American Journal of Clinical Microbiology and Antimicrobials

Research Article
Published: 06 Feb, 2023

Identification and Antibiotic Susceptibility of *Neisseria cinerea* and *Neisseria meningitidis* in Patients with Suspect of Gonorrhea in Cuba, 2014-2021

Llanes R[1]*, Gutiérrez O[1], Feliciano O[1], Sánchez J[2], De Baetselier I[3], Cuylaerts V[3], Vuylsteke B[4] and Crucitti T[5]

[1]*Department of Bacteriology-Mycology, National Reference Laboratory of Neisseria & Helicobacter, Institute of Tropical Medicine Pedro Kourí (IPK), Cuba*

[2]*Program for Control of Sexually Transmitted Infections, Ministry of Public Health, Cuba*

[3]*Department of Clinical Sciences, Clinical Reference Laboratory, Institute of Tropical Medicine Antwerp, Belgium*

[4]*Department of Public Health, Sexual and Reproductive Health, Institute of Tropical Medicine Antwerp, Belgium*

[5]*Experimental Bacteriology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar*

Abstract

Background: There are a growing incidence of urogenital and anorectal infections caused by *Neisseria meningitidis* and commensal *Neisseria* species. Prevalence of Antimicrobial Resistance (AMR) of commensal *Neisseria* is steadily increasing.

Aims: To confirm the identification of presumptive *N. gonorrhoeae* isolates and other *Neisseria* species from patients with genital or extragenital symptoms in Cuba, during the period 2014-2021. We also assessed the antibiotic susceptibility of commensal *Neisseria* spp. and *N. meningitidis* isolates.

Methods: during the study period, 520 *Neisseria* spp isolates from patients with suspicion of gonorrhea were received at the *Neisseria-Helicobacter* reference laboratory for definitive identification and the study of the AMR. The identification of gonococci included Gram staining, oxidase and catalase tests, glucose utilization on CTA medium and a PCR amplifying a fragment of the OPA gene. Further identification comprised colistin susceptibility, growth on Mueller-Hinton agar at 22ºC, automated Vitek 2 system and an in-house PCR that amplifies a 127 bp fragment of the *N. meningitidis* sodC gene. The antibiotic susceptibilities to penicillin, ceftriaxone, ciprofloxacin, azithromycin, rifampicin, tetracycline and gentamicin were determined by the E-test method (for meningococci) and by agar dilution for commensal *Neisseria*.

Results: the 48.5% of the *Neisseria* spp isolates (252/520) were viable; of them 241 were *N. gonorrhoeae*. Of the 11 non-gonorrhoeae isolates, six and three isolates were identified as *N. cinerea* and *N. meningitidis*, respectively and two *Neisseria* spp could not be identified at species level. The AMR profiles in six isolates of *N. cinerea* revealed that all isolates except one were at least resistant to one antibiotic, with the exception of gentamicin, and four isolates exhibited combined resistance to two or more antibiotics. This behavior is quite different to the AMR observed in *N. meningitidis* isolates in which, only one isolate showed intermediate susceptibility to penicillin.

Conclusion: It represents one of the largest studies that report of *N. cinerea* from patients with genital symptomatology, combined with multiple resistance to several antibiotics, and the demonstration of high resistance to azithromycin/ceftriaxone. This behavior is troublesome as these organisms constitutes a reservoir of AMR and may transfer mutations encoding resistance to *Neisseria gonorrhoeae* and *N. meningitidis.* Further investigations are required to know about the role of MALDI-TOF or genomic tools in identification of non-pathogenic *Neisseria* at the reference laboratory and if enhanced antibiotic stewardship activities could reduce the MIC of these organisms.

Keywords. ***Neisseria Cinerea;*** **Prevalence of Antimicrobial Resistance (AMR); Antibiotic susceptibility in *N. meningitidis***

OPEN ACCESS

Correspondence:
Rafael Llanes, Department of Bacteriology-Mycology, National Reference Laboratory of Neisseria & Helicobacter, Institute of Tropical Medicine Pedro Kourí (IPK), Havana, Cuba, Tel: 53 59804433; E-mail: llanes@ipk.sld.cu

Received Date: *29 Dec 2022*
Accepted Date: *01 Feb 2023*
Published Date: *06 Feb 2023*

Citation:
Llanes R, Gutiérrez O, Feliciano O, Sánchez J, De Baetselier I, Cuylaerts V, et al. Identification and Antibiotic Susceptibility of Neisseria cinerea and Neisseria meningitidis in Patients with Suspect of Gonorrhea in Cuba, 2014-2021. Am J Clin Microbiol Antimicrob. 2023; 6(1): 1063.

