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The Prevalence and Antimicrobial Sensitivity Patterns of Neisseria gonorrhoeae Isolated from Suspected Patients in a Tertiary Level Hospital in Bangladesh

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Abstract

Background: Neisseria gonorrhoeae is one of the most common sexually transmitted diseases in developing countries. It causes infections particularly of the urethra in men and the endocervix in women. Over the last decade, Neisseria gonorrhoeae strains have developed a significant level of resistance against several antimicrobial agents such as penicillin, tetracycline, and quinolones in several countries including Bangladesh, which is causing an increasing difficulty in the management of gonorrhoea. **Aims:** Our purpose was to identify the risk factors related to gonorrhoea infection and the trend of antimicrobial resistance of N. gonorrhoeae isolated from gonorrhoea patients. **Materials and Methods:** This prospective observational study was conducted at Rajshahi Medical College Hospital (RMC) between March 2018 and February 2019 where a total of 974 patients were included. Neisseria gonorrhoeae was identified microscopically, by culture and by PCR. An antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion test, according to CLSI. **Results:** Twenty-nine (2.98%) were positive to have N. gonorrhoeae. Antibiotic susceptibility pattern for ceftriaxone was found 62.07% sensitive and 37.93% resistant. Surprisingly, cefuroxime was found to be 100% sensitive whereas ciprofloxacin was sensitive for 51.73%, intermediately sensitive for 27.58%, and was resistant for 20.69%. Similarly, it was 20.69% sensitive, 13.79% intermediately sensitive and 65.52% resistant to tetracycline. In contrast, it was alarmingly resistant to penicillin at 68.97% and 13.79% was intermediately sensitive**. Conclusion:** In this stud, penicillin and tetracycline were shown the highest level of drug resistance which is most commonly used for treating this infection in Bangladesh. For this reason, proper laboratory diagnosis and antimicrobial susceptibility testing, are highly recommended to manage this infection properly.

Keywords: Neisseria gonorrhoeae, Antimicrobial susceptibility, Multidrug resistance.

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Introduction

Gonorrhoea is a sexually transmitted disease (STD) caused by Neisseria gonorrhoeae, and for this disease, human is the only natural host. N. gonorrhoeae is one of the most common sexually transmitted diseases in developing countries (Lessmana, 2001). It causes infections particularly of the urethra in men and the endocervix in women. In these regions, the columnar epithelium of the endocervix is susceptible to infection and having any sign and such symptom as pain during sexual intercourse, during urinating painful or burning sensation, and abnormal vaginal discharge, as well as, during disseminated infection, it causes cramps and pain to the lower abdomen (Ward, 1972).

The prevalence of gonorrhoea in males aged from 15 to 49 years was estimated to be 2% in Sub-Saharan Africa, about 1% in South and Southeast Asia, and only 0.6% in South and Central America, whereas the prevalence of it is almost one-tenth in the industrialized countries (Gerbase, 1998). Over the last decade, Neisseria gonorrhoeae strains have developed a significant level of resistance against several antimicrobial agents such as penicillin, tetracycline, and quinolones in several countries including Bangladesh (Stathi, 2006; Wang, 2006; Enders, 2006; Shethi, 2006; Bhalla, 2002, Bhalla, 1998, Ray, 2000; Bala, 2003 and Tapsaal, 2005), which is causing an increasing difficulty in the management of gonorrhoea.

In this article, we aimed at identifying the risk factors related to gonorrhoea infection and the trend of antimicrobial resistance of N. gonorrhoeae isolated from gonorrhoea patients between March 2018 and February 2019.

Materials and Methods

A total number of 974 patients - out of which 521 male patients clinically presenting with acute urethritis and 453 female patients presenting with cervical/vaginal discharge attended at Rajshahi Medical College Hospital (RMC), Rajshahi, Bangladesh between March 2018 and February 2019 - were enrolled in this prospective observational study. This study was conducted with the ethical clearance of the authority and written informed consent was obtained from each respondent.

Isolation and identification of Neisseria gonorrhoeae were performed after proper collection of urogenital specimens. Firstly, a Gram stain was done to observe the presence of N. gonorrhoeae under the microscope. It was identified by the presence of intracellular and extracellular gram-negative diplococci.

