Short Communication

Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in HIV-Seropositive Patients and Gonococcal Antimicrobial Susceptibility: an Update in Thailand

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SUMMARY: We surveyed the rate of chlamydial and gonococcal infections among human immunodeficiency virus (HIV)-seropositive patients in Thailand as well as the current status of antimicrobial resistance of *Neisseria gonorrhoeae* and determined the prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) in Thailand. A total of 1,158 endocervical swabs from 824 HIV-seropositive patients were collected to detect both organisms by Gen-Probe. The prevalences of chlamydial and gonococcal infection were 9.7 and 1.3%, respectively. Susceptibility of 122 gonococcal isolates to 6 drugs was determined by the disk diffusion method. None of the isolates was susceptible to penicillin or tetracycline. With respect to fluoroquinolones, more than 90% of the isolates were resistant to ciprofloxacin and ofloxacin. No gonococcal isolate with resistance to cepotaxime and ceftriaxone was detected. Among the 122 isolates, 83.6% or 102 isolates were PPNG, and most (79.5%) of these 122 isolates were resistant to ciprofloxacin and ofloxacin. No gonococcal isolate was resistant to cefotaxime and ceftriaxone. All of the 102 isolates identified as PPNG contained the *bla*<sub>TEM</sub> gene. We then performed a preliminary molecular study and identified, for the first time in Thailand, a PPNG isolate producing beta-lactamase and containing the *bla*<sub>TEM</sub> gene which was identical to the beta-lactamase TEM protein of *Salmonella enterica* identified as TEM-135.

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isolates from different patients were collected during June 2005 to May 2007 from Siriraj Hospital and Bangrak Hospital. The latter is a Ministry of Public Health STD hospital that serves a population suspected of having STD infections in Thailand. *N. gonorrhoeae* was isolated and identified according to standard microbiological techniques (8). Isolates were kept at –80°C in 5% trypticase soy broth plus 20% v/v glycerol until use.

The bacterial susceptibility of the isolates to penicillin, cefotaxime, ceftriaxone, tetracycline, ciprofloxacin, and ofloxacin was evaluated by disk diffusion (9). *N. gonorrhoeae* ATCC 49226 was used as a control.

To detect PPNG, we performed a beta-lactamase test (10) using a nitrocefin disk (cefinate; Becton-Dickinson Ltd., Sparks, Md., USA). *Staphylococcus aureus* ATCC 25923 and *N. gonorrhoeae* ATCC 49226 were used as positive and negative controls, respectively. A positive reaction yields a pink color.

The molecular study of gonococcal resistance to penicillin was performed using a loopful of colonies of each isolate taken from a culture on blood agar after 24 h for plasmid extraction by a NucleoSpin Plasmid DNA Purification kit (Macherey-Nagel Ltd., Bethlehem, Pa., USA). The purified plasmid DNA was used as a template to detect the gene coding for beta-lactamase (*blaTEM*) by PCR amplification reaction. The primers for the *blaTEM* gene (11) were amplification primer 1 (TEM-F) 5’-ATGAGTATTCAACATTCCCC-3’ and amplification primer 2 (TEM-R) 5’-CTGACAGTTACCAATGCCTA-3’, leading to a product of 868 bp. The amplification protocol consisted of denaturation at 95°C for 5 min, followed by 25 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, with a final extension at 72°C for 7 min. The pUC19 plasmid DNA containing the ampicillin-resistant gene (*blaTEM*) was used as a positive control. The purified PCR product was verified by DNA sequencing with TEM-F and TEM-R primers in order to confirm the sequence of the *blaTEM* gene (First Base Laboratory Ltd., Selangor, Malaysia). The sequences of the PCR product were compared to those in the GenBank database by means of a homology search for best matches using the nucleotide blast (blastn) and protein blast (blastx) programs at http://ncbi.nlm.nih.gov/blast/Blast.cgi.

Antimicrobial agent No. (%) of isolates
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Penicillin      –      17 (13.9) 105 (86.1)
Tetracycline   –      6 (4.9) 116 (95.1)
Cefotaxime     122 (100) –      –
Ceftriaxone    122 (100) –      –
Ciprofloxacin  2 (1.6) 10 (8.2) 110 (90.2)
Ofloxacin      6 (4.9) 5 (4.1) 111 (91)

The success of the HIV Control Program in Thailand has led to a more than 80% decrease in the prevalence of gonorrhea (13). However, the high prevalence of *C. trachomatis* infection found in this study warrants more attention during gynecologic examination. We found that the mean age of HIV-positive patients with a positive Gen-Probe result for *C. trachomatis* was 30 ± 9.5 years (range, 14 - 53 years), whereas it was 26.5 ± 7.6 years for HIV-positive patients with *N. gonorrhoeae* (range, 14 - 53 years). These mean ages were not significantly different. As in other investigations (14,15), it was found that the infection rates of both *C. trachomatis* and *N. gonorrhoeae* were high in patients aged ≤35 years.