Introduction

The genus Neisseria contains the two Gram-negative cocci, which are established human pathogens, *Neisseria meningitidis* (meningococcus) which is a major cause of meningitis and bacteremia, and *Neisseria gonorrhoeae* (gonococcus) that causes gonorrhea. The genus also contains many commensal species, most of which are harmless inhabitants of the upper respiratory and alimentary tracts [1].

Neisseria gonorrhoeae causes urogenital symptoms in humans, i.e., urethritis and dysuria in men and cervicitis in females, and affect the conjunctiva in neonates and adult patients. This organism also produces extra-genital infections in rectum and oropharynx, especially in Men and Transgender Individuals who seeks Sex with Men (MTSM) [1]. These extra-genital infections are mostly asymptomatic [1,2]. According to the World Health Organization (WHO), 87 million new gonorrhea cases occurred among 15- to 49-year-old people in 2016, globally [3]. Recently, there are a growing number of infections caused by *N. meningitidis* located in the urogenital and anorectal tract, with evidence of transmission between sexual partners [4]. These genital and extra-genital transmission pathways may have facilitated the occurrence of meningitis outbreaks among MTSM in North America and European countries [5].

Although rare, other non-pathogenic Neisseria species such as *Neisseria cinerea*, *Neisseria mucosa*, *Neisseria lactamica*, *Neisseria oralis*, *Neisseria sicca* and *Neisseria subflava* may cause genital symptoms [6-9]. Prevalence of Antimicrobial Resistance (AMR) of commensal *Neisseria* is steadily increasing, which is troublesome as these organisms constitutes a reservoir of AMR and may transfer mutations encoding resistance to *N. gonorrhoeae* and *N. meningitidis* [10-12]. However, the resistant phenotypes of non-pathogenic *Neisseria* have been described infrequently [10,12-13]. Due to the concerns of the increased antibiotic multi-resistance of *N. gonorrhoeae* isolates, WHO included this organism its list of "priority pathogens" posing the greatest threat to human health in terms of its Antimicrobial Resistance (AMR) [14].

Pathogenic and non-pathogenic *Neisseria* can be differentiating in species by phenotypic methods, such as carbohydrate utilization and enzymatic substrate tests [1,6]. However, misclassification is not uncommon using these methods [6,7]. To avoid misclassification of *Neisseria* species, nucleic acid amplification tests [1,2] and other genome tools as multi-locus sequence typing and DNA sequencing are useful techniques [15].

As such, the overall aim of the present study was to confirm the identification of presumptive *Neisseria gonorrhoeae* isolates from patients with genital or extragenital symptoms in Cuba, during the period 2014-2021. In addition, we also assessed the antibiotic susceptibility of commensal *Neisseria* spp. and *N. meningitidis* isolates, which would assist in prospective surveillance for novel resistance mechanisms that may be rapidly acquired by *N. gonorrhoeae*.

Material and Methods

Study design

A cross-sectional study was developed at the National Reference Laboratory of *Neisseria* and *Helicobacter* (NRL-NH), Tropical Medicine Institute Pedro Kourí (IPK) between the years 2014 to 2021.

Neisseria spp. isolates

During the period 2014-2021, five hundred and twenty isolates of *Neisseria* spp. from urethra, endocervix, vagina, vulva, conjunctiva and pharyngeal exudates from patients with suspicion of gonorrhea were received at the NRL-NH, from all 15 Cuban provinces.

Since 2002, Cuban National Guidelines for STI recommends syndrome management without the use of diagnostics of STIs, with the exception of syphilis and HIV, for which laboratory diagnosis is used [16]. For men with urethral symptoms, it is recommended that at primary health care level a Gram stain of the urethral exudate is examined to diagnose gonorrhea before providing antimicrobial treatment. Hospitals and provincial microbiology laboratories are competent to culture *N. gonorrhoeae* from different anatomical sites (including urogenital, anogenital and pharyngeal tract) using Thayer Martin or chocolate agar media. The microbiology laboratories identify presumptively *N. gonorrhoeae* based on the colony morphology, Gram staining of the culture, production of oxidase and the catalase test [16]. In addition, a few hospital laboratories located in La Habana province use the automatic system Vitek 2 compact (BioMérieux Marcy-l'Étoile, France) to identify *N. gonorrhoeae* and other 28 taxa belonging to the genera *Actinobacillus, Campylobacter, Capnocytophaga, Cardiobacterium, Eikenella, Gardnerella, Haemophilus, Kingella, Moraxella, Neisseria, Oligella*, and *Suttonella*, using the card NH [17]. All laboratories isolating *N. gonorrhoeae* or *Neisseria* spp. are instructed to send the isolates to the NRL-NH, for confirmation and antibiotic susceptibility testing [16].

