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Changing trends in antimicrobial susceptibility of *Neisseria gonorrhoeae* in a regional centre for sexually transmitted infections in central India from 2012-2021

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Abstract

Background: Emergence of extensively drug resistant (XDR) *N. gonorrhoeae* in Europe and Australia from patients who had travelled to south - east Asia strengthens the need for strict antimicrobial resistance (AMR) surveillance. Based on the on the ongoing surveillance data, the present study compares AMR profile of *N. gonorrhoeae* between two time-frames, A and B, viz 2012-16 and 2017-21, respectively, and also ascertains the susceptibility of *N. gonorrhoeae* to the currently recommended first line antimicrobials.

Material and Methods: *N. gonorrhoeae* isolated from patients attending the regional center for diagnosis and treatment of sexually transmitted infections (STIs) in Government Medical College & Hospital, Nagpur, India. Between 2012 and 2021, were conventionally identified. Antimicrobial susceptibility testing was performed using Australian Gonococcal Surveillance Programme (AGSP)¹⁰ method based on Calibrated Dichotomous Sensitivity (CDS) test using azithromycin, ceftriaxone, cefixime, cefpodoxime, ciprofloxacin, gentamicin, nalidixic acid, penicillin, spectinomycin and tetracycline.

Statistical analysis: Using Chi square test and Fisher Exact test for small sized samples, p value compared the trends between the two time frames.

Results: The ten year surveillance data was compared between two time-frames 2012-16 and 2017-21. In both time-frames all isolates were sensitive to ceftriaxone, spectinomycin and gentamycin and resistant to ciprofloxacin and nalidixic acid. Changing trends were observed with emergence of 10% isolates with decreased susceptibility to cefixime. Increased in resistance to azithromycin from 7.1% to 17.5% (p=0.664), to tetracycline from 7.1% to 20% (p=0.418) & to penicillin from 28.6% to 40% (p=0.533) was observed. Susceptibility to cefpodoxime was incongruous.

Conclusion: The study highlights emergence of *N. gonorrhoeae* with decreased susceptibility to cefixime and increasing resistance to azithromycin in our region which is an indicator of impending threat to recommended first line treatment of gonorrhoea.

Keywords: Antimicrobial susceptibility, disease surveillance, Gonorrhoea, India, *Neisseria gonorrhoeae*

Introduction

Gonorrhoea is the second commonest bacterial STI ^[1] and a major public health concern. WHO estimated that in 2020 there were 82.4 million new cases infected among adolescents and adults aged 15- 49 years world wide with a global incident rate of 19 per 1000 women and 23 per 1000 men ^[2].

Gonorrhoea has progressively developed resistance to the antimicrobials prescribed to treat it ^[3, 4]. Multi drug resistance (MDR) in *N. gonorrhoeae* is common. With the spread of fluoroquinolone resistance in gonococci, the cephalosporins have been recommended for treatment of gonorrhoea ^[5].

Extensively drug resistant (XDR) *N. gonorrhoeae* have been documented from Canada between 2012-16 accounting for 0.1% of 18768 isolates ^[6]. Three cases of XDR *N. gonorrhoeae* have been reported from United Kingdom (one case) and from Australia (two cases) ^[7, 8] in February and March 2018 respectively. All the three cases had history of travel to south-east Asia necessitating strict surveillance.

In the present study we compared AMR profile of *N. gonorrhoeae* between two time-frames viz 2012-16 and 2017-21, and have documented changing trends in the on-going surveillance of antimicrobial resistance in *N. gonorrhoeae* from patients attending the regional center for diagnosis and treatment of STIs in central India.

Material and Methods

Surveillance for gonorrhoea is an on-going activity at the regional center for diagnosis and treatment for STIs at Government Medical College & Hospital, Nagpur, central India. The center also undertakes training, research and referral responsibilities.

Urethral discharge from male patients and endocervical swabs and urethral discharge from female patients attending the regional center between 2012 and 2021 were collected. The samples were processed by conventional methods^[9] for isolation of *N. gonorrhoeae*. Identification was based on colony morphology, Gram stain and oxidase, superoxol and carbohydrate utilization tests. Antimicrobial sensitivity was performed using Australian Gonococcal Surveillance Programme (AGSP)^[10] method based on Calibrated Dichotomous Sensitivity (CDS) test. Low potency antimicrobial discs used were procured from Oxoid. They

were azithromycin (15µg), ceftriaxone (0.5 µg), cefixime (5 µg), cefpodoxime (10 µg), ciprofloxacin (1 µg), gentamycin (30 µg), nalidixic acid (30 µg), spectinomycin (100 µg), penicillin (0.5 IU) and tetracycline (10 µg). Nalidixic acid was used only to identify isolates less sensitive to ciprofloxacin and results of susceptibility to this antimicrobial were not used for treatment.

