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Frequency and Sensitivity Pattern of *Neisseria gonorrhoae* Isolated from Women at a Tertiary Care Hospital in Dhaka City

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Abstract

Background: Antibiotic sensitivity pattern of *Neisseria gonorrhoae* is changing frequently. **Objective:** The purpose of the present study was to see the antibiotic sensitivity pattern of *Neisseria gonorrhoae* isolated from women presented with cervicitis. **Methodology:** This cross sectional study.carried out in the Department of Microbiology at Dhaka Medical College, Dhaka from July 2011 to December 2012 for a period of one and half year. All the women presented with or without the clinical features of cervicitis at child bearing age (15 to 49 yrs) with or without pregnancy were enrolled as study population. Endocervical swabs were collected for Gram staining, culture, biochemical test from clinically diagnosed patients of cervicitis for presumptive identification of *Neisseria gonorrhoeae*. The isolates with presumptive identification were confirmed by rapid carbohydrate utilization test (RCUT), which differentiates *N.gonorrhoeae* from other *Neisseria.* Antimicrobial susceptibility test was done according CLSI guideline. **Result:** A total 245 females were recruited of which *N. gonorrhoeae* were detected from 39(15.9%) cases. Among 39 positive cases of gonococcal cervicitis, 19 (48.71%) were detected in the age group of 21-30 years. 56.41% were sensitive and 30.76% were resistant to ceftriaxone. Similarly 51.28% were sensitive and 23.07% were resistant to cefuroxime; 23.07% sensitive, 30.76% moderately sensitive and 46.15% resistant to ciprofloxacin. **Conclusion:** Gonococcal cervicitis is found in a high frequency rate with a reduced sensitivity to antibiotics especially ciprofloxacin, second and third generation cephalosporins. [*Bangladesh J Infect Dis 2014;1(1):3-7*]

Keywords: Sensitivity pattern; *Neisseria gonorrhoae*; cervicitis

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Introduction

Cervicitis is more common in the women of childbearing age that is between 15 to 49 years[1]. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* account for about half of the cervicitis cases[2]. Among these two diseases, gonococcal infection is one of the oldest known bacterial diseases[3]; however, it still causes a significant morbidity among the sexually active individuals[4]. In Bangladesh, the prevalence of gonococcal cervicitis is 0.2% to 0.5%[5]. Gonococcal cervicitis can be diagnosed by detecting gram negative intracellular diplococcic in cervical exudates but it's sensitivity is <55%[6]. Culture has a sensitivity of 85 to 95% but it needs special care as *N. gonorrhoeae* is a fastidious organism and very much susceptible to drying[7]. Although culture has advantage to observe the antibiotic susceptibility of the isolated bacteria, it needs selective media and at least 3 days period is needed to provide a positive result[7-8]. Antimicrobial resistance in *N. gonorrhoeae* is the most significant challenge to control gonorrhea. Gonococcal strains are resistant to penicillins, tetracyclines and recently resistant strains to fluroquinolones and azithrtomycin have emerged[9]. Ceftriaxone retains its activity but recently isolates are detected with decreased sensitivity in various regions of the world[10].

In Bangladesh, the non-selective culture methods are being used in the laboratory practices for isolation of *Neisseria gonorrhoeae*, the sensitivity of which is very low. So, the usage of selective media can make better isolation of the organism. Few studies have been carried out in Bangladesh regarding diagnosis of bacterial causes of cervicitis including *Neisseria* gonorrhoeae[5,11]. Therefore, the study is designed to isolate and identify *Neisseria gonorrhoeae* from endocervical swab specimens of suspected patients of cervicitis attending Dhaka Medical College Hospital by culture.