Confirmation of *Neisseria* spp. at the NRL-NH

The NRL-NH confirmed the identification of the *Neisseria* spp. isolates by Gram staining, oxidase and superoxol tests. The identification of *N. gonorrhoeae* was done by glucose utilization on Cystine Tryptic Agar medium [1], immediately after reception at the reference laboratory. Further confirmatory identification was developed after freezing conservation at- 70°C. When Neisseria strains were identified other than gonococcus, an in-house real-time PCR (qPCR) that amplified an OPA gene fragment of *N. gonorrhoeae,* based on the publication by Hopkins et al. was used [18]. For the further identification of the strains lacking the OPA gene fragment, the following algorithm was followed: Colistin susceptibility, growth on Mueller-Hinton agar at 22ºC [8], and Vitek 2 system using NH card, which contains 30 biochemical tests [17]. Moreover, for the identification of *N. meningitidis*, an in-house qPCR that amplifies a 127 bp fragment of the sodC gene was used [19]. Serogroup determination in meningococci strains was performed by end-point PCR according to the methodology described by Taha et al. [20]. All DNA extractions were performed as previously published [19].

Antibiotic susceptibility in *N. meningitidis* and other commensal Neisseria isolates

The NRL-NH determined the antibiotic susceptibility of *N. meningitidis* using the E-test method (BioMérieux) on Mueller Hinton agar (Biolife, Italy) supplemented with 5% sheep blood. The Minimal Inhibitory Concentrations (MICs) of penicillin, ceftriaxone, rifampicin, ciprofloxacin and azithromycin were established. Breakpoints recommended by the Clinical Laboratory Standard International (CLSI) to penicillin (≥ 0.5 µg/mL), ciprofloxacin (≥ 0.12 µg/mL), rifampicin (≥ 2 µg/mL), ceftriaxone (≤ 0.12 µg/mL) and azithromycin (≤ 2 µg/mL) were applied [21]. *Streptococcus pneumoniae* ATCC 49619 and *Escherichia coli* ATCC 25922 were used as internal controls. MICs of other commensal *Neisseria* isolates to penicillin, ceftriaxone, azithromycin, ciprofloxacin, tetracycline, and gentamicin were determined by agar dilution method on GC

agar medium (Biolife), supplemented with 1% Vitox (Biolife) [21]. As established breakpoints for non-pathogenic *Neisseria* spp. are lacking, we used those for *N. gonorrhoeae* as recommended by CLSI [21]. However, CLSI did not define the breakpoint for gentamicin, thus, the cut-off values recommended by the Gonococcal Isolate Surveillance Project, of the Centers for Disease Control and Prevention, were employed [22]. The *N. gonorrhoeae* strains ATCC 49226, WHO M, WHO P and WHO K were used as internal control.

Studies on sexual contacts of patients and data analysis

At provinces, epidemiological investigation conducted on sexual partners, before submission of *Neisseria* spp. isolates to the NRLNH was developed, and genitourinary cultures on Thayer Martin or chocolate agar were obtained [16].

A descriptive data to explore the demographic and sexual behavior characteristics of patients in whom commensal *Neisseria* spp. or *N. meningitidis* isolates were recovered from genital and extragenital sites was collected and the presence of symptomatology. Information included sex, age, sexual practices, sexual preference in men, year of isolation, and site of collection of samples for a better characterization of patients.

Results

During the period 2014-2021, 520 *Neisseria* spp. isolates were received at the NRL-NH, IPK. The *Neisseria* spp. were obtained from different samples collected from mainly men (N=472) and less from women (N=48), all suspected to be infected with *N. gonorrhoeae.* Isolates were sent from every Cuban province. Of the total number of isolates received, less than half were viable (252/520 (48.5%) of them 241 were confirmed as *N. gonorrhoeae.* Of the 11 non-gonorrhoeae isolates, six isolates were identified as *N. cinerea* and three isolates as *N. meningitidis.* The isolates were obtained in 2014 (n=1), 2015 (n=6), 2018 (n=1) and 2021 (n=1) in five laboratories. Two *Neisseria* spp. isolates could not be identified at species level.

Sociodemographic and behavioral characteristics of the nine patients in which *N. cinerea* and *N. meningitidis* was isolated revealed a median age 30.6 years (range 19 to 67 years), three were females and six were males (Table 1). According to sexual preference in males, five were heterosexual and one was MTSM. The majority of isolates was obtained from urethral exudates (five samples), followed by endocervix exudates (two samples), and a pharyngeal exudate. None of these patients were co-infected with *N. gonorrhoeae.*

Results of epidemiological investigation in provinces on sexual partners of eight of nine patients, in which meningococci or *N. cinerea* was identified in genitourinary samples, revealed no growth of *Neisseria* species.

