

***Neisseria gonorrhoeae* antimicrobial resistance
surveillance report of Belgium – 2023**



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1. Rationale – Background

The Institute of Tropical Medicine (ITM) is the National Reference Centre of Sexually Transmitted Infections (NRC-STI) of Belgium since 2010. Besides chlamydia, gonorrhoea is the second most detected bacterial STI in Belgium.^{1,2} However, more worrying is the fact that *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, is evolving into a superbug. It acquired resistance to all classes of antimicrobials used for treating infections.³ Given the extraordinary capacity of *N. gonorrhoeae* to acquire resistance mechanisms, it is likely that *N. gonorrhoeae* may become untreatable in the not-too-distant future. As such, an important surveillance activity of the NRC-STI is the follow-up of antimicrobial resistance of *N. gonorrhoeae* to anticipate resistance of *N. gonorrhoeae* to certain antimicrobials. The results may lead to an adaptation of the treatment guidelines when necessary. Here, we present the data from 2023 along with historical data spanning the past decade up to 2014.

2. Methods

The NRC-STI receives presumable *N. gonorrhoeae* isolates from every district of Belgium. At the NRC-STI, confirmation of the identification of *N. gonorrhoeae* is performed by Gram stain, oxidase test, and confirmation via molecular detection or via MALDI-TOF MS. When *N. gonorrhoeae* is confirmed, additional antimicrobial susceptibility testing is performed to determine the minimal inhibition concentration or MIC to a certain antibiotic. The MIC allows interpretation of resistance to a specific antibiotic based on breakpoints available in the EUCAST guidelines version 13.0. Since 2023, the breakpoint of tetracycline was adapted from >1mg/L to >0.5mg/L. **Table 1** lists the antibiotic tested, the method, the breakpoint for resistance, and the frequency at which antimicrobial susceptibility testing (AST) is performed. Multidrug resistance is defined to be resistant to azithromycin, ciprofloxacin and ceftriaxone. Resistance to azithromycin can be low-level (LL) resistant (MIC 1.5-3 mg/L), intermediate level (IL) resistant (MIC 4-192 mg/L) or high-level resistant (MIC >=256 mg/L).⁴

Table 1: Antibiotic susceptibility testing of *N. gonorrhoeae* at the NRC-STI

Antibiotic	Test method	Resistance breakpoint (EUCAST)	AST frequency
Azithromycin	Etest (Biomerieux, France)	>1 mg/L	Yearly
Ciprofloxacin	Etest (Biomerieux, France)	> 0.064 mg/L	Yearly
Ceftriaxone	Etest (Biomerieux, France)	>0.125 mg/L	Yearly
Cefixime	Agar dilution method	>0.125 mg/L	Yearly*
Penicillin	Agar dilution method	>1 mg/L	Every three years - 2025
Tetracycline	Agar dilution method	>0.5 mg/L	Yearly*
Spectinomycin	Agar dilution method	> 64 mg/L	Every three years - 2025
Gentamicin	Agar dilution method	No breakpoint defined	Every three years - 2025

*MIC of cefixime and tetracycline is only tested on a maximum of 200 isolates collected between September till December as required by the European Gonococcal Antimicrobial Surveillance Programme if the year is not a snapshot year (Euro-GASP). AST: Antimicrobial susceptibility testing

3. Results

3.1 Characteristics of *N. gonorrhoeae* isolates

In 2023, the NRC-STI received 888 samples originating from all Belgian regions (Flanders, Brussels and Wallonia). However, 5.2% (46/888) of the isolates did not survive the transport, three isolates were contaminated (0.3%) and three isolates (0.3%) were not confirmed to be *N. gonorrhoeae* which brings the number of *N. gonorrhoeae* isolates to 836. Annex one lists the number of *N. gonorrhoeae* isolates by the 72 different laboratories. After review, 17 isolates were found to be duplicates (infections within one month) and were not included in the analysis (n=819). Since the start of our surveillance activities, this is the highest number of isolates confirmed and compared with 2022 (n=675), we note an increase of 21% (**Figure 1**). The isolates were detected in 797 individuals, 16 individuals had more than one infection in 2023.

