ANTIMICROBIAL SUSCEPTIBILITY OF NEISSERIA GONORRHOEAE ISOLATED FROM PATIENTS ATTENDING PRIVATE CLINICS IN ZARIA.

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ABSTRACT

A total of 125 Neisseria gonorrhoeae strains were isolated from patients attending private clinics in Zaria, Kaduna State, Nigeria. Out of the 125 gonococcal isolates, 90 (72%) were resistant to penicillin G, 85 (68%) to ampicillin, 70 (56%) to tetracycline, 55 (44%) to erythromycin and 26 (22%) isolates were resistant to gentamicin. All the 125 Neisseria gonorrhoeae isolates were susceptible to ceftriaxone, cefuroxime and ofloxacin. Out of the 90 Neisseria gonorrhoeae isolates resistant to penicillin, 65 (72.2%) were positive for β-lactamase production (PPNG). The remaining 25 (27.7%) penicillin resistant strains were β-lactamase negative. The findings of this study have shown high prevalence of multi-drug resistant strains of Neisseria gonorrhoeae amongst attendes of private clinics in Zaria.

Key words: Neisseria gonorrhoeae, antibiotic susceptibility, resistance, PPNG.

INTRODUCTION

Parts of West Africa have been reported to be one of the two origins of penicillin resistant strains of Neisseria gonorrhoeae. The penicillinase-producing Neisseria gonorrhoeae (PPNG) strains were first reported in both England and the U.S.A. in 1976 (1; 2). The origins of these PPNG strains were traced to West Africa and the Far East Asia (3). Joosse et al. (4), reported substantial increase in resistance to penicillin (89%) and tetracycline (98%) in Surabaya, Indonesia. Quinoline-resistant N. gonorrhoeae strains are very common in East Asia (5). In Nigeria, Bảkare et al (6) reported that 59 (92%) of 64 isolates from male patients at Special Treatment Clinic, University College Hospital, Ibadan were penicillinase-producing N. gonorrhoeae (PPNG) and 5 (7.8%) were non-penicillinase-producing N. gonorrhoeae (NPPNG). In 1987, it was estimated that PPNG constituted 70-80% of gonococcal isolates in Nigeria (7). Jatau ef al. (8) reported that 189 (84%) of 225 penicillin-resistant strains of N. gonorrhoeae clinical isolates obtained from various locations of Kaduna state were positive for β-lactamase production.

Based on these reports, we decided to determine the anti-microbial susceptibility patterns of Neisseria gonorrhoeae isolates cultured from patients attending private clinics in Zaria.

MATERIALS AND METHODS

Bacterial isolates
One hundred and twenty-five *Neisseria gonorrhoeae* strains isolated from 35 female and 90 male patients presenting at various private clinics in Zaria with arthritis and cervicitis were screened for their susceptibility patterns against eight antibiotics, using the agar diffusion plate method.

**Antibiotic susceptibility testing**

Pure *Neisseria gonorrhoeae* isolates were screened against eight antibiotics made up of penicillin G (2.4mcg), ampicillin (10 mcg), tetracycline (10 mcg), erythromycin (5mcg), gentamicin (5 mcg), ceftriaxone (0.1 mcg), cefuroxime (10 mcg) and ofloxacin (10 mcg) purchased from Oxoid Unipath, U.K. Reference *Neisseria gonorrhoeae* strains were obtained from WHO collaborating Centre Reference and Research in Gonococci, Copenhagen, Denmark. An inoculum of $10^9$ c.f.u. per ml of each *Neisseria gonorrhoeae* was made from a 24 hours growth on modified Thayer-Martin agar plates. The inoculated plates were left for 10 minutes at room temperature before the antibiotic discs were aseptically placed on them. The inverted plates were incubated in an atmosphere of 5% CO$_2$ at 36°C for 24 hours. The zone of inhibition was measured and interpreted using the guidelines of the National Committee on Clinical Laboratory Standards (NCCLS).

**Determination of Beta-lactamase production and Minimum Inhibitory Concentrations**

Beta-lactamase production was detected using the Rapid Iodometric Method (9). Minimum Inhibitory Concentrations (MICs) of penicillin G, ampicillin, tetracycline, erythromycin, gentamicin, ceftriaxone, cefuroxime and ofloxacin were determined on each *N. gonorrhoeae* isolate using tube dilution methods (10).

