Full Length Research Paper

Antibiotic resistance profile of *Neisseria gonorrhoeae* strains isolated in Dakar, Senegal

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Abstract

Background: *Neisseria gonorrhoeae*, causative agent of gonorrhea, has emerged in recent years by acquiring resistance to antimicrobials recommended for treatment. The aim of this study was to determine antibiotic resistance profile of *N. gonorrhoeae* strains isolated in Dakar. Methods: This retrospective study involved strains of *N. gonorrhoeae* isolated from pathological samples of patients received at medical biology laboratory of Pasteur Institute in Dakar. Samples were inoculated on Chocolat PVX and Chocolate+VCN agar plates incubated at 37°C under 5% CO_2 for 24-48 hours. Species identification was performed using Api-NH® identification gallery. Minimum inhibitory concentration was determined by E-test method with 0.5 McFarland bacterial suspension prepared from few colonies of pure culture with NaCl 0.85% Medium according to EUCAST 2022 criteria. Results: 41 strains of *N. gonorrhoeae* were isolated from patients (40 men and one woman). Median age was 38 years and age group most represented was [20-29 years] with 36.6%. Isolates were sensitive to ceftriaxone, cefixime and gentamycin (100%) and to azyhtromycin (95.1%). However, they were resistant to tetracycline (92.7%) and ciprofloxacin (95.1%). Conclusion: This study revealed third-generation cephalosporins (ceftriaxone and cefixime), gentamicin and azyhtromycin are still effective, and are drugs of choice in current recommendations for treatment of gonorrhea.

Key words: *Neisseria gonorrhoeae*, Sexually transmitted infection, Antimicrobial resistance, MIC, Senegal.

INTRODUCTION

Gonorrhea is a sexually transmitted infection (STI) caused by *N. gonorrhoeae*, which remains a major public health problem worldwide due to its ability to acquire resistance to antimicrobial agents recommended for treatment (Unemo et *al.,* 2019; WHO, 2021). Untreated or inadequately treated genital infection with *N. gonorrhoeae* can lead to severe complications such as epididymitis in men, pelvic inflammatory syndrome in women, which can result in infertility and intrauterine pregnancies. In newborns, this pathogen can cause gonococcal conjunctivitis, which can lead to blindness (WHO, 2012; WHO, 2016). In 2016, World Health Organisation (WHO) estimated that 87 million new infections occurred in people aged between 15 and 49, with highest incidence in sub-Saharan Africa (WHO, 2018; Rowley et *al.*, 2019). Every year, there are an estimated 50 and 100 new infections per 1,000 women and men respectively in African region (Lowe et *al.*, 2019). In absence of a vaccine against gonorrhea, effective, affordable and accessible antimicrobial agents are essential to reduce substantial morbidity and spread of superbug in the era of incurable gonorrhea (Alirol et *al.*,

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2017; Wi et *al.*, 2017).*N. gonorrhoeae* is on WHO global priority list of antibiotic-resistant bacteria, having developed resistance to all antimicrobials recommended for treatment since introduction of sulfa drugs in the 1930s (Tacconelli et *al.*, 2017; Unemo and Shafer, 2014). In recent decades, *N. gonorrhoeae* has dramatically developed plasmid-and/or chromosome-mediated antimicrobial resistance (AMR), leading to abandonment of use of antimicrobial agents as standard first-line treatment (Unemo and Shafer, 2014). The recent emergence of gonococcal strains resistant to ceftriaxone and azithromycin in Australia and the UK has raised serious concerns about untreatability of gonorrhea (Jennison et *al.*, 2019). As a result, dual therapy with cephalosporins and azithromycin or doxycycline is currently recommended (Unemo et *al.*, 2019).

However, little is known about antibiotic resistance profile and mechanisms of *N. gonorrhoeae* strains circulating in sub-Saharan Africa, the most affected region by gonorrhea (Unemo and Shafer, 2014 ; Rowley et *al.*, 2016 ; Cehovin et *al.*, 2018). Insufficient data is due to the lack of access to laboratory diagnostic services and use of syndromic management for the treatment of STIs in this region (Ndowa et *al.*, 2013). Syndromic management has a number of limitations, including absence of antibiotic susceptibility testing, inability to identify asymptomatic infections, limited possibilities for large-scale surveillance, and failure to record data on people with treatment failure (White et al., 2008; Garrett et *al.*, 2017). Data on antimicrobial resistance patterns in *N. gonorrhoeae* are scarce in Senegal. The aim of this study was to characterize antimicrobial resistance profile by determining MICs of *N. gonorrhoea*e strains isolated from gonococcal patients in Dakar.

