Original article

Emergence of penicillin resistant *Neisseria gonorrhoeae*.

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Abstract

Introduction: Since the beginning of the modern chemotherapeutic era, the treatment of gonorrhea has been dogged by the shifting antimicrobial susceptibility of *Neisseria gonorrhoeae.* Though penicillins have been recommended for treatment of gonorrhoeae, there have been reports of treatment failure with penicillins. In this study, we report the emergence of penicillin resistance *N. gonorrhoeae* and its antimicrobial susceptibility pattern.

Methods: Antibiotic sensitivity pattern of *N. gonorrhoeae* isolates from the male patients was determined by Kirby Bauer's Disc Diffusion method. The zone size was interpreted according to the Zone size interpretative chart. β-lactamase production was detected by Paper iodometric method.

Results: A total of 30 isolates of *N. gonorrhoeae* were obtained from male patients with suspected acute gonococcal urethritis. Out of the 30 isolates, 18 (60.0%) were resistant to penicillin. Most of the penicillin resistant isolates were from the patients of age group 31 to 40 years (77.8%) followed by 21 to 30 years (53.3%) and 41 to 50 years (50.0%) ($P>0.05$). All isolates were sensitive to ceftriaxone. Similarly, 6 isolates were resistant to ciprofloxacin and 10 isolates were resistant to tetracycline. β-lactamase producer isolates were 16 in number.

Conclusions: Penicillin resistant *N. gonorrhoeae* is on the rise in and around Birgunj. Thus, systematic surveillance of antibiotic sensitivity pattern of *N. gonorrhoeae* could be effective in detecting emergence of newly resistant strains before they become widespread in the community.

Keywords: Antimicrobial susceptibility, *Neisseria gonorrhoeae*, penicillin

Introduction

Gonorrhoea is one of the classical sexually transmitted disease (STD) with human as the host for the causative agent, *N. gonorrhoeae*. According to a global estimate from World Health organization (WHO), around 62 million new cases occurred in 1995 and the highest rate was found in South and Southeast Asia, Sub Saharan Africa and South and Central America.[1] According to Family Health Division, Ministry of Health, Nepal, gonorrhoea is one of the prevalent STD in Nepal.[2] The problem is further compounded by the emergence of resistance to antimicrobial agents that are commonly used against *N. gonorrhoeae*, making the treatment expensive and prolonged.

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failure. Antibiotic sensitivity pattern for *N. gonorrhoeae* in different part of the world is also not uniform, and the emergence of resistance to the commonly used drugs, such as penicillin, tetracycline and ciprofloxacin is frequent in different part of the world including South East Asian Region (SEAR).[3-9] The development and spread of resistance is probably due to suboptimal doses of penicillin and irrational use of antibiotics due to easy accessibility of the antibiotics. The

present study reports the emergence of penicillin resistant *N. gonorrhoeae* in NMCTH, Birgunj, Nepal.

Methods

A total of 30 consecutive male patients attending Skin and Venereal disease OPD at National Medical College and Teaching Hospital during February 2009 to May 2009 and clinically suspected of having acute gonococcal urethritis were included in the study.

Urethral swabs were taken from the study subjects for direct microscopy and culture for *N. gonorrhoeae*. Gram stained smears were prepared from the urethral swab to look for pus cells and intracellular Gram negative diplococci. Specimens were immediately inoculated on selective Modified Thayer Martin Agar Media (Himedia, Mumbai, India) and incubated for 24–48 hours at 35^{0}C in humid atmosphere containing 5–10 percent CO_2. Isolates were identified by colony morphology, Gram staining, oxidase test, superoxol[10] and rapid carbohydrate utilization test.[11] Antimicrobial discs containing penicillin (10 I.U.), ciprofloxacin (5 mcg), tetracycline (30 mcg) and ceftriaxone (30 mcg) were used for disc diffusion on Muller Hinton Agar plates. The isolates were defined as susceptible, partially susceptible and resistant using the zone size interpretative chart, following the Kirby Bauer disc diffusion method. Beta-Lactamase production was detected by Rapid penicillinase paper strip test.[12] Statistical analysis was performed using Chi-square test.

Results

A total of 30 isolates of *N. gonorrhoeae* were obtained from patients with suspected acute gonococcal urethritis(Fig.1).