Secondly, the species were inoculated on Blood agar, Chocolate agar, and Modified Thayer Martin medium, and were incubated in a candle jar at 35–37°C for 48 hours, and then the plate was examined for colony growth. If there was growth, a further biochemical test was done to

confirm N. gonorrhoeae. The organism is oxidase-positive and ferments glucose but not maltose, sucrose, or lactose. Species identification was done by carbohydrate utilization test which distinguished N. gonorrhoea from other Neisseria species. Eventually, N. gonorrhoeae was confirmed by DNA detection by PCR using species-specific primers.

Antimicrobial Susceptibility Testing.

Antimicrobial susceptibility testing of isolated N. gonorrhoeae was performed using the Kirby–Bauer disk diffusion test, according to CLSI. From the pure culture, 3–5 colonies of bacteria were transferred to a tube with sterile normal saline to prepare a suspension which is comparable with 0.5McFarland standards. A sterile swab was used to distribute the bacteria evenly over the entire surface of the agar with a 1% selective supplement. The susceptibility patterns isolates were tested against the following antimicrobial agents: penicillin (P 10 IU); tetracycline (TE 30μg); ciprofloxacin (CIP 5μg); ceftriaxone (CRO 30μg) and cefuroxime (CR 30μg). The standard reference strain of N. gonorrhoeae ATCC 49226 was used as recommended by the Clinical and Laboratory Standards Institute (CLSI) for controlling the entire quality (CLSI, 2016). After 24 hours of incubation, the disk was examined to measure the zone of inhibition by scale to identify the antibiotic's sensitivity.

Results

A total of 974 patients were included in this study. Out of those majority, 521 (53.49%) were males with the age ranging from 25 to 38 years whereas 453(46.51%) was female, age ranged from 23 years to 33 years. Out of the total respondents, 759(77.93%) were rural population, in contrast, only 215(22.07%) were from urban areas. Regarding their income level, low socio-economic condition patient was by far the highest number with 884(90.76%) followed by middle socio-economic and higher socio-economic groups with 82(8.42%) and 08(0.82%) respectively. At the same time, the highest number of the population was illiterate 853(87.58%), in contrast, 96(9.86%) had completed their primary/secondary education and only 25(2.56%) had completed their higher degrees (Table 1).

Among 974 respondents, 29 (2.98%) were culturally and microscopically confirmed to have N. gonorrhoeae (Table 2).

In our study, N. gonorrhoeae's antibiotic susceptibility pattern for ceftriaxone was found 62.07% sensitive and 37.93% resistant. Surprisingly, cefuroxime was found to be 100% sensitive for N. gonorrhoea whereas ciprofloxacin was sensitive for 51.73%, intermediately sensitive for 27.58%, and was resistant for 20.69%. Similarly, it was 20.69% sensitive, 13.79% intermediately sensitive and 65.52% resistant to tetracycline. In contrast, it was alarmingly resistant to penicillin at 68.97% and 13.79% was intermediately sensitive (Figure 1).

Table 1: Socio demographic data of the study population (n= 974)

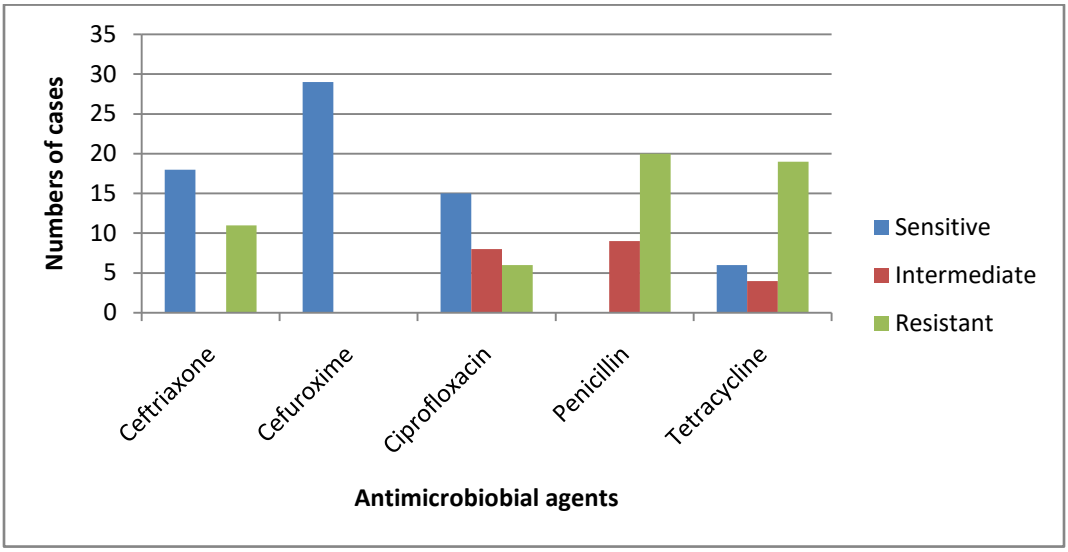
Variables		Total Number	Percentage (%)
Sex	Male	521	53.49
	Female	453	46.51
Age	23-27	657	67.45
	28-32	284	29.16
	33-38	33	3.39
Residence	Rural	759	77.93
	Urban	215	22.07
Socio-economic condition	Low	884	90.76
	Middle	82	8.42
	High	08	0.82
Educational level	Illiterate	854	87.58
	Primary to Secondary	96	9.86
	Up to graduation	25	2.56