METHODOLOGY

Bacterial strains and study population

This retrospective study involved 41 strains of *N. gonorrhoeae* isolated from genital specimens and first-draft urine of patients with gonococcal disease received at medical biology laboratory of Institute Pasteur in Dakar (Senegal) for bacteriological analysis.

Culture and antibiotic susceptibility testing

Specimens (swabs or first-draft urine) were inoculated onto Chocolate PVX and Chocolat + VCN agar (Becton Dickinson, Sparks, MD, USA), incubated at 37°C in a humid atmosphere with 10% CO_2 for 24-48 hours. Species identification was performed using the Api NH identification gallery (Biomérieux, Marcy-l'Étoile, France). A 0.5 McFarland bacterial suspension of each isolate was prepared using 0.85% Medium NaCl physiological water

API® (Biomérieux, Marcy-l'Étoile, France). Inoculum from each isolate was inoculated onto Chocolate agar incubated at 35°C in an atmosphere of 10% CO_2 for 24 hours. MICs of 6 antibiotics (ceftriaxone, cefixime, ciprofloxacin, gentamycin, azithromycin, tetracycline) were determined by E-test method (Biomérieux, Marcy-l'Étoile, France) according to manufacturer's recommendations. Strains were categorized sensitive, intermediate or resistant based on EUCAST (European Committee on Antimicrobial Susceptibility Testing) 2022 criteria.

The control strain of *N. gonorrhoeae* ATCC 4926 was used as a reference.

Statistical analysis

Verified raw data were exported from Excel to Whonet 5.6 version 2022 software for analysis of MICs data and determination of MIC_{50} and MIC_{90} of antibiotics tested.

RESULTS

Bacterial strains and study population

A total of 41 strains of *N. gonorrhoeae* were analyzed during study. Strains were isolated from 40 men and 1 woman, with a median age of 35 years (extremes 19 and 62 years). Age groups [20-29 years] and [30-39 years] were most represented, with 36.6% and 26.8% respectively (Table I). Majority of strains were isolated from urethral swabs (n=39), with two strains isolated from an endocervical swab and one from a first-draft urine.

Antibiotic susceptibility profile

All isolates were sensitive to ceftriaxone (MIC ≤ 0.023 mg/L), cefixime (MIC ≤ 0.016 mg/L) and gentamycin (MIC ≤ 8 mg/L) with very low MIC_{50} (0.016 mg/L) and MIC_{90} (0.016 and 0.023 mg/L) for third-generation cephalosporins (Table II).Similarly, majority of isolates (95.1%, n= 39) were sensitive to azithromycin (MIC ≤ 1 mg/L) with a MIC_{50} of 0.064 mg/L and a MIC_{90} of 1 mg/L.

Isolates were highly resistant to ciprofloxacin (92.7%, n=39) and tetracycline (90.25%; n=38) with respective MIC ranges of 0.002 to 6 mg/L and 0.38 to 64 mg/L and high values of MIC_{50} (1 and 12 mg/L) and MIC_{90} (3 and 48 mg/L) (Table II).

Two strains were resistant to azithromycin (MICs = 1.5 mg/L), ciprofloxacin (MICs = 1.5 mg/L) and tetracycline (MICs = 1.5 and 16 mg/L).Thirty-five strains were resistant to ciprofloxacin (MICs ranging from 0.25 to 4 mg/L) and tetracycline (MICs ranging from 0.75 to 64 mg/L). Five strains were resistant to only one antibiotic, including three to ciprofloxacin and two to tetracycline. Only one isolate was sensitive to all antibiotics tested.

Table I: Main socio-demographic characteristics of patients.

	Number	%
Women	1	2.4
Men	40	98.6
Medianage	38 years	
Extremes	[19 – 62 years]	
Age range (years)		
[< 20]	1	2.4
[20-29]	15	36.6
[30-39]	11	26.8
[40-49]	9	22
[> 50]	5	12.2
Total	41	100

Table II: MICs, MIC_{50} and MIC_{90} values for *N. gonorrhoeae* isolates (n=41).