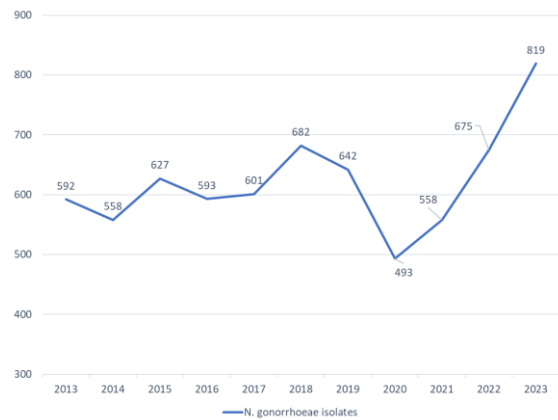


Figure 1: Number of *N. gonorrhoeae* isolates identified by NRC-STI over the years.

In 2023, most of the isolates came from Flanders (661/819; 80.7%), followed by Wallonia (111/819; 13.6%) and Brussels (46/819; 5.6%) (**Figure 2**). The increase in the number of isolates was only noted in Flanders. One isolate was found in an individual who was not domiciled in Belgium.

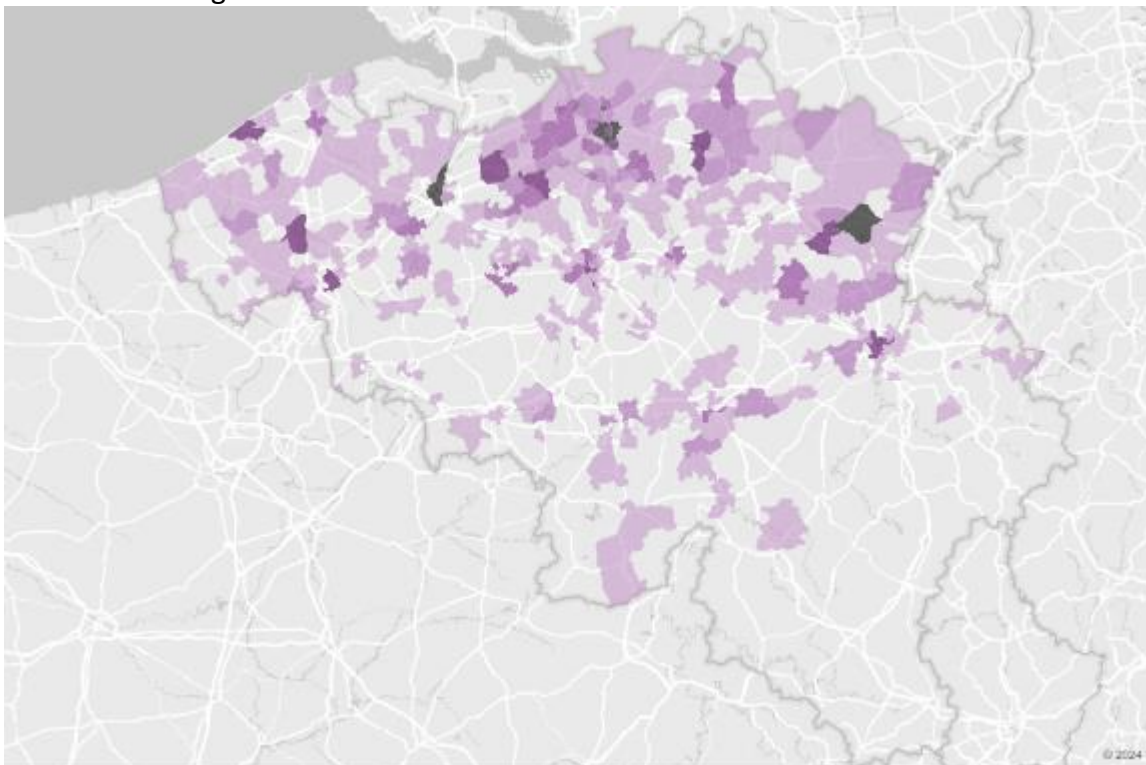


Figure 2: Geographical location of identified gonococcal isolates. The shading intensity indicates the number of isolates, ranging from light purple for 1-3 isolates to very dark purple for 11-20 isolates. Black areas represent more than 21 gonococcal isolates).