WHO strains K and O were used for quality control.

Beta-lactamase production was determined by the chromogenic cephalosporin method using nitrocefin slide (Becton Dickinson and Company, Sparks, MD, USA)^[9].

Confirmation of identification and antimicrobial susceptibility of each isolate was done by the Apex STD Laboratory, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi.

p value was determined using Chi square test and Fisher Exact test for small sized samples to compare the trends between the two time-frames.

Results

The ten year *N. gonorrhoeae* AMR surveillance data was compared between two time-frames: A (2012-16) with 14 isolates and B (2017- 21) with 40 isolates (Table 1).

Table 1: Antimicrobial susceptibility of *N. gonorrhoeae* from 2012 to 2021

	Time - frame A: 2012-16 (n=14)			Time - frame B: 2017-21 (n=40)		
	S ²	DS ³	R ⁴	S	DS	R
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Ceftriaxone	14 (100)	0	0	40 (100)	0	0
Cefixime	14 (100)	0	0	36 (90)	4 (10)	0
Spectinomycin	14 (100)	0	0	40 (100)	0	0
Gentamycin ¹	-	-	-	36 (100)	0	0
Azithromycin	13 (92.2)	0	1 (7.1)	33 (82.5)	0	7 (17.5)
Tetracycline	13 (92.2)	0	1 (7.1)	32 (80)	0	8 (20)
Cefpodoxime	12 (85.7)	2 (14.7)	0	39 (97.5)	1 (2.5)	0
Penicillin	4 (28.6)	6 (42.8)	4 (28.6)	17 (42.5)	7 (17.5)	16 (40)
Ciprofloxacin	0	0	14 (100)	0	0	40 (100)
Nalidixic acid	0	0	14 (100)	0	0	40 (100)

¹ Gentamycin was added to the panel of antimicrobials in January 2018. It was tested on 36 strains isolated thereafter. ² S: sensitive, ³ DS: Decreased susceptibility, ⁴ R: Resistant.

All isolates (100%) were sensitive to ceftriaxone, spectinomycin and gentamycin and resistant to ciprofloxacin and nalidixic acid consistently in both time-frames A and B. Changing trends were observed for cefixime, azithromycin,

tetracycline and penicillin. Four (10%) isolates with decreased susceptibility to cefixime (p=0.563) emerged in time-frame B, one in each of the years 2017, 2018, 2020, & 2021 (Table 1, Fig 1).