Methodology

This was a cross sectional study.carried out in the Department of Microbiology at Dhaka Medical College, Dhaka from July 2011 to December 2012 for a period of one and half year. All the women presented with or without the clinical features of cervicitis at child bearing age (15 to 49 yrs) with or without pregnancy who were attended at Gynaecology and Obstetrics out patient department (OPD) of Dhaka Medical College Hospital were enrolled as study population. Women with per vaginal bleeding or menopause women or receiving any antimicrobial drugs in the past 15 days were excluded from this study. It was approved by the research review committee (RRC) and ethical review committee of Dhaka Medical College. Informed written consent was taken from each patient before sample collection. Three endocervical swabs were collected from clinically diagnosed patients of cervicitis[12]. One swab was immediately used for Gram staining. Another swab was used for culture and the third one was mixed with 2 ml sterile phosphate buffer saline and kept at -20˚C until it was used for PCR[13]. In Gram stained smear, pus cells were observed and *Neisseria gonorrhoeae* was identified by presence of intracellular and extracellular bean shaped gram negative diplococci. The specimen of endocervical swab was inoculated on blood agar, chocolate agar and Modified Thayer Martin (MTM) media with proper labeling. The inoculated plates were then kept into CO_2 extinction jar with soaked paper towel at the bottom and the lid of the jar was closed tightly. Each time the jar was opened to keep a new inoculated plate, the candle was flamed. The CO_2 jar was kept at 37˚C for over night incubation. Culture plates were examined after 24 hours of incubation for growth of *Neisseria gonorrhoeae. N. gonorrhoeae* was grown both in chocolate agar and selective Modified Thayer Martin media. Presumptive identification of *Neisseria gonorrhoeae* was done by colony morphology, oxidase test, superoxol test and Gram staining and species identification was done by carbohydrate utilization test and PCR. The isolates with presumptive identification were confirmed by rapid carbohydrate utilization test (RCUT), which differentiates *N.gonorrhoeae* from other *Neisseria.* Finally *N. gonorrhoeae* was confirmed by detecting DNA of *N. gonorrhoeae* by PCR using species specific primers. Antimicrobial susceptibility test was done for all *N.gonorrhoeae* isolates with five antimicrobial agents such as penicillin (10unit), tetracycline (30μg), ceftriaxone (30μg), ciprofloxacin (5μg) and cefuroxime (30μg) following CLSI guideline[14]. The susceptibility was done by disc diffusion method to find out inhibitory zone diameters to the discs of recommended strength following CLSI guideline[14]. The plates were then incubated in a humid atmosphere with 5 10% CO_2 inside a candle jar at 37^0 C for 24 hours. A representative of each batch of discs was tested with WHO reference strains of *N. gonorrhoeae* ATCC 49226 and *Staphylococcus aureus* ATCC 25923 to verify their optimum performance before disc diffusion test was done with the clinical isolates. After 18 to 24 hours of incubation each plate was examined and the diameter of each zone of inhibition was measured with a scale[12]. Statistical analysis was performed by SPSS 19. The qualitative data were expressed as frequency and percentage and the quantitative data were expressed as mean and standard deviation.

Result

A total 245 females of child bearing age (15 49 yrs), both pregnant and non pregnant were recruited after fulfiling the inclusion and exclusion criteria of which *N. gonorrhoeae* were detected from 39(15.9%) cases.

Table 1: Rate of Isolation of *N. gonorrhoeae* among Study Population (n=245)

N. gonorrhoeae	Frequency	Percentage
Positive	39	15.9
Negative	206	84.1
Total	**245**	**100.0**

Among 39 positive cases of gonococcal cervicitis, 19 (48.71%) were detected in the age group of 21-30 years followed by 10 (25.64%) in 31-40 years. Only 3 (7.70%) cases were found in 15-20 years of age group.

Table 2: Distribution of Study Population According to age (n=245)

Age Group (Years)	*N. gonorrhoeae*		Total
	Positive	Negative	
15 to 20	3	17	20
21 to 30	18	108	126
31 to 40	8	56	64
41 to 49	6	29	35
Total	**39**	**206**	**245**

*Mean±SD=29.4±8.45; p value=0.938

Regarding antibiotic susceptiblty pattern of *N. gonorrhoeae*, 56.41% were sensitive and 30.76% were resistant to ceftriaxone. Similarly 51.28% were sensitive and 23.07% were resistant to cefuroxime; 23.07% sensitive, 30.76% moderately sensitive and 46.15% resistant to ciprofloxacin. The organism was 28.20% sensitive, 46.15% moderately sensitive and 25.64% resistant to penicillin; 38.46% sensitive and 35.89% resistant to tetracycline (Figure I).

Discussion

The causative organism of cervicitis are related to sexually transmitted infections[15] which continues to present major health, social and economic problems in the developing world, leading to considerable morbidity, mortality and stigma[16]. The prevalence rates apparently are far higher in developing countries where STI treatment is less accessible[15]. An estimated 448 million new cases of curable STIs occur each year[14] and the incidence was about 70.80 million in South-East Asian region[17].