The majority of the samples was found among men (648/819; 79.1%). One-fifth of the isolates was detected among women (168/819; 20.5%), two among transgender individuals. **Figure 3** shows the number of isolates received per semester from 2013 to 2023 stratified by sexual transmission. The number of isolates of female origin did not increase since the previous year (160 in 2022; 168 in 2023).

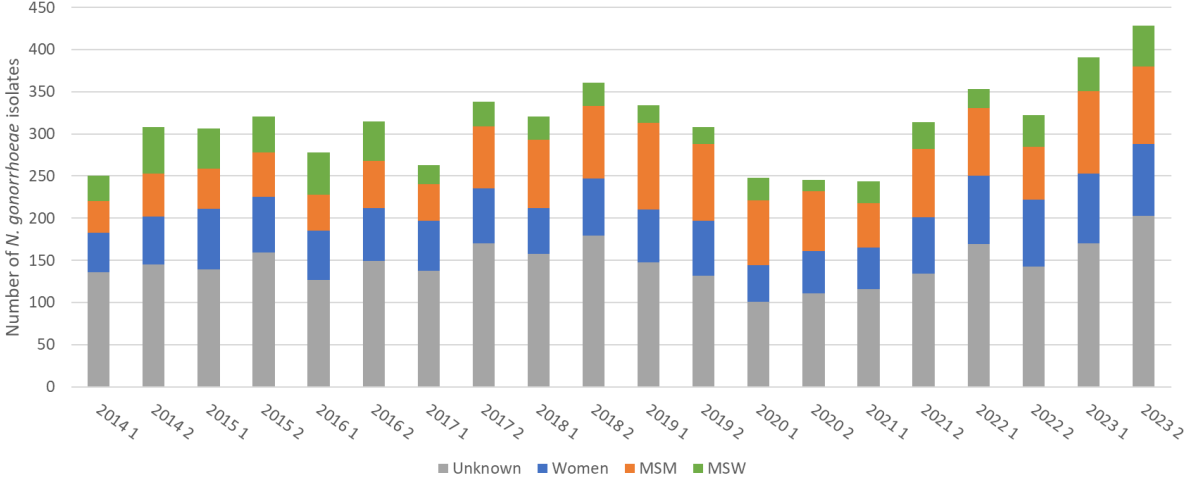


Figure 3: Percentage of *N. gonorrhoeae* isolates received by the NRC-STI from 2013 to 2023 per semester stratified by sexual transmission. MSM: Men who have sex with men and includes all anorectal male samples. MSW: Men who have sex with women. Unknown: Unknown gender or men with unknown sex of sex partners

Table 1 presents the locations of infection for *N. gonorrhoeae* isolates in 2023. Most isolates originated from the urogenital anatomical sites followed by the anorectum. Interestingly, seven isolates were associated with bloodstream infections and another subset (n=7) with articular infections. This observation shows the occurrence of disseminated gonococcal infection (DGI), a rare condition caused by untreated *N. gonorrhoeae* infections (14/819; 1.7%). In the previous ten years, the number of *N. gonorrhoeae* isolates from suspected DGI cases was on average 4 per year (min 0, max 6).

Table 1: Infection site of the *N. gonorrhoeae* isolates of 2023

Biological origin	Female (n=168)		Male (n=648)		Other/Unknown(n=3)		Total (n=819)	
	N	%	N	%	N	%	N	%
Urogenital	155	92.3	551	85.0	2	66.7	708	86.5
Anorectal	2	1.2	60	9.3	0	0.0	62	7.6
Throat	1	0.6	14	2.2	0	0.0	15	1.8
Pooled Sample (urine, anorectal & throat)	0	0.0	5	0.8	0	0.0	5	0.6
Eye	4	2.4	2	0.3	0	0.0	6	0.7
Articular origin	3	1.8	4	0.6	0	0.0	7	0.9
Blood	2	1.2	5	0.8	0	0.0	7	0.9
Unknown	1	0.6	7	1.1	1	33.3	9	1.1

3.2 Antimicrobial resistance of *N. gonorrhoeae*

Table 2 lists the MICs of the *N. gonorrhoeae* isolates of 2023 for the following antibiotics: azithromycin, ciprofloxacin, ceftriaxone, cefixime and tetracycline.