The MIC to the different antibiotics of the non-gonorrhoeae *Neisseria* spp. is shown in Table 2. A N. meningitidis isolate showed a decreased susceptibility to penicillin (MIC of 0.25 µg/mL). All meningococcal isolates remained susceptible to all other antibiotics tested. The serogroup distribution in meningococci demonstrated that one of each isolate investigated belong to the serogroups C, B, or were not-typeable (Table 2).

Of the six *N. cinerea* isolates analyzed four showed a combined resistance to two antibiotics. Two isolates had fully resistance (MIC 2 µg/mL), and three had an intermediate susceptibility to penicillin, two isolates had a decreased susceptibility to ceftriaxone (MIC 0.5 and 1 µg/mL), four isolates were resistant to ciprofloxacin (MIC range: 2 µg/mL to 32 µg/mL), and three isolates demonstrated resistance to azithromycin (MIC range: 2 µg/mL to 16 µg/mL). All isolates were susceptible to gentamicin (MIC range: 4 µg/mL to 8 µg/mL) (Table 2).

Discussion

In the current study, we identified six isolates as *N. cinerea* and three isolates as *N. meningitidis* (3.6%) among viable *Neisseria* spp. received at NRL-NH during the period 2014 to 2021, in Cuba. These isolates had colonies resembling gonococci: Small, smooth, and non-pigmented after 24 h of incubation or meningococci: Small, round, greyish and smooth on 24 h incubation [1]. In this study, the majority of isolates (eight of nine) were obtained from patients with genital symptoms. Five of these patients confirmed to practice oral, which might explain the probable way of acquisition of *N. meningitidis* or *N. cinerea* in the genital tract, as previously has been described in the literature [23]. The act of oral sex facilitates transmission of *N. meningitidis* from the oropharynx to the urogenital and anorectal tracts. Consequently, *N. meningitidis*, may occasionally colonize or infect the mucosal layers of receiver's body tract [24]. However, we did not identify any report in the literature about the relationship between oral sex and genitourinary *N. cinerea* infection.

Epidemiological investigation conducted at the provinces of the sexual partners of patients, in which meningococci or *N. cinerea* was detected revealed no growth of *Neisseria* species from genital specimen. Unfortunately, oropharyngeal cultures were not performed on their sexual contacts. Then, the hypothesis of oro-genital transmission cannot be confirmed. Moreover, we did not investigate the presence of other STIs agents such as *Chlamydia trachomatis* or *Mycoplasma genitalium*, which may be the cause of the genital symptoms. Indeed, *Chlamydia* and *M. genitalium* represent common causes of urethritis

Table 1: Results of identification of non-gonococcal Neisseria from urogenital and pharyngeal samples of patients with suspicion of gonorrhea in Cuba, 2014 to 2021.

Isolate	Specie	Specimen	Age	Sex	Symptoms	Province	Sexual preference	Sexual practice	Year of isolation
1	*N. meningitidis* serogroup B	Urethra	30	M	Urethritis	CFG	MTSM	Oral, anal, genital	2014
2	*N. meningitidis* serogroup C	Endocervix	67	F	Cervicitis	GTM	Not- applicable*	Oral, genital	2015
3	*N. meningitidis* not-groupable	Pharynx	19	F	Pharyngeal symptoms	ART	Not- applicable*	Oral, genital	2018
4	*N. cinerea*	Endocervix	31	F	Cervicitis	GTM	Not- applicable*	Anal, genital	2015
5	*N. cinerea*	Urethra	19	M	Urethritis	GTM	Heterosexual	Oral, genital	2015
6	*N. cinerea*	Urethra	40	M	Urethritis	VCL	Heterosexual	Genital	2015
7	*N. cinerea*	Urethra	18	M	Urethritis	VCL	Heterosexual	Oral, genital	2015
8	*N. cinerea*	Urethra	23	M	Urethritis	SCU	Heterosexual	Oral, genital	2015
9	*N. cinerea*	Urethra	29	M	Urethritis	GTM	Heterosexual	Genital	2021

*Not applicable for females, only for males

CFG: Cienfuegos; GTM: Guantánamo; ART: Artemisa; VCL: Villa Clara; SCU: Santiago de Cuba; MTSM: Men and Transgender Individuals who seeks Sex with Men

Table 2: Minimal Inhibitory Concentrations (MIC; mg/L) and interpretive category of *N. meningitidis* and *N. cinerea* isolated from patients with suspicion of gonorrhea in Cuba, 2014 to 2021.

