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A Study Of The Antimicrobial Susceptibility Pattern Of Neisseria Gonorrhoeae

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Abstract: Background and objectives: *Rapidly emerging antimicrobial resistance of Neisseria gonorrhoeae isolates to currently recommended antibiotics is a setback for effective treatment and control of disease. This study was carried out to determine the antimicrobial susceptibility profile in the isolates obtained from female sexual workers and male patients attending STI clinics in Hyderabad.*
Materials and methods*: Isolates were tested for sensitivity pattern of penicillin, tetracycline, ciprofloxacin azithromycin, cefixime and spectinomycin. Minimum inhibitory concentration (MIC) and zones of inhibition were interpreted according to CLSI guidelines. β lactamase production was detected by chromogenic cephalosporin method using nitrocefin disc.*
Results and conclusion: *20 isolates of Neisseria gonorrhea were identified and tested for antimicrobial susceptibility profile. Of them 35%, 35%, 20%, 45% and 5% were resistant to penicillin, tetracycline, ciprofloxacin, azithromycin and cefixime respectively. 30% of isolates were penicillinase-producing N. gonorrhoeae. More of less susceptible strains to these antibiotics were identified. Emergence of less susceptible (10%) and resistant strains (5%) to cefixime, which is the current drug of choice, is a cause of concern. Thus periodic surveillance of susceptibility levels of N. gonorrhoeae is essential to prevent the dissemination of drug resistant strains in the community.*
Keywords: *Antimicrobial susceptibility, MIC values, Neisseria gonorrhoeae, PPNG*

I. Introduction

Gonorrhea remains one of the most common sexually transmitted infection (STI) in developing countries. Two important factors, which are responsible for the high rate of prevalence of gonorrhea, are increasing antimicrobial resistance of N. gonorrhoeae and presence of a large reservoir of asymptomatic carriers that unknowingly transmit the disease to their sexual contacts.

Strategies for the control of gonorrhoea have relied on the use of highly effective and often, single-dose therapy administered at the time of diagnosis [1]. Therapeutic options for gonococcal infection are nowadays limited due to the spread of gonococci resistance to a wide variety of antimicrobials. The dramatic increments in the rates of quinolone-resistant gonococci and the current shortage of spectinomycin leave the newer cephalosporins as the last safe choice among the drugs that are currently recommended as firstline treatment. Therefore, increasing reports of reduced in vitro activities of expanded-spectrum cephalosporins against Neisseria gonorrhoeae are of serious concern [2].

Since the failure to cure a case of gonorrhoea has public health implications due to the potential for continued transmission and rapid emergence of antimicrobial resistance and facilitating the spread of HIV, Sentinel surveillance and close monitoring of the in vitro antimicrobial susceptibility of clinical isolates of N. gonorrhoeae has a crucial role in preventing spread of resistant strains and monitoring effective antimicrobial therapy for gonorrhoea thereby providing guidance for appropriate case management [3,4].

II. Materials And Methods

Study population: A total of 50 male patients clinically presenting with acute urethritis at STI OP at Gandhi hospital and 125 consecutive female sexual workers (FSW) either asymptomatic or presenting with cervical/vaginal discharge to a sentinel STI clinic in Hyderabad from November 2008 to July 2009 were included in the study.

Study procedure: Informed consent was obtained from all the participants. Three swabs were collected from each patient for Direct Microscopy, culture and Gen -Probe APTIMA GCassay (a second generation Nucleic acid amplification test- NAAT, which utilizes transcription mediated amplification). All samples were processed by standard bacteriological techniques. Bedside inoculation was done on Modified Thayer Martin medium (consisting of GC agar base supplemented with 2% Horse hemoglobin, Isovital X and antibiotics such as Vancomycin, Colistin, Nalidixic acid, Trimethoprim) and incubated at 35-36.5˚c in candle extension jar for 24-48hrs. Isolates were identified by standard biochemical tests and confirmed by Phadebact Monoclonal Co agglutination and APTIMA GC assay which were performed according to the manufacturer's instructions.

Colonies were subculture from primary selective medium to a non Selective medium, such as GC-chocolate agar with 1% supplement, to obtain pure culture for antimicrobial susceptibility testing. Isolates were preserved in trypticase soy broth with 20% glycerol at -70◦c.