Table 2: Minimal Inhibitory Concentration (MIC) and final interpretation of the *N. gonorrhoeae* isolates 2023

	S		MIC limit value	I		MIC limit value	R		MIC limit value
	N	%	mg/L	N	%	mg/L	N	%	mg/L
Azithromycin (n=819)	523	63.9	≤ 1	-	-	-	296	36.1	> 1
Ciprofloxacin (n=819)	297	36.3	≤ 0.032	1	0.1	0.047-0.064	521	63.6	> 0.064
Ceftriaxone (n=819)	818	99.9	≤ 0.125	-	-	-	1	0.1	> 0.125
Cefixime (n=197)	196	99.5	≤ 0.125	-	-	-	1	0.5	> 0.125
Tetracycline (n=197)	42	21.3	≤ 0.5				155	78.7	> 0.5

MIC Breakpoints according to EUCAST guidelines . S: Susceptible; I: Susceptible, Increased Exposure; R: Resistant

Figure 3 shows the antimicrobial resistance of *N. gonorrhoeae* to the different antibiotics over a span of ten years.

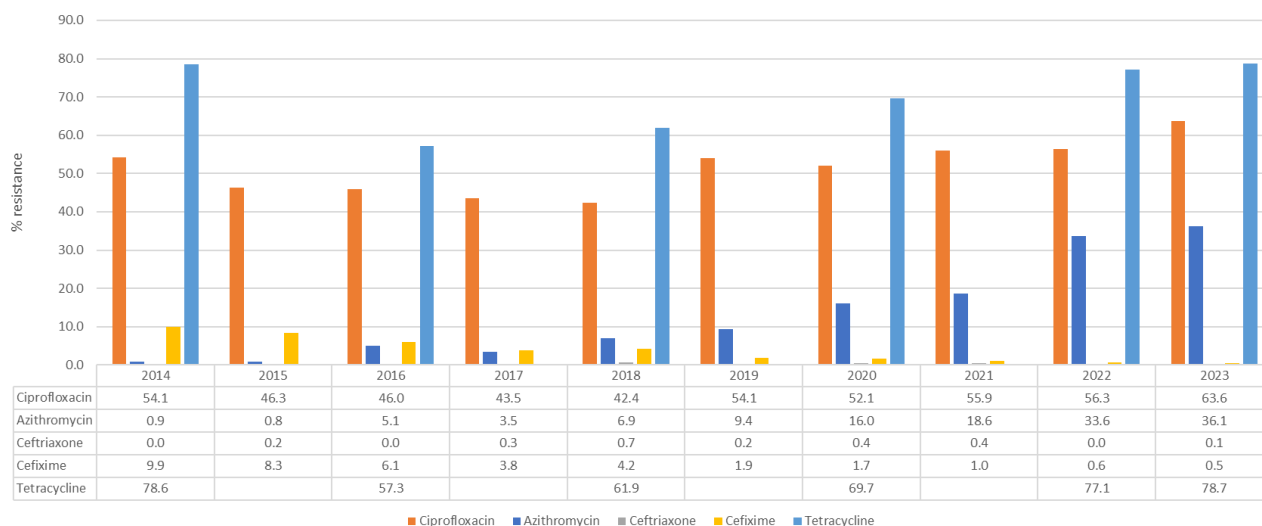


Figure 4: Antimicrobial resistance of *N. gonorrhoeae* to ciprofloxacin, azithromycin, ceftriaxone, cefixime, tetracycline over 10 years (2014-2023) in Belgium.

3.2.1 Azithromycin resistance of *N. gonorrhoeae*

Resistance of *N. gonorrhoeae* to azithromycin is stabilizing, but still over one in three of the isolates is resistant to azithromycin (36.1%). Stratifying per sexual transmission, azithromycin resistance is still the highest among MSM but remains stable. In contrast, resistance in female isolates is still increasing to 31.0% (**Figure 4**).