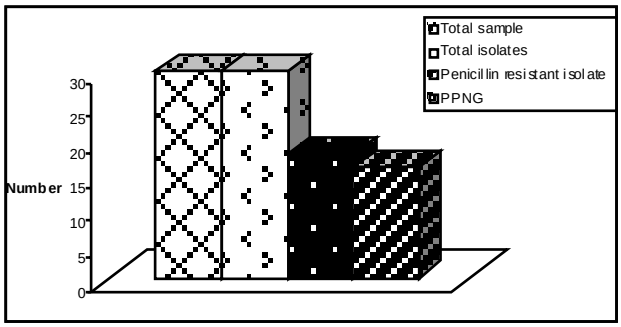

Fig. 1: Number of various isolates of *N. gonorrhoeae* in total sample

Out of the 30 isolates, 26.6% of isolates were sensitive, 31.3% were partially sensitive and 60.0% isolates were resistant to penicillin (Table 1). Most of the penicillin resistant isolates were from the patients of age group 31 to 40 years (77.8%), followed by 21 to 30 years (53.3%) and 41 to 50 years (50.0%) (P>0.05) (Table 2). Similarly, 50.0% isolates were sensitive, 16.6% isolates were partially sensitive and 33.3% isolates were resistant to tetracycline. Likewise, towards ciprofloxacin, 43.3% isolates were sensitive, 36.6% isolates were partially sensitive and 20.0% isolates were resistant. All (100%) isolates were found to be sensitive towards ceftriaxone. Further, Beta-lactamase test (paper iodometric) showed 53.3% of isolates to be penicillinase producing.

Table 1: Antimicrobial susceptibility pattern of *N. gonorrhoeae* isolates (n= 30)

Antibiotics	Susceptibility pattern for *N. gonorrhoeae*	Number of isolates (%)
Penicillin	Resistant	18 (60.0)
	Partially Sensitive	4 (31.3)
	Sensitive	8 (26.6)
Tetracycline	Resistant	10 (33.3)
	Partially Sensitive	5 (16.6)
	Sensitive	15 (50.0)
Ciprofloxacin	Resistant	6 (20.0)
	Partially Sensitive	11 (36.6)
	Sensitive	13 (43.3)
Ceftriaxone	Resistant	0 (0.00)
	Partially Sensitive	0 (0.00)
	Sensitive	30 (100.0)

Table 2: Age-wise distribution of penicillin resistant isolates

Age group	Total isolates	Penicillin resistant isolates	Percentage	P-value
21-30 years	15	8	53.3	P>0.05
31-40 years	9	7	77.8	
41-50 years	6	3	50.0	

Discussion

The present study highlighted the emergence of penicillin resistant *N. gonorrhoeae* in Birgunj. In our study, resistance to penicillin was detected in 60.0% (18/30) of isolates, partially sensitive to penicillin was seen in 13.3% isolates and sensitive to 26.6% of isolated cases. The isolation rate (53.3%) of PPNG (Penicillinase producing *Neisseria gonorrhoeae*) in our study was higher than that reported in Tribhuvan University Teaching Hospital (TUTH) in 2001.[7] This may be attributed to the lower socio-economic status and insufficient awareness among the people in the outskirts as compared to the capital of the country leading to indiscriminate use of antibiotics and delay in seeking medical treatment. Study was conducted in different focal point laboratories in SEAR including Nepal under GASP;

most of the laboratory reported significant percentage of PPNG among the isolates.[13]

Though the higher number of Penicillin resistant isolates were recovered from the age group 31 to 40 years as compared to other age group, the difference was statistically insignificant.

Tetracycline and ciprofloxacin resistance detected among 33.3% (10/30) and 20.0% (6/30) of the positive isolates, respectively may be attributed to the overuse of these drugs since these drugs can be orally taken and also prescribed for various infection, and moreover, in this part of the world over-the-counter availability of drugs without prescription is a common practice.[14]

In our study, ceftriaxone was found to be sensitive against all isolates. Therefore, based on our research finding, we would like to recommend ceftriaxone as the first line of drug for the treatment of gonorrhoea. Center for Disease Control and Prevention (CDC) has already recommended single dose therapy with a broad spectrum cephalosporin (cefixime or ceftriaxone) or a fluroquinolone (ciprofloxacin or ofloxacin) for uncomplicated gonorrhea.[15]

Gonorrhoea remains a significant disease globally. While it is more frequent in poorer countries (and in marginalized groups in all countries), disease rates remain unacceptably high in developed countries, and appear to be increasing at present.[16] Increasing resistance in *N. gonorrhoeae* is an increasing problem in the United States and presents a challenge in the design of control strategies and the selection of antimicrobial therapies.[17]

In our study too, the detection of drug resistance among commonly used drugs for the treatment of *N. gonorrhoeae* is significantly high. At this time, *N. gonorrhoeae* must be grown in culture to conduct tests for antimicrobial susceptibilities. The increasingly widespread use of nonculture methods for diagnosis is a major challenge to monitoring of resistance pattern.[18] Furthermore, routine susceptibility testing is not performed in most settings because of cost and reproducibility problems.

The knowledge of antimicrobial susceptibility of *N. gonorrhoeae* is a prerequisite for proper treatment and control of the disease. In Nepal, there is no established antimicrobial susceptibility surveillance for *N. gonorrhoeae*. In the absence of laboratory data and an established monitoring system, selection of appropriate antibiotics for empirical treatment of gonorrhea is difficult.

Conclusions

Systemic surveillance of antibiotic sensitivity test should be carried out for continuous monitoring of the antibiotic susceptibility in *N. gonorrhoeae* for determination of optimal treatment regimen.

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