Antimicrobial Susceptibility Testing: It was performed on GC susceptibility test medium, a standard quality controlled GC agar base medium with 1% defined supplement (Isovital X). The following commercially available disks (Himedia) were tested by Kirby-Bauer disc diffusion method; Penicillin (10 unit), Tetracycline (30-µg), Spectinomycin (100-µg), Cefixime (5-µg), Ciprofloxacin (5-µg), Azithromycin (15-µg). MICs of Spectinomycin, Cefixime, Ciprofloxacin, and Azithromycin were determined by the Etest method (AB Biodisk, Sweden). Multiresistant isolates were defined as: quinolone-resistant N. gonorrhoeae (QRNG) + penicillinase-producing N. gonorrhoeae (PPNG); QRNG+tetracycline-resistant N. gonorrhoeae (TRNG); QRNG + PPNG + TRNG and resistance to any four antibiotics.

The interpretative criteria for all antibiotics except azithromycin were as recommended by the Clinical and Laboratory Standards Institute (CLSI) [5]. The Criteria for interpretation of azithromycin was as recommended by the Neisseria Reference Laboratory (NRL) at CDC [6]. Isolates were tested for β- lactamase production by Chromogenic Nitrocefin disk method (BBL Cefinase; Becton Dickinson). ATCC 49226 was used as a positive control strain as recommended by CLSI.

Ethical approval: Study was approved by ethical committee of Gandhi Medical College and Hospital, Hyderabad.

III. Results

During the period of eight months, 125 FSW and 50 males attending STI OP were enrolled among whom 13(10.4%) and 7(14%) isolates of Neisseria gonorrhea were identified by culture respectively. By NAAT, 18.4% FSW and 40% males had infection. A total of 20 isolates were put up for antimicrobial susceptibility testing. The susceptibility pattern is as shown in Table 1.

All the isolates were sensitive to spectinomycin with MIC< 4 µg/ml, 85% were sensitive to cefixime with MIC 0.023µg/ml. 30% were β lactamase positive (PPNG). Among the β lactamase negative isolates, all had reduced susceptibility to penicillin. Among the 6 PPNG strains, two were resistant to ciprofloxacin and azithromycin, one was resistant to tetracycline and cefixime, one was resistant to ciprofloxacin and tetracycline, one was resistant only to cefixime and one was resistant to all the three.

Of the three strains which were less susceptible and resistant to cefixime, one was sensitive to all other drugs, one was resistant to pencillin(PPNG) and ciprofloxacin and one was resistant to pencillin(PPNG), tetracycline and ciprofloxacin. 10% of strains were resistant to 4 antibiotics, 20% were resistant to 3 antibiotics, 35% were resistant to 2 antibiotics and 15% were resistant to only one antibiotic. 20% strains were sensitive to all antibiotics except pencillin. Of the multiresistant strains 20%, 10% and 10% were QRNG+PPNG, QRNG+TRNG and QRNG+PPNG+TRNG.

TABLE -1; Antibiotic susceptibility profile; no and % (n=20)

Penicillin	S ; ≥47 LS ; 27-46 R ; ≤ 26	0 13(65%) 7(35%)
Tetracycline	S ; ≥38 LS ; 31-37 R ; ≤ 30	4(20%) 9(45%) 7(35%)
Ciprofloxacin	S ; ≤ 0.06 LS ; 0.125-0.5 R ; ≥1	7(35%) 9(45%) 4(20%)
Azithromycin	S ; 0.125-0.5 LS ; 0.5-1 R ; ≥1	7(35%) 4(20%) 9(45%)
Cefixime	S ; ≤ 0.25 LS ; 0.25-1 R ; ≥1	17(85%) 2(10%) 1(5%)
Spectinomycin	S ; ≤ 32 LS ; 64 R ; ≥128	20(100%) 0 0

(Percentages are according to disk diffusion (mm) for Penicillin and Tetracycline and according to MIC (µg/ml) values for Ciprofloxacin, Azithromycin, Cefixime and Spectinomycin)

IV. Discussion

Resistance of *N. gonorrhoeae* to traditional antimicrobial agents, e.g., penicillin and tetracycline, in the 1980s led to discontinuation of their use in treating gonococcal infections [7]. Our observation also reinforces the emergence of penicillin, tetracycline and quinolone resistance in *N. gonorrhoeae* isolates.