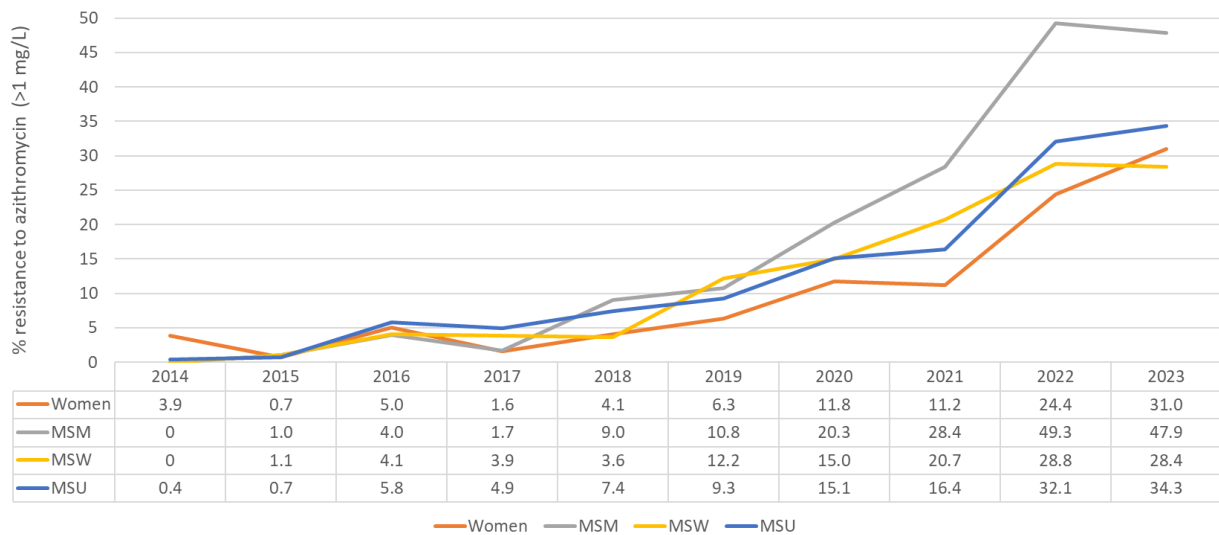


Figure 5: Resistance to azithromycin of *N. gonorrhoeae* stratified by gender and sexual transmission over 10 years. MSM: Men who have sex with men. MSW: Men who have sex with women. MSU: Men with unknown sexual orientation or unknown gender.

Stratifying per azithromycin MIC, we see a clear shift over time from susceptible isolates (≤ 0.125 mg/L) to less susceptible isolates (0.19-1 mg/L). Interestingly, all, except one, azithromycin resistant isolates from MSM were LL resistant. More diversity in the resistance pattern was found among women (43/52 Low Level, 8/52 Intermediate Level and 1/52 High Level Resistance) (Figure 5). This HL resistant isolate was found in a woman with cervicitis.