Isolate	Specie and specimen	PEN	CRO	CIP	AZI	GEN	RIF
1	*N. meningitidis* (urethra)	0.25 (I)	0.016(S)	0.016 (S)	0.5 (S)	ND	0.125 (S)
2	*N. meningitidis* (endocervical)	0.032 (S)	0.032 (S)	0.032 (S)	0.125 (S)	ND	0.25 (S)
3	*N. meningitidis* (pharyngeal)	0.016 (S)	<0.016 (S)	0.016 (S)	0.25 (S)	ND	0.50 (S)
4	*N. cinerea* (endocervical)	0.5 (I)	0.023 (S)	32 (R)	16 (R)	4 (S)	ND
5	*N. cinerea* (urethra)	2 (R)	0.016 (S)	16 (R)	0.125 (I)	4 (S)	ND
6	*N. cinerea* (urethra)	0.06 (S)	0.016 (S)	0.25 (I)	0.06 (S)	8 (S)	ND
7	*N. cinerea* (urethra)	0.25 (I)	0.5 (I)	2 (R)	2 (R)	4 (S)	ND
8	*N. cinerea* (urethra)	2 (R)	1 (I)	2 (R)	0.5 (I)	8 (S)	ND
9	*N. cinerea* (urethra)	1 (I)	0.125 (S)	0.06 (S)	4 (R)	4 (S)	ND

ND: Non-Defined; S: Susceptible; I: Intermediate; R: Resistant; PEN: Penicillin; CRO: Ceftriaxone; CIP: Ciprofloxacin; AZI: Azithromycin; GEN: Gentamicin; RIF: Rifampicin

and cervicitis in Cuba [25,26], and elsewhere [3]. As such, we cannot ensure that *N. meningitidis* or *N. cinerea* are the real cause of the symptomatology.

In the current study, the *N. meningitidis* isolate serogroup B was recovered from a MTSM patient. Meningococcal colonization and infection of the urogenital and anorectal tracts are more common than previously recognized, and more prevalent among MTSM than men or women who have heterosexual sex [5,27]. During the periods 2004 to 2005 and 2014 to 2015, *N. meningitidis* was investigated in 1,143 and 544 MTSM, respectively, who consulted the STI program in Argentina. The prevalence of this organism was 17.8% and 28.1% in the first and second period, respectively [27].

Two of the cases of genital meningococci (belonging to serogroup C and non-typeable) were found in women. Serogroup C is frequently found in urogenital meningococci, especially those belonging to the hypervirulent ST-11 complex lineage 11.2 [4]. Recently, the Cuban NRL-NH through the Latin America Network of Surveillance for Meningitis reported the first cases and deaths by IMD caused by serogroup C [28], in more than 20 years of surveillance [29].

Two of the identified *N. meningitidis* isolates had reduced susceptibility to penicillin, and all isolates exhibited a whole susceptibility to the other antimicrobials used in the treatment and prevention of IMD, which has been reported before in Cuba [28,30], and other countries [31,32]. The Cuban NRLNH already notified meningococcal strains resistant to penicillin, with MIC values ≥ 0.5 μg/mL [30]. It is vital that meningococci recovered from the urogenital tracts is closely monitored, particularly in terms of emergence of any new genes associated with AMR, and whether these strains are preventable through vaccination [5,31].

Neisseria cinerea is most likely misidentified as *N. gonorrhoeae* because they are phenotypically similar [33,34]. There are few reports in the literature about identification of *N. cinerea* in patients with genital infections. In 1985, an 8-years-old boy with prolonged proctitis and perianal inflammation caused by *N. cinerea* was notified [6]. Knapp et al. detected this organism in a woman with cervicitis attending an arthritis clinic in the USA. [7]. Meanwhile, in China, this commensal *Neisseria* spp. was identified in four males with urethritis and prostatitis [8]. Also in China, Wang et al. detected three isolates of *N. cinerea* in male patients with genitourinary symptoms [9]. In addition, *N. cinerea* has been involved in several infections as septicemia and meningitis [35], urinary infections [36], and eye infections [37].

Phenotypic identification of *N. cinerea* is difficult due to the limited number of expressed characteristics. Certain features (i.e., no growth of *N. cinerea* on chocolate agar at 22°C or golden-brown pigmentation in many of the strains) allow identification, but none of these are reliable for accurate species identification [34]. Thus, a gram-negative, oxidase-positive diplococcus isolated on non-selective media should be identified by definitive methods [11,38], including Matrix-Assisted Laser Desorption Ionization mass spectrometry (MALDI-TOF), which is not 100% certain for *Neisseria* spp. [39]. Some genomic methods, as multi-locus sequence typing; nucleotide sequencing and whole genome sequencing are expensive and require experienced personnel [40,41]. In the current study, we opted for the use of Vitek II method combined with colistin susceptibility and growth on chocolate agar at 22°C, followed by a negative result of q-PCR for gonococci. This algorithm allowed for a definitive identification in all *N. cinerea* without the need of a MALDI-TOF or sequencing instruments. However, two *Neisseria* spp. isolates were un-identifiable by using this algorithm, and requires additional identification by MALDI-TOF or genomic methods.