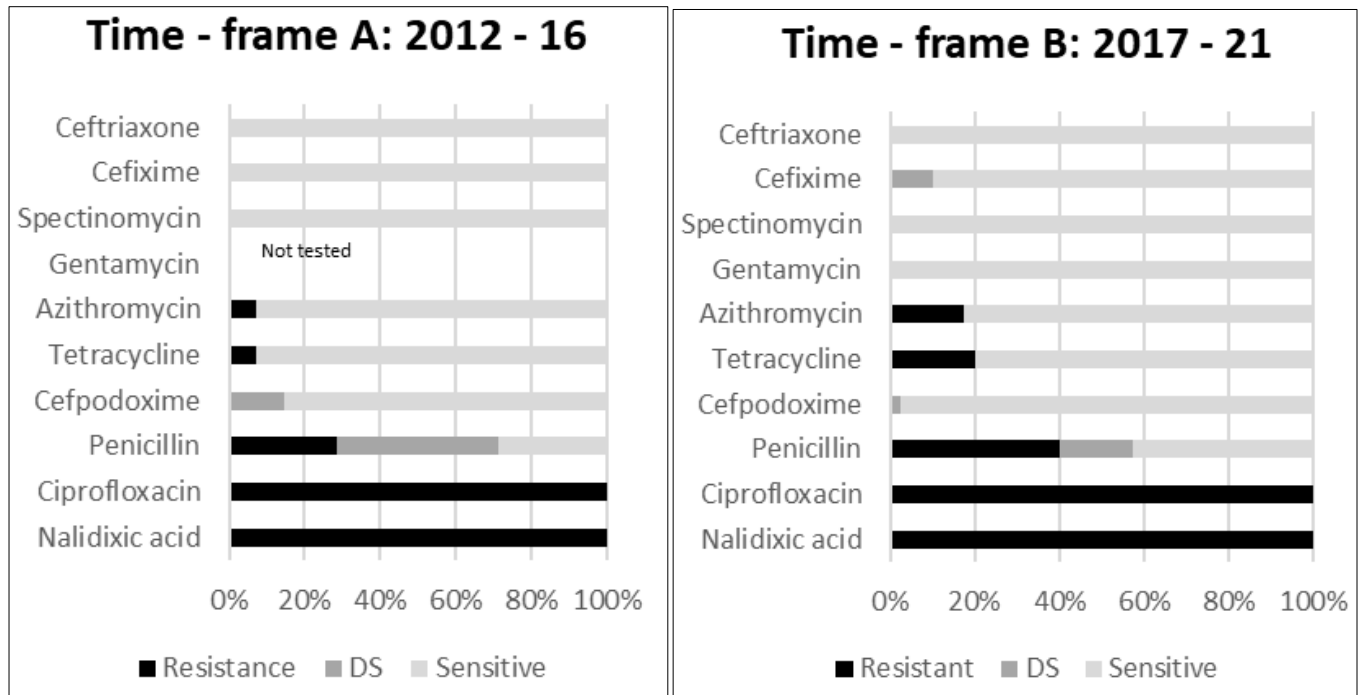


Fig 1: Changing trends in Antimicrobial Susceptibility of *N. gonorrhoeae*

Resistance to azithromycin increased from 7.1% to 17.5% ($p=0.664$), to tetracycline from 7.1% to 20% ($p=0.418$) and to penicillin from 28.6% to 40% ($p=0.533$)

(Table 1, Fig 1). Amongst all the penicillin resistant isolates, 15 (27.8%) were penicillinase producers.

Response of *N. gonorrhoeae* to cefpodoxime testing was incongruous. Susceptible isolates increased from 85.7% to 97.5% and isolates with decreased susceptibility decreased from 14.7% to 2.5% in time-frames A and B respectively.

Discussion

In the global scenario, sulphonamides were the first chemotherapeutic agents used for gonorrhoeae in 1930s. In 1940s penicillin became the drug of choice due to low minimum inhibitory concentration (MIC) of $\leq 0.5 \mu\text{g/ml}$ for *N. gonorrhoeae*. Other drugs like aminoglycosides, tetracyclines and macrolides were used in penicillin sensitive patients or those infected with penicillin resistant strains. By 1980s ciprofloxacin amongst the fluoroquinolones became a better option and by early 2000 ciprofloxacin resistant strains were predominant in Europe [3, 4]. Fortunately extended spectrum cephalosporines were effective in low concentrations and WHO recommended dual therapy with ceftriaxone and azithromycin for gonorrhoea [5].

N. gonorrhoeae with MDR are prevalent in many countries. XDR *N. gonorrhoeae* have also emerged with low levels of resistance to ceftriaxone (MIC = $0.5 \mu\text{g/ml}$) and high level of resistance to azithromycin ($>256 \mu\text{g/ml}$) [6, 7, 8]. Ceftriaxone continues to be the drug of choice for gonorrhoea in our region. In the present study 10% isolates with decreased susceptibility to cefixime emerged in our region in 2017. A number of member states across WHO regions have reported *N. gonorrhoeae* isolates with decreased susceptibility or resistance to ceftriaxone and cefixime [11, 12]. This is worrisome as resistance to third generation cephalosporins could substantially limit the treatment options for gonorrhoea.

Increasing resistance to azithromycin in our region in the recent years is also disturbing. Till 2019 a single sporadic azithromycin resistant *N. gonorrhoeae* was isolated. Between 2020 - 2021, there was a spurt in azithromycin resistant *N. gonorrhoeae* with the isolation of a total of seven isolates in two years. Coincidentally all these patients gave history of taking azithromycin during the covid -19 pandemic although only 3 out of 7 patients were laboratory confirmed cases of covid-19 infection. Whether development of resistance to azithromycin was a natural course in the evolution of the bacterium or it was due to rampant use of azithromycin during covid-19 pandemic cannot be ascertained. Dual therapy with Azithromycin and an extended spectrum cephalosporin had revolutionized gonococcal therapy as azithromycin shortened treatment time to half and improved patient compliance due to high tissue levels and long half life [13].

We found a gradual increase in resistance to penicillin and tetracycline over the years. Plasmid mediated penicillin and tetracycline have been documented [14]. These drugs are widely available to public and their inappropriate use needs to be checked.

In India a continuous increase in resistance to penicillin, tetracycline and ciprofloxacin has been reported in spite of their disuse for treatment. The increase in number of strains with decreased susceptibility to extended spectrum cephalosporins and azithromycin resistance, currently recommended management of gonorrhoeae has also been documented [15, 16].

In the present study although the emergence of *N. gonorrhoeae* with decreased susceptibility to cefixime and increase in resistance to azithromycin, penicillin and tetracycline between 2017-21 were all statistically insignificant, yet they are early indicators of a serious concern. Although sensitivity to ceftriaxone is maintained, emergence of *N. gonorrhoeae* with decreased susceptibility to cefixime and increased resistance to azithromycin is an indicator to impending threat in the treatment of gonorrhoea. An active, continuous and comprehensive surveillance and

judicious use of extended spectrum cephalosporins is warranted.

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