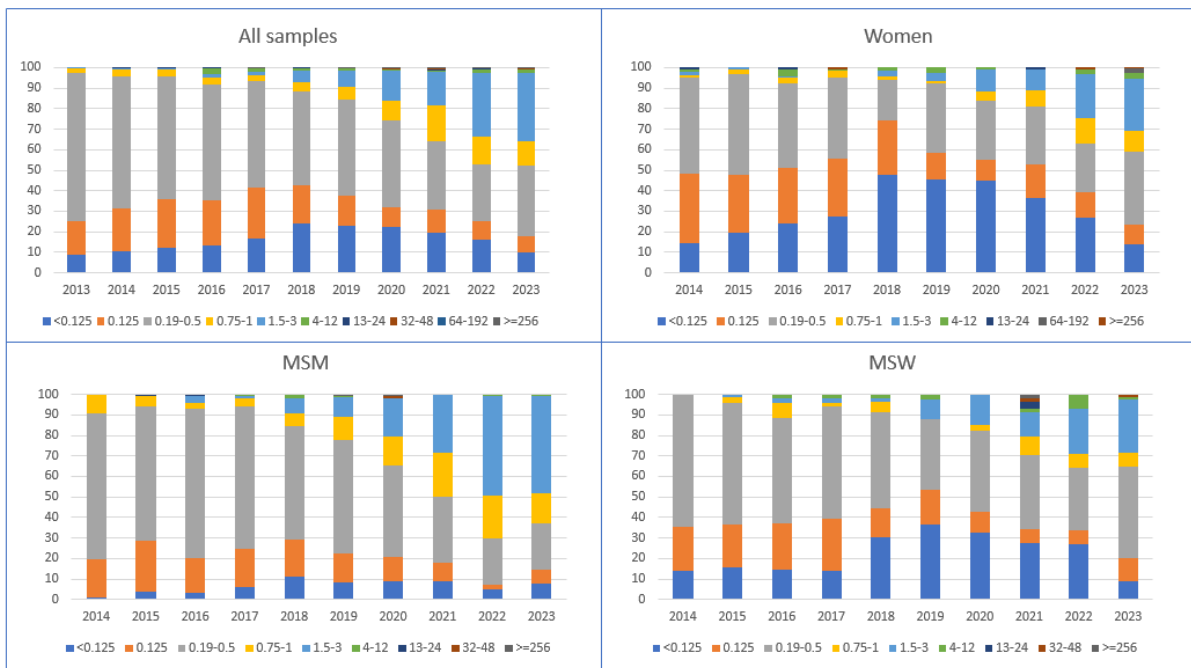


Figure 6: Minimal inhibitory concentration (MIC) distribution (mg/L) for azithromycin over ten years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

3.2.2 Ciprofloxacin resistance of *N. gonorrhoeae*

While resistance to ciprofloxacin had remained stable over the past years, we observed a notable 13% increase compared to the previous year, reaching the highest reported levels in the last decade. In 2023, nearly two-thirds of the isolates were resistant to ciprofloxacin (63.6%). When stratifying by sexual transmission, and comparing to data from 2014, we

observed a discernible increase exclusively among men. Specifically, this increase was statistically significant among MSM and MSU (Figure 7). The MIC distribution over the years for ciprofloxacin in the different population groups can be found in Figure 8.

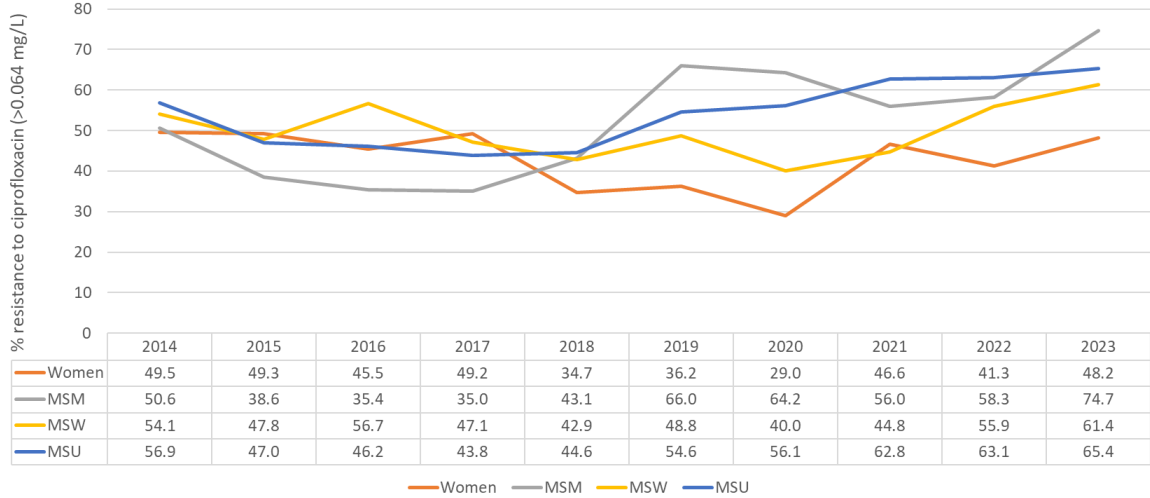


Figure 7: Resistance to ciprofloxacin of *N. gonorrhoeae* stratified by gender and sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women. MSU: Men with unknown sexual orientation or unknown gender.