The AMR phenomenon may emerge earlier and spread more extensively in commensals than in pathogenic *Neisseria* spp., because it's considerably higher prevalence of commensals in human populations. In consequence, a calling for surveillance of AMR in commensal *Neisseria* is actually recommended [10,13]. The characterization of AMR profiles in six isolates of *N. cinerea* reveals that all isolates except one were at least resistant to one antibiotic, with the exception of gentamicin, and four isolates exhibited combined resistance to two or more antibiotics. This behavior is quite different to the AMR observed in *N. meningitidis* isolates in the current study and in invasive strains recovered during the last 5 years in Cuba [28,30]. A similar pattern has been found for penicillin and other antibiotics in *N. gonorrhoeae* isolates globally [14] and in Cuba (Llanes, unpublished data, 2022). The presence of higher MICs for ceftriaxone and azithromycin associated with resistance in our circulating *N. cinerea* are of concern, as they represent the drugs of choice for treatment of gonorrhea in Cuba [16]. Much of these resistance mechanisms in *N. gonorrhoeae* to these antimicrobials are acquired *via* Horizontal Gene Transfer (HGT) from commensal *Neisseria*, including *N. cinerea* [10,42].

AMR in *N. gonorrhoeae* has frequently emerged in core-groups with high rates of partner turnover and high antimicrobial consumption, as MTSM [11,12]. Recently, Cuba initiated HIV Pre-exposure Prophylaxis (PrEP) medication for MTSM, managed

by the national program of control of STI-HIV [43]. In this study, we detected a *N. meningitidis* isolate with reduced susceptibility to penicillin in a MTSM patient. In addition, the combination of elevated antimicrobial consumption and problems with adherence to antibiotic treatment by the Cuban population has been recently notified [44], which might explain the elevated AMR observed in some bacterial species in Cuba [45], and perhaps in commensal *Neisseria*.

The major limitation of the present investigation is the uncertainty that commensal *Neisseria* or *N. meningitidis* causes symptoms described by patients, as the main causal agents of STIs were non-investigated. In addition, the study in sexual partners was restricted to the genital tract [11,13], there was no investigation on previous consumption of antibiotics [12], or about strain characterization of mutations encoding resistance or reduced susceptibility to antibiotics, which prevented to identify putative genes involved in AMR [10,13]. The number of key populations as MTSM evaluated was also limited.

Conclusion

The current represents one of the largest studies that report of *N. cinerea* from patients with genital symptomatology [6-9], combined with multiple resistances to several antibiotics, and the demonstration of high resistance to azithromycin/ceftriaxone. Larger studies are necessary to analyze phenotypic and genomic antibiotic resistance patterns in other commensal *Neisseria* species, and in populations at high risk for the emergence of AMR in Cuba, as PrEP users. Further investigations are required to know about the role of MALDI-TOF or genomic tools in further identification of non-pathogenic *Neisseria* at the NRLNH and if enhanced antibiotic stewardship activities in this and other populations could reduce the MIC of these organisms [12,13].