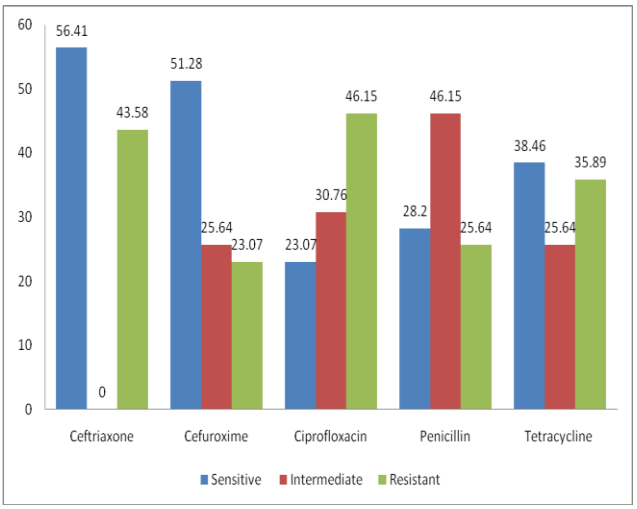

Figure 1: Antibiotic susceptibility pattern of *N. gonorrhoeae*

Gonococcal infection is one of the oldest diseases of the world that causes cervicitis in women[18]. About 30-50% of women have gonococcal infection[19]. In this study, among 245 suspected cases of cervicitis, 15.91% were positive for gonococcal infection and among them, 19 (48.71%) were detected in the age group of 21-30 years. The mean age with SD of gonococcal infection was 29.4±8.45 years. Desai et al[20] observed the mean age of gonococcal. Chowdhury et al[21] showed that 60% of sexually transmitted diseases were in the age group of 20-30 years which were in accordance with the study. Young women were particularly susceptible to STIs because they had fewer antibodies to fight pathogens and greater cervical ectopy[19]. The biologic basis for this association is thought to be anatomic differences in cervix of younger women and cervical change during puberty[22].

In the present study, regarding antibiotic susceptiblty pattern of *N. gonorrhoeae*, 56.41% were sensitive, and 43.58% were resistant to ceftriaxone. In contrast to the present study, Olsen et al[23] showed that 95% *N. gonorrhoeae* were sensitive to this drug and 5% were resistant. However, Bala et al[24] summarized some results and showed that less susceptible strains of *N. gonorrhoeae* to ceftriaxone emerged in some countries. Therefore, currently only ceftriaxone is not the drug of choice for treatment of gonococcal infection. In this study, among the isolates of *N. gonorrhoeae*, 23.07% were sensitive, 30.76% were moderately sensitive and 46.15% were resistant to ciprofloxacin. Shirin et al[25] observed that 48.9% *N. gonorrhoeae* were resistant to ciprofloxacin which is in accordance with the present study. In contrast,

90.5% resistance to ciprofloxacin was observed in 2002 by ICDDR,B[26]. Singh et al[27] and Malar et al[28] reported that >70% of isolated *N. gonorrhoeae* were resistant to ciprofloxacin in India and South Asian countries. This emergence of resistance was probably the consequence of multiple factors, which include syndromic treatment of STDs particularly gonorrhea by ciprofloxacin, self medication by the patients, over use of this drug by clinicians in various other clinical conditions that might coexist with gonococcal infection. In this study, among the isolates of *N. gonorrhoeae*, 74.3% were susceptible and 25.6% were resistant to penicillin. Of them, 28.2% of *N. gonorrhoeae* were sensitive and 46.1% were moderately sensitive. Bala and Sood[29] observed that 42.9% *N. gonorrhoeae* showed reduced susceptibility to penicillin and Malar[28] showed that 29.6% *N. gonorrhoeae* were resistant to this drug which is in accordance with the present study. On the other hand, Putnam et al[30] showed in a study in South East Asia that 26.6% *N. gonorrhoeae* were moderately sensitive and 60.6% were resistant to penicillin. A decade later, Singh et al[27] reported 39.7% *N. gonorrhoeae* were resistant to penicillin. So it is shown that the resistance to penicillin did not rise significantly over the last few years and the susceptibility of penicillin is increasing. This might be due to limited use of penicillin to treat gonorrhea cases in the recent years.

Conclusion

Gonococcal cervicitis is found in a high frequency rate with a reduced sensitivity to antibiotics especially ciprofloxacin, second and third generation cephalosporins. Therefore, clinician should aware of prescribing the antibiotics to the patients presented with gonococcus.

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