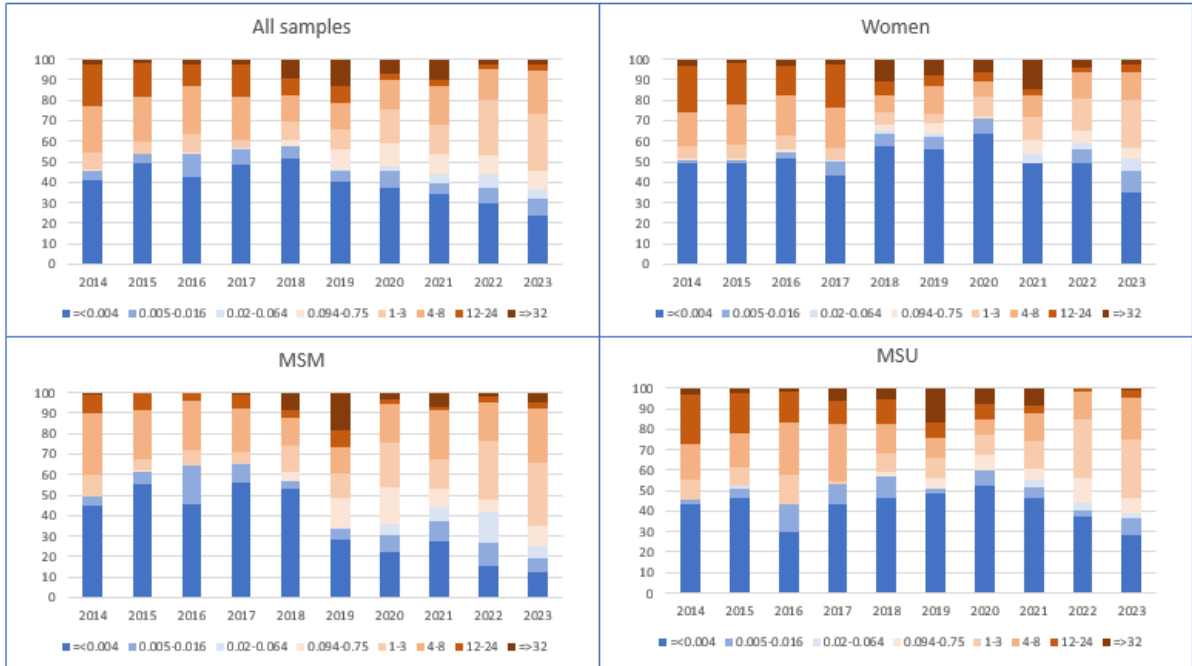


Figure 8: Minimal inhibitory concentration (MIC) distribution (mg/L) for ciprofloxacin over the last ten years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women.

3.2.3 Ceftriaxone resistance of *N. gonorrhoeae*

Resistance to ceftriaxone has been detected in 14 cases since 2014. In 2023, one ceftriaxone resistant isolate was detected. The urogenital sample was from a heterosexual man with urethritis. The MIC was just above the breakpoint (0.19 mg/L) and there was no clinical resistance. The isolate was also resistant to ciprofloxacin (6mg/L). For ceftriaxone, we did not observe any increase in MIC (Figure 9). On the contrary, the majority of isolates exhibited MIC values of 0.016 or lower (92.3%) in 2023. It is noteworthy that we transitioned our antimicrobial susceptibility testing (AST) methodology for ceftriaxone

from agar dilution to E-test, with a minimum concentration of MIC <0.016 mg/L, in May 2018. However, to better visualize shifts from highly susceptible *N. gonorrhoeae* isolates to less susceptible ones, we transitioned to E-test with a minimum concentration of <0.002 mg/L in May 2023.



Figure 9: Minimal inhibitory concentration (MIC) distribution (mg/L) for ceftriaxone over the years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

3.2.4 Tetracycline resistance of *N. gonorrhoeae*

We visualise tetracycline resistance according to the old breakpoint (>1 mg/L) and new breakpoint (>0.5 mg/L) for comparison purposes (Figure 10). As a result of the change in breakpoint, a large shift towards tetracycline resistance has been noted. However, using the same breakpoint over the years, tetracycline resistance has remained relatively stable over the years.