References

1. Pottinger P, Reller LB, Ryan KJ, Weissman S. Neisseria. Chapter 30, Part III. Pathogenic Bacteria. In: Sherris Medical Microbiology. 7th Ed. New York: Mc Graw-Hill Education; 2018. p. 567-82.
2. Meyer T, Buder S. The laboratory diagnosis of *Neisseria gonorrhoeae*: Current testing and future demands. Pathogens. 2020;9(2):91.
3. Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, et al. Chlamydia, gonorrhoeae, trichomoniasis and syphilis: Global prevalence and incidence estimates, 2016. Bull World Health Organ. 2019;97(8):548-62.
4. Toh E, Williams JA, Qadadri B, Ermel A, Nelson DE. Development of a simple probe real-time PCR assay for rapid detection and identification of the US novel urethrotropic clade of *Neisseria meningitidis* ST-11 (US_NmUC). PLoS One. 2020;15(2):e0228467.
5. Ladhani SN, Lucidarme J, Parikh SR, Campbell H, Borrow R, Ramsay ME. Meningococcal disease and sexual transmission: Urogenital and anorectal infections and invasive disease due to *Neisseria meningitidis*. Lancet. 2020;395(10240):1865-77.
6. Dossett JH, Appelbaum PC, Knapp JS, Totten PA. Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. J Clin Microbiol. 1985;21:575-7.
7. Knapp JS, Totten PA, Mulks MH, Minshew BH. Characterization of *Neisseria cinerea*, a nonpathogenic species isolated on Martin-Lewis medium selective for pathogenic *Neisseria* spp. J Clin Microbiol. 1984;19(1):63-7.
8. Wang DN, Luo ZH, Wang H. Diagnosis and treatment of genitourinary infection with non-gonococcal *Neisseria* in men. Zhonghua Nan Ke Xue. 2009;15(6):499-504.
9. Wang H, Luo ZL, Yi X, She X, Wang D, Wang Y, et al. Gene detection of *Neisseria* spp. isolated from male patients with genitourinary tract infections. Zhonghua Nan Ke Xue. 2012;18(1):39-43.
10. Fiore MA, Raisman JC, Wong NH, Hudson AO, Wadsworth CB. Exploration of the *Neisseria* resistome reveals resistance mechanisms in commensals that may be acquired by *Neisseria gonorrhoeae* through horizontal gene transfer. Antibiotics. 2020;9(10):656.
11. Laumen JGE, Van Dijck C, Abdellati S, Manoharan-Basil SS, De Baetselier I, Martiny D, et al. Markedly reduced azithromycin and ceftriaxone susceptibility in commensal *Neisseria* species in clinical samples from Belgian men who have sex with men. Clin Infect Dis. 2021;72(2):363-4.
12. Dong HV, Pham LQ, Nguyen HT, Nguyen MXB, Nguyen TV, MayF, et al. Decreased cephalosporin susceptibility of oropharyngeal *Neisseria* species in antibiotic-using men who have sex with men in Hanoi, Vietnam. Clin Infect Dis. 2020;70(6):1169-75.
13. Vanbaelen T, Van Dijck C, Laumen J, González N, De Baetselier I, Manoharan-Basil S, et al. Global epidemiology of antimicrobial resistance in commensal Neisseria species: A systematic review. Int J Med Microbiol. 2022;312(3):151551.
14. Unemo M, Golparian D, Eyre DW. Antimicrobial resistance in *Neisseria gonorrhoeae* and treatment of gonorrhea. Methods Mol Biol. 2019;97:37-58.
15. Bennett JS, Jolley KA, Earle SG, Corton C, Bentley SD, Parkhill J, et al. A genomic approach to bacterial taxonomy: An examination and proposed reclassification of species within the genus Neisseria. Microbiology (Reading). 2012;158(Pt 6):1570-80.
16. Colectivo de autores. Plan Estratégico Nacional para la Prevención y Control de las ITS, el VIH y las hepatitis. 2019-2023. MINSAP, Prosalud. 2019.
17. Valenza G, Ruoff C, Vogel U, Frosch M, Abele-Horn M. Microbiological evaluation of the new VITEK 2 *Neisseria-Haemophilus* identification card. J Clin Microbiol. 2007;45(11):3493-7.
18. Hopkins MJ, Ashton LJ, Alloba F, Alawattegama A, Hart IJ. Validation of a laboratory-developed real-time PCR protocol for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine. Sex Transm Infect. 2010;86(3):207-11.
19. Dolan TH, Hatcher CP, Satterfield DA, Theodore MJ, Bach MC, Zhao X, et al. sodC-based real-time PCR for detection of *Neisseria meningitidis*. PLoS One. 2011;6(5):e19361.
20. Taha MK. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J Clin Microbiol. 2000;38(2):855-7.
21. Clinical Laboratory Standard International. Performance Standards for Antimicrobial Susceptibility Testing. 26th Ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.
22. Mann LM, Kirkcaldy RD, Papp JR, Torrone EA. Susceptibility of *Neisseria gonorrhoeae* to gentamicin-gonococcal isolate surveillance project, 2015-16. Sex Transm Dis. 2018;45(2):96-8.
23. Jannic A, Mammeri H, Larcher L, Descamps V, Tosini W, Phung B, et al. Orogenital transmission of *Neisseria meningitidis* causing acute urethritis in men who have sex with men. Emerg Infect Dis. 2019;25(1):175-6.
24. Keys TF, Hecht RH, Chow AW. Endocervical *Neisseria meningitidis* with meningococcemia. N Engl J Med. 1971;285(9):505-6.
25. Mondeja BA, Rodríguez NM, Blanco O, Fernández C, Jensen JS. Mycoplasma genitalium infections in Cuba: Surveillance of urogenital syndromes, 2014-15. Int J STD AIDS. 2018;29(10):994-8.
26. Guilarte E, Soto Y, Kourí V, Limia CM, Sánchez ML, Rodríguez AE, et

al. Circulation of Human Papillomavirus and *Chlamydia trachomatis* in Cuban Women. MEDICC Rev. 2020;22(1):17-27.