(Source: Bangladesh Bureau of Statistics – 2020)

Table 2: Rate of Isolation of N. gonorrhoeae among the study group (n=974)

N. gonorrhoeae	Total number	Percentage(%)
Positive	29	2.98
Negative	945	97.02
Total	974	100

Figure 1: Antimicrobial sensitivity patterns of N. gonorrhoeae observed from the patient having a gonococcal infection (n=29).



Discussion

Documentation of Neisseria gonorrhoeae resistance to multiple antibiotics over the entire globe has given rise to major concerns regarding a future of untreatable gonorrhoea. World Health Organization and the Centers for Disease Control and Prevention have recently recommended ciprofloxacin for the treatment of uncomplicated gonorrhoea infection in countries where multidrug-resistant strains have been found such as Southeast Asia and Central Africa (WHO, 2001).

In this study, among 974 suspected cases of N. gonorrhoeae, 29(2.98%) were positive for gonococcal infection, and among them, males were predominant 521(53.49%). The majority of the cases were detected between the age group 23 and 27 years which was 657(67.45%). In the study of Desai *et al* (2009), they observed that the average age of gonococcal infection was 26-34 years. Chowdhury et a**l** (2010) showed that about 60% of sexually transmitted diseases were between 20 and 30 years which were in accordance with our study. Illiterate young men with low-income levels were particularly more susceptible to gonorrhoea because they had no proper knowledge and information about the route of transmission of this infection as well as for their poor nutritional status for their poor income leads to produce fewer antibodies to fight pathogen**s** (Farley, 2003; Prasad, 2005 and Desai, 2009).

In our study, a high level of resistance to penicillin 20(68.97%) and also tetracycline 19(65.52%) was observed which is comparable with some of the previous studies in the USA (Paterson, 2000), Australia (Workowski, 2015, and Romania (Unemo, 2014). It may be due to the emergence of penicillin-resistant beta-lactamase-producing strains. A high prevalence of plasmid-mediated high-level or chromosomally mediated resistance to penicillin or tetracycline has been reported in Southeast Asia (Lahra, 2018) and South Africa (Ray, 2005) Also, it is known that gonorrhoea and drug resistance vary greatly from country to country due to the different treatment protocols of the countries.

At the same time, ciprofloxacin was sensitive for 15(51.73%). It was probably as ciprofloxacin has been used extensively in Bangladesh, as it is relatively cheaper and effective. As a consequence of the large-scale use of this group of antibiotics in our country, this drug is gradually developing resistance.

Similarly, ceftriaxone was found sensitive in 18(62.07%) cases which is an alarming sign for all of us. The drastic increase of resistant strains is occurring in those countries where drugs are being sold without a prescription (Ison, 1998 and Ison, 1992). Self-medication without having proper knowledge about substances has been found to play a significant role in the development of antimicrobial resistance (Klausner, 1999).

Conclusion

In our study, the prevalence of gonococcal infection was found significantly high in rural populations than urban. At the same time, people from low socio-economic status were more prone to this infection. Lack of proper education, as well as lack of information about sexually

transmitted infections (STIs), were the crucial key factors for N. gonorrhoeae infection. In this stud, penicillin and tetracycline were shown the highest level of drug resistance which is most commonly used for treating this infection in Bangladesh. For this reason, proper and prompt laboratory diagnosis, as well as antimicrobial susceptibility testing, are highly recommended to manage this infection properly.

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