Antibiotics	Critic values (mg/L)		Sensitive strains	mg/L		
	S ≤	R >	N (%)	Range	MIC_{50}	MIC_{90}
Ceftriaxone	0.125	0.125	41 (100)	0.002 – 0.023	0.016	0.023
Cefixime	0.125	0.125	41 (100)	0.016	0.016	0.016
Gentamycin	16	-	41 (100)	2 - 8	8	8
Azithromycin	1	-	39 (95.1)	0.016 – 1.5	0.064	1
Ciprofloxacin	0.03	0.06	2 (4.9)	0.002 - 6	1	3
Tetracycline	0.5	0.5	3 (7.3)	0.38 - 64	12	48

S: sensitive, R : resistant

DISCUSSION

The aim of this study was to determine antibiotic resistance profile of *N. gonorrhoeae* strains isolated in Dakar by determining MIC in order to study efficacy of antibiotics recommended for treatment of gonorrhea. Antibiotic resistance in *N. gonorrhoeae* strains has become a global problem. It is therefore important to moni-

tor emergence and spread of resistant strains. In addition, gonococcal infections are on the increase and can be attributed to various factors in different parts of the world (WHO, 2012).

The rate of *N. gonorrhoeae* isolation was higher in young adult males than in females of the same age, this result was reported in a study conducted in South Africa (Rambaran et *al.*, 2019).Median age was 38 years (extremes 19 and 62 years), and age group [30-39 years] was themost represented (36.6%). Although our study population is not representative of general male population, it nevertheless constitutes a sentinel population for studying emergence of *N. gonorrhoeae* resistance.

All isolates were sensitive to ceftriaxone, cefixime and gentamicin. MIC_{50} and MIC_{90} of ceftriaxone (0.016 mg/L and 0.023 mg/L respectively) and cefixime (0.016 mg/L respectively) were very low. This good sensitivity of isolates to ceftriaxone and cefixime has been reported for strains isolated in Ivory Coast (Yéo et *al.*, 2018).

Ceftriaxone and cefixime are effective and remain drugs of choice in first-line treatment recommendations. Gentamicin could also be an alternative for treatment of gonococcal disease. Only two strains (4.9%) were resistant to azithromycin with an MIC>1 mg/L. A resistance rate of 18.6% to this antibiotic has been reported in China(Yin et *al.*, 2018).With spread of resistance, dual therapy (ceftriaxone and azithromycin) has been recommended as first-line treatment for uncomplicated gonorrhea in many countries around the world (WHO, 2018). This drug is still active on majority of isolates tested, with a MIC_{90} equal to 1 mg/L (corresponding to the sensitivity threshold). Nevertheless, resistance to azithromycin could continue to increase in our country, especially as consumption of the drug is rising sharply. WHO has suggested that an antimicrobial should not be used when more than 5% of strains are resistant to it (WHO, 2012).The rate of resistance to ciprofloxacin was 95.1% with MIC_{50} and MIC_{90} values of 1 and 3 mg/L respectively. A resistance rate of 83.9% has been reported in Ivory Coast (Yéo et *al.*, 2018). In United States, a resistance rate of 13.5% has been reported (Kirkcaldy et *al.*, 2013). The use of ciprofloxacin, which was previously recommended for treatment of gonorrhea, is no longer appropriate in view of the reported rates of resistance. Resistance to tetracycline was very high (92.7%) with high MIC_{50} and MIC_{90} values of 12 and 48 mg/L respectively. A resistance rate of 92.8% has been reported in North Africa (Hançali et *al.*, 2013). Resistance rates of over 95% have been reported in Bhutan and Indonesia (Bala et *al.*, 2013)**,** while in United States, South America and the Caribbean, around 22% of isolates were resistant to this antibiotic (Kirkcaldy et *al.*, 2013; Dillon et *al.*, 2013). Given this high rate of resistance to tetracycline, this antibiotic is not appropriate for the treatment of gonorrhea in Senegal and many other countries. In view of these results, it is necessary to set up surveillance of antibiotic resistance in *N. gonorrhoeae* isolates to third-generation cephalosporins (ceftriaxone, cefixime), gentamicin and particularly azithromycin, in order to monitor emergence of strains resistant to these antibiotics.

However, the study has some limitations, notably the small sample size which limits the generalizability or representativeness of the findings to a larger population.

CONCLUSION

This study showed that ceftriaxone, cefixime and gentamicin were effective against isolates of *N. gonorrhoeae*. Azithromycin remains effective even though two isolates were resistant to this antibiotic. High rates of resistance were observed with tetracycline and ciprofloxacin. These results demonstrate efficacy of antibiotics proposed in gonorrhea treatment algorithm in our country, but raise the issue of monitoring emergence of antimicrobial resistance in order to adapt gonorrhea treatment recommendations to local data. However, the study reveals perspectives on resistance of *Neisseria gonorrhoeae* with broader sampling and molecular characterization of isolates.

Authors' contributions

All authors contributed to article and approved submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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