The characteristics of the patients from whom the gonococci were isolated were as follows: the mean age was 26.07 ± 9.24 years (range, 3 days - 68 years), and 74.47% were male and 25.53% female. HIV-positive patients were included. Commercial sex workers were included as 22.13% of total patients (male prostitutes, 16.39%; female prostitutes, 5.74%). In the antimicrobial susceptibility test (Table 2), no isolate was susceptible to penicillin and tetracycline. However, *N. gonorrhoeae* retained 100% susceptibility to both cefotaxime and ceftriaxone, the broad spectrum cephalosporins. With respect to fluoroquinolones, only 2 isolates (1.6%) were susceptible to ciprofloxacin, and 6 isolates (4.9%) were susceptible to ofloxacin. Noticeably, 86.1% of isolates were resistant to penicillin. The high percentage of penicillin resistance observed in our study may have been due to unnecessary use of drugs for unrelated illnesses or self-medication with penicillin or other beta-lactams by patients. Recent reports have shown prevalences of penicillin resistance in *N. gonorrhoeae* which vary geographically from approximately 6.5% in the United States (16) to 81.8% in China (17) and 68% in Indonesia (18). For tetracycline resistance, the prevalences have been reported to be as high as 100% in Indonesia in 2001 (18) and 57% in Bangladesh in 2005 (10). In the present study, 95.1% of isolates were tetracycline-resistant, which was higher than the previous value of 89.1% reported for isolates in Thailand in 1994 - 1995 (19). The ciprofloxacin resistance in Thailand increased from 0.3% in 1992 (20) to 21.8% in 1997 (19). A total of 90.2 and 91% of *N. gonorrhoeae* isolates were respectively resistant to ciprofloxacin and

Table 1. Prevalence of *N. gonorrhoeae* and *C. trachomatis* by Gen-Probe method according to patients’ age groups

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>No. of patients tested</th>
<th>No. (%) of patients with positive test</th>
<th><em>C. trachomatis</em></th>
<th><em>N. gonorrhoeae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>≤15</td>
<td>6</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td></td>
</tr>
<tr>
<td>16 - 25</td>
<td>146</td>
<td>20 (13.7)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
<tr>
<td>26 - 35</td>
<td>443</td>
<td>37 (8.4)</td>
<td>6 (1.4)</td>
<td></td>
</tr>
<tr>
<td>36 - 45</td>
<td>168</td>
<td>17 (10.1)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>46 - 55</td>
<td>37</td>
<td>2 (5.4)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>3</td>
<td>1 (33.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Not accessible</td>
<td>21</td>
<td>1 (4.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>824</td>
<td>80 (10.8)</td>
<td>11 (1.3)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial susceptibility of *N. gonorrhoeae* by disk diffusion method

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Penicillin</td>
<td>–</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>–</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>122 (100)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>122 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>6 (4.9)</td>
</tr>
</tbody>
</table>
showed 100% identity to amino acid sequences with accession no. CAG25427 from S. enterica without the official name of this beta-lactamase being found in the database. However, according to the associated report (22), mutation in amino acid position 182 from methionine to threonine in amino acid of TEM-1 sequence will change this sequence to TEM-135, which is still a restricted spectrum beta-lactamase. The DNA sequence from this study is deposited in the GenBank database under accession no. GQ896333.

In general, N. gonorrhoeae carries indigenous plasmids, including cryptic plasmids. However, at least 3.2, 3.3, and 4.4 megadalton (Md) beta-lactamase plasmids have been described (19,23,24). Furthermore, the genes involved in beta-lactamase production, such as \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{SHV}} \), and \( \text{bla}_{\text{TEM}} \)-like, have been reported (7,8,23). In Thailand in 1977, Knapp et al. reported that 26 of 101 (25.7%) isolates they tested were PPNG, and that most of these PPNG isolates (96.2%, 25/26) possessed the 4.4 Md, with only a single isolate possessing the 3.2 Md beta-lactamase plasmid (19), without specifying TEM-type of beta-lactamase. Until now, however, there has been no report concerning the genetic study of the \( \text{bla}_{\text{TEM}} \) gene of \( N. \) gonorrhoeae in Thailand. This study is an example of an \( \text{bla}_{\text{TEM}} \)-like isolate in other \( N. \) gonorrhoeae isolates in order to elucidate both the precise molecular characterization and the clonal spread.

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Use of trade names is for laboratory test only and does not imply products’ endorsement by CDC or Ministry of Public Health, Thailand.

REFERENCES