**RESULTS**

Male patients dominated clinic attendance during this investigation (Figure 1). Out of the 125 *Neisseria gonorrhoeae* isolates, 90 (72%) were resistant to penicillin, 85 (68%) to ampicillin, 70 (56%) to tetracycline, 55 (44%) to erythromycin and 26 (22%) were resistant to gentamicin. All the 125 *N. gonorrhoeae* isolates were susceptible to ceftriaxone, cefuroxime and ofloxacin (Table 1, Figure 2). Out of 90 gonococcal isolates, 65 (72.2%) were β-lactamase (penicillinase) positive. The remaining 25 penicillin resistant strains were β-lactamase negative. The Minimum Inhibitory Concentrations (MICs) of penicillin, ampicillin and tetracycline were higher than 0.125mcg/ml. Penicillin had an MIC of 32.0 mcg/ml against four (4) isolates, ampicillin had 32.0 mcg/ml against two (2) isolates and tetracycline had 32 mcg/ml against one (1) isolate. Cefuroxime, ceftriaxone and ofloxacin had the value of 0.125 mcg/ml against all the isolates tested. The MICs of most drugs, in common use for treatment of gonorrhoea, such as penicillin, ampicillin and tetracycline were high (Table 1).
TABLE 1. Minimum Inhibitory Concentrations of various antibiotics tested against *Neisseria gonorrhoeae* isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% of isolates with MIC values (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.0</td>
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<tr>
<td>Erythromycin</td>
<td>0.0</td>
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<tr>
<td>Gentamicin</td>
<td>0.0</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>100</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>100</td>
</tr>
</tbody>
</table>

FIGURE 1. Distribution of *Neisseria gonorrhoeae* infections among male and female patients.

1. Males
2. Females
FIGURE 2. Antibiogram of *Neisseria gonorrhoeae* showing susceptibility and resistance to various antibiotics.

Key:
- PenG = penicillin G
- Amp = ampicillin
- Te = tetracycline
- E = erythromycin
- Gen = gentamicin
- Cef = ceftriaxone
- Cefu = cefuroxime
- Oflo = ofloxacin

**DISCUSSION**

The anti-microbial susceptibility patterns of the *Neisseria gonorrhoeae* isolates showed that most were resistant to antibiotics that are commonly used as primary therapy against gonorrhoea. There were fewer isolates that were resistant to gentamicin than to penicillin, ampicillin, tetracycline and erythromycin (Figure 2). All the *Neisseria gonorrhoeae* isolates were susceptible to ceftriaxone, cefuroxime and ofloxacin. The resistance exhibited by these *Neisseria gonorrhoeae* isolates could be attributed to indiscriminate use of anti-microbial agents and self-medication that results in sub-therapeutic dosage, thereby stimulating the development of resistant mutants (7), a phenomenon that is empirically known to be common among men. This may account for the dominance of male patients in this study.

Penicillin had MIC of 32.0 mcg/ml against four *Neisseria gonorrhoeae* isolates, ampicillin had MIC of 32.0 mcg/ml against two isolates while tetracycline had the same MIC against one isolate (Table 1). The high MICs of penicillin, ampicillin and tetracycline confirmed the high level of resistance by the isolates to
these antibiotics. It also confirmed our earlier report on the high prevalence rate of *Neisseria gonorrhoeae* strains resistant to commonly used antibiotics for the treatment of gonorrhoea in Zaria (8).

The high penicillin and tetracycline resistance is similar to such increase in resistance by *N. gonorrhoeae* that resulted in C.D.C. recommending the use of newer fluoroquinolones and selected extended-spectrum cephalosporins as primary therapy against uncomplicated gonococcal infections in the U.S.A. (11). The susceptibility of the 125 *Neisseria gonorrhoeae* clinical isolates to quinolones is, however, contrary to reports that *Neisseria gonorrhoeae* strains resistant to quinolones (QRNG) are wide spread in Asia (5), and that their prevalence is endemic in California and Hawaii, U.S.A. (12).

Beta-lactamase production was detected in 65 (72.2%) of the 90 penicillin resistant strains. The remaining 25 (27.8%) penicillin resistant strains were β-lactamase negative. The high prevalence rate of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) found in our study is in agreement with earlier report that PPNG constitutes 70-80% of *N. gonorrhoeae* isolates in Nigeria.

Anti-microbial resistance mechanisms are of two types:

1. chromosomal resistance which results from serial changes in the structure of penicillin-binding proteins and/or outer membrane permeability, and
2. the production of β-lactamase by plasmid bearing strains of *N. gonorrhoeae* that was first reported in 1976 (13).

The initial epidemiologic report on PPNG strains showed that there was a spread of these strains from Asia and Africa to all other parts of the world.

The high prevalence of PPNG strains among patients attending private clinics in Zaria poses serious public health problems because the β-lactamase has been found to be identical with that found in *Haemophilus influenzae* which is reported to have acquired resistant plasmid from other Gram-negative bacilli (13).

*Gonorrhoeae* has a high prevalence rate in Zaria. The situation should attract public health officials' attention because Zaria is an academic center and should not be allowed to serve as a source of PPNG and T2NG spread-particularly now that the world is a global village that travelers can be sources of spread of sexually transmitted diseases (14). All STD should be treated promptly and screened for (for cure). All *Neisseria gonorrhoeae* isolates should be tested for susceptibility to drugs in common use. Specialized clinics for STD's should be established to ensure proper control and prevention of such diseases.

**REFERENCES**


