive to spectinomycin.

DISCUSSION
The increasing number of reports of decreased susceptibility to ESCs, the first-line treatment for gonorrhoea along with the verified reports of treatment failures with cefixime worldwide,\textsuperscript{6–9} have accentuated the problem of decreasing treatment options for gonorrhoea. To accommodate strains with increased MICs, the recommended dose for ceftriaxone has been increased from 125 mg to 250 mg in various parts of the world while in Japan the current recommendation is to use 1000 mg.\textsuperscript{11} The Centers for Disease Control and Prevention (CDC) treatment guidelines recommend the use of single-dose injectable (ceftriaxone 250 mg i.m.) or oral cephalosporin (cefixime 400 mg) plus azithromycin 1 g orally in a single dose or doxycycline 100 mg orally twice a day for 7 days in case of uncomplicated gonococcal infections of the cervix, rectum and urethra to be administered on site,\textsuperscript{18} whereas the National AIDS Control Organization (NACO) guidelines recommend the use of cefixime 400 mg orally with azithromycin.

\begin{table}[h]
\centering
\caption{Comparison of susceptibility of \textit{Neisseria gonorrhoeae} isolates (n=18) using disc-diffusion method and minimum inhibitory concentration by Etest}
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
\textbf{Antibiotic} & \textbf{Disc diffusion} & \multicolumn{3}{c|}{\textbf{Minimum inhibitory concentration}} \\
& & \textbf{Resistant} & \textbf{Less sensitive} & \textbf{Sensitive} & \textbf{Resistant} & \textbf{Less sensitive} & \textbf{Sensitive} \\
\hline
Penicillin & 6\textsuperscript{*} & 12 & 0 & 6 & 12 & 0 \\
Ciprofloxacin & 16 & 2 & 0 & 16 (6+10 HLR) & 2 & 0 \\
Tetracycline & TRNG: 3 & Not TRNG: 15 & – & TRNG: 3, CMRNG\textsuperscript{2}: 4 & 4 & 7 \\
Spectinomycin & 0 & 0 & 18 & 0 & 0 & 18 \\
Azithromycin & 1 & 0 & 17 & 1 & 0 & 17 \\
Ceftriaxone & 0 & 3 (DS) & 15 & 0 & 3 (DS) & 15 \\
Cefpodoxime & 0 & 3 (DS) & 15 & nd & nd & nd \\
\hline
\end{tabular}
\textsuperscript{*4} penicillinase producing \textit{Neisseria gonorrhoeae} (PPNG)  \\
HLR high-level resistance  \\
TRNG tetracycline-resistant \textit{Neisseria gonorrhoeae}  \\
CMRNG\textsuperscript{2} chromosomally-mediated tetracycline-resistant \textit{Neisseria gonorrhoeae}  \\
DS decreased susceptibility  \\
nd not done
\end{table}

\begin{table}[h]
\centering
\caption{Antibiotic sensitivity pattern of strains with decreased susceptibility to ceftriaxone}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Isolate} & \textbf{Ceftriaxone} & \textbf{Ciprofloxacin} & \textbf{Penicillin} & \textbf{Tetracycline} & \textbf{Spectinomycin} & \textbf{Azithromycin} & \textbf{Cefpodoxime} \\
\hline
\textbf{AR} & \textbf{MIC} & \textbf{AR} & \textbf{MIC} & \textbf{AR} & \textbf{MIC} & \textbf{AR} & \textbf{MIC} & \textbf{AR} & \textbf{MIC} & \textbf{ZD} & \textbf{MIC} & \textbf{AR} \\
\hline
1 & 8 & 0.125 & 0 & 32 & 1 & 1 & 7 & 1 & 10 & 12 & 34 & 0.25 & 10 \\
2 & 7 & 0.064 & 3 & 1 & 0 & 16 & 11 & 1 & 9 & 8 & 34 & 0.125 & 11 \\
3 & 8 & 0.064 & 0 & 32 & 0 & 1 & 10 & 1 & 10 & 4 & 32 & 0.125 & 11 \\
& DS & HLR & R & N-TRNG (LS) & & & & & & S & S & & & \\
\hline
\end{tabular}
\textsuperscript{AR annular radius (mm)}  \\
\textsuperscript{MIC minimum inhibitory concentration (μg/ml)}  \\
\textsuperscript{ZD zone diameter (mm)}  \\
\textsuperscript{DS decreased susceptibility}  \\
\textsuperscript{HLR high-level resistance}  \\
\textsuperscript{CMR chromosomally mediated resistance}  \\
\textsuperscript{N-TRNG not-tetracycline-resistant \textit{Neisseria gonorrhoeae}}  \\
\textsuperscript{LS less-sensitive}  \\
\textsuperscript{S sensitive}  \\
\textsuperscript{R resistant}  \\
\textsuperscript{PPNG penicillinase producing \textit{Neisseria gonorrhoeae}}
\end{table}
1 g orally as a single dose in case of uncomplicated gonococcal infections of the cervix and urethra.16

There has been an increase in the proportion of strains (3 of 18 isolates) showing decreased susceptibility to ceftriaxone as compared to an earlier report from India by Bala et al. (2007), where 9 of 382 isolates were reported with decreased susceptibility to ceftriaxone. Also, in a previous study, a maximum MIC of 0.094 μg/ml was reported,12 whereas in our study a higher MIC (0.125 μg/ml) was observed which may indicate a potential drift towards resistance to ESCs. The past experience with other antibiotics, e.g. penicillin, where a rise in MIC required numerous escalations in the recommended effective dose finally leading to development of resistance to penicillin,11 increases the concern that a similar trend might be observed with ESCs, with sequential increase in MICs preceding the emergence of resistance. It is therefore imperative to monitor the MIC changes in ESCs, which may serve as a warning sign to alert treating venereologists to the problem of resistance.

ESCs are extensively used in our setting to manage other infectious diseases, particularly enteric fever, which is endemic in India. As antibiotics are a shared resource, the indiscriminate use of ESCs creates an ‘antibiotic soup’ ideal for emergence of antibiotic resistance in the microbial world and more so in the case of N. gonorrhoeae that is genotypically and phenotypically versatile.20

Even though all isolates were sensitive to spectinomycin, its non-availability in India precludes its use. Further, as reported in other microorganisms, resistance to azithromycin and spectinomycin develops rapidly after use.

ESCs form the mainstay of gonorrhoea therapy and rise of MICs to the same could necessitate a search for more effective antimicrobials in the long term. Dual therapy may provide better treatment options if it can be provided globally and at a low cost. However, this is unlikely to occur in many situations such as resource-poor settings and in these situations it will not improve containment of antimicrobial resistance.18 As ESC resistance is chromosomally-mediated, the chances of its spreading horizontally are reduced. However, it is essential to limit unnecessary use of this vital drug so that its efficacy is not further decreased.

The correlations between the results of in vitro antimicrobial susceptibility and clinical outcome are extremely close in N. gonorrhoeae. Thus, a periodic monitoring of the antimicrobial susceptibility pattern of the organism in different parts of the country for the prescribed antimicrobials is essential to generate an annual summary of trends and changes to guide therapy recommendations.

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