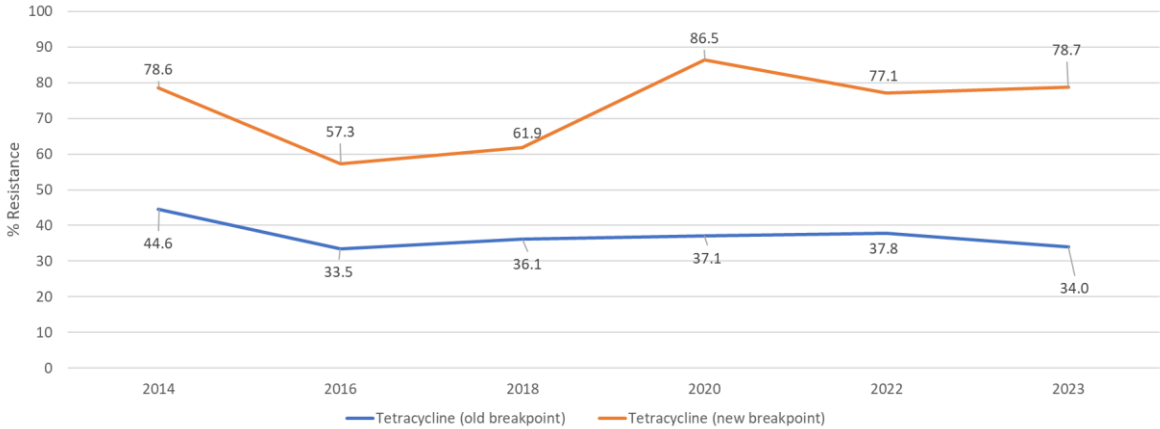


Figure 10: Antimicrobial resistance (%) of *Neisseria gonorrhoeae* to tetracycline old breakpoint (>1mg/L) and to tetracycline new breakpoint (>0.5 mg/L) over ten years in Belgium.

Due to the implementation of doxycycline post-exposure prophylaxis (doxy-PEP) to prevent STI's among MSM in several countries (intake of doxycycline 200 mg within 24-72h after condomless sex), tetracycline susceptibility testing is now performed yearly on a subset of maximum 200 isolates to estimate doxy-PEP effectiveness in reducing gonorrhoeae cases. Previously, we performed tetracycline resistance every other year.

Figure 11 depicts the resistance patterns per sexual transmission. Again, the highest resistance pattern is found among MSM with over 80% of isolates resistant to tetracycline. The MIC distribution stratified per transmission route, can be found in **Figure 12**. Notably, 23.9% (11/56) of tetracycline-resistant isolates from MSM had MICs above 4 mg/L, compared to 8.7% (2/23) among women.

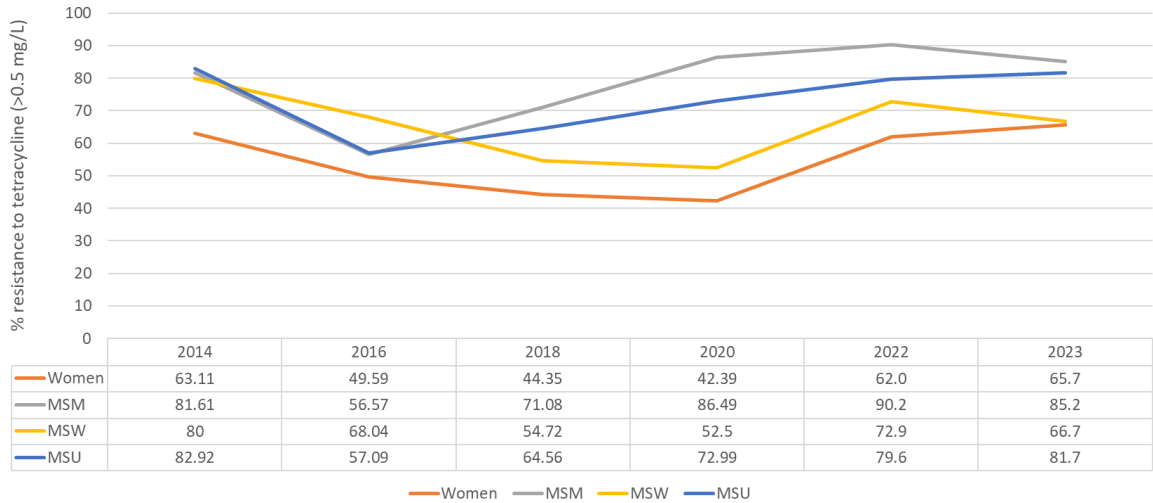


Figure 11: Resistance to tetracycline (breakpoint >0.5 mg/L) of *N. gonorrhoeae* stratified by gender and sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women. MSU: Men with unknown sexual orientation or unknown gender