There were more of less susceptible strains than resistant strains for penicillin, tetracycline and ciprofloxacin in the present study similar to a study conducted by Manju bala et al in Delhi [8]. This may reflect the loss of selective pressure from the disuse of these antibiotics as treatment for gonorrhoea. In the present study, PPNG were 30% which compared well with other studies as shown in Table 2 except for a study from Indonesia which reported 79%. In WHO Western Pacific Region (WPR), the prevalence of PPNG varied from 1% to 90% [8]. PPNG isolates from Asia are proline auxotrophs and carry a 4.4 MDa plasmid [9]. There is an additional conjugative 24.5 MDa large plasmid associated with it. The presence of the associated conjugative plasmid in Asian strain is responsible for rapid spread of resistance to other gonococci. Tetracycline resistance in gonococci may be mediated by chromosomal or plasmid determinants. The location of the tet M gene on the transferable plasmid has perhaps served to enhance the transmission efficiency of tetracycline resistance in *N. gonorrhoeae* strains, causing the rapid spread of TRNG.

The percentage resistance of *N. gonorrhoeae* isolates to a panel of antibiotics in the present study is compared with various studies in Table 2. Presently, the recommended first-line treatments for gonorrhea in most countries include antibiotics such as cefixime, ceftriaxone, spectinomycin, and in some cases azithromycin or ciprofloxacin. However, resistance to fluoroquinolones and azithromycin is rapidly increasing [8, 10]. The use of the quinolone group of antibiotics for the treatment of gonorrhoea has been discontinued in India for quite some time because of reported high levels of resistance. However, there were considerable differences in rates of quinolone resistance in different studies in India. Incidence of QRNG (MIC >1 µg/ml) was much higher in other studies unlike in the present study (20%) and in a study by Shilpee et al (10%) [11] as shown in Table 2. QRNG is also widely distributed in other WHO SEAR and WPR countries [12, 13, 14, 15, and 16]. Recently and most disquieting, the emergence of N. gonorrhoeae isolates with reduced susceptibility or resistance in vitro to broad-spectrum cephalosporins such as cefixime [7, 17, 18, 19, 20, 21, 22] and ceftriaxone [8] have been reported. In occasional cases, treatment failures when using cefixime have been reported [22]. In the present study also resistant and less susceptible strains to cefixime were noted which is a cause of concern.

It is fortunate that spectinomycin resistance has not been reported often as it is an alternative drug of choice for cases having hypersensitivity to cephalosporins. Spectinomycin is not easily available in India and this may explain the retention of efficacy of this antibiotic. However, some a small number of spectinomycin-resistant strains were found in China, Vietnam [12] and also from India [23]. A study from Indonesia [24] reported 18.1% resistant strains. Therefore, effective surveillance of antimicrobial resistance is required to monitor trends in established types of resistance and promptly identify new types of resistance.

TABLE 2 – Resistance of antibiotics in various studies

		ANTIBIOTICS WITH THEIR RESISTANCE IN %					
Study	Region	penicillin	tetracycline	ciprofloxacin	cefixime	spectinomycin	PPNG
Manju bala 2002-2006	Delhi	54-18	6.7-18.2	80-83	1.7-5.5 (LS)	0	21.2
Sunil sethi 2006	Delhi	46.6	51	77.7	0	Nt	22
DoneganEA 2006	Indonesia	81	100	40	0	0	79
Yang.Y 2006	Shanghai	93	56	98	0	0	37.8
Dejongh H 2007	South Africa	32	36	7	0	Nt	16
Khakhi P 2007	Delhi	33.3	23	98	0	0	20
Shilpee 2007	Delhi	20	10	10	nt	0	20
Present study	Hyderabad	35	35	20	5 (10 -LS)	0	30

LS; Less susceptible, nt; not tested

V. Conclusion

Our study highlights the need for assessment of antimicrobial susceptibility pattern which is of great importance in monitoring the emergence and spread of resistance and planning of appropriate treatment regimens and control programmes. This also helps in preventing the resistant strains becoming endemic especially when commercial sex workers (high frequency transmitters) have been infected.

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