27. García SD, Sorhuet-Pereira C, Perazzia BE, Losada ME, Cabellos G, Casco RH, et al. *Neisseria meningitidis* aislada de muestras de hombres que tienen sexo con hombres. Rev Argent Microbiol. 2019;52(2):101-6.

28. Organización Panamericana de la Salud. Informe regional de SIREVA II, 2018. Datos por país y por grupos de edad sobre las características de los aislamientos de Streptococcus pneumoniae, *Haemophilus influenzae* y *Neisseria meningitidis*, en procesos invasivos bacterianos. 2021.

29. Climent Y, Yero D, Martínez I, Martín A, Jolley KA, Sotolongo F, et al. Clonal distribution of disease-associated and healthy carrier isolates of *Neisseria meningitidis* between 1983 and 2005 in Cuba. J Clin Microbiol. 2010;48(3):802-10.

30. Suárez N, Martínez I, Feliciano O, Gutiérrez O, Valdés MJ, Villasusa I, et al. Caracterización de cepas de *Neisseria meningitidis* causantes de enfermedad invasiva en Cuba. Panorama Cuba y Salud. 2014;9(1):13-9.

31. Siqueira R, Gomes AP, Dutra JL, Balbino PS, Santana LA, Oliveira L, et al. Meningococcal disease, a clinical and epidemiological review. Asian Pacific J Trop Med. 2017;10(11):1019-29.

32. Moreno J, Sanabria O, Saavedra SY, Rodríguez K, Duarte C. Phenotypic and genotypic characterization of Neisseria meningitidis serogroup B isolates from Cartagena, Colombia. Biomedica. 2015;35(1):138-43.

33. Public Health England. UK standards for microbiology investigations. Identification of *Neisseria* species. Bacteriology-Identification ID 6. 2015;3(29).

34. Kochi CV, Guibourdenche M, Lemeland JF, Riou JY. *Neisseria cinerea*, a bacterium whose bacteriological identification is difficult. Clin Microbiol Infect. 1999;5(10):647-50.

35. Kirchgesner V, Plesiat P, Dupont MJ, Estavoyer JM, Guibourdenche M, Riou JY, et al. Meningitis and septicemia due to *Neisseria cinerea*. Clin Infect Dis. 1995;21(5):1351.

36. Garcia SD, Descole EM, Famiglietti AM, Lopez EG, Vay CA. Infection of the urinary tract caused by *Neisseria cinerea*. Enferm Infecc Microbiol Clin. 1996;14(9):576.

37. Fiorito TM, Noor A, Silletti R, Krilov LR. Neonatal conjunctivitis caused by *Neisseria cinerea*: A case of mistaken identity. J Pediatric Infect Dis Soc. 2019;8(5):478-80.

38. Knapp JS, Hook EW 3rd. Prevalence and persistence of *Neisseria cinerea* and other *Neisseria* spp. in adults. J Clin Microbiol. 1988;26(5):896-900.

39. Anderson N, Weber C. An illustrative example of the need for ongoing clinical microbiology competency in the era of MALDI-TOF MS microorganism identification: *Neisseria* spp. 2022.

40. Van Dijck C, Laumen JG, Manoharan-Basil SS, Kenyon C. Commensal *Neisseria* are shared between sexual partners: Implications for gonococcal and meningococcal antimicrobial resistance. Pathogens. 2020;9(3):228.

41. Mechergui A, Achour W, Ben-Hassen A. Laboratory identification methods of nonpathogenic *Neisseria* species. Rev Med Microbiol. 2014;25(4):85-92.

42. Manoharan-Basil SS, Laumen JGE, Van Dijck C, De Block T, De Baetselier I, Kenyon C. Evidence of horizontal gene transfer of 50S ribosomal genes rplB, rplD, and rplY in *Neisseria gonorrhoeae*. Front Microbiol. 2021;12:683901.

43. PrEP Watch. Explore data and information on prep across the globe: Where do you fit on the map?

44. Castillo D, Alfonso I, Lambert JM. Consumo de antimicrobianos seleccionados en el Cardiocentro Pediátrico "William Soler" durante el periodo 2011-2015. Rev Cubana Salud Pub. 2019;45(1):e1136.

45. Oliva A, Carmona Y, de La C. López E, Álvarez R, Aung MS, Kobayashi N, et al. Characterization of neonatal infections by gram-negative bacilli and associated risk factors, Havana, Cuba. Infect Dis Rep. 2021;13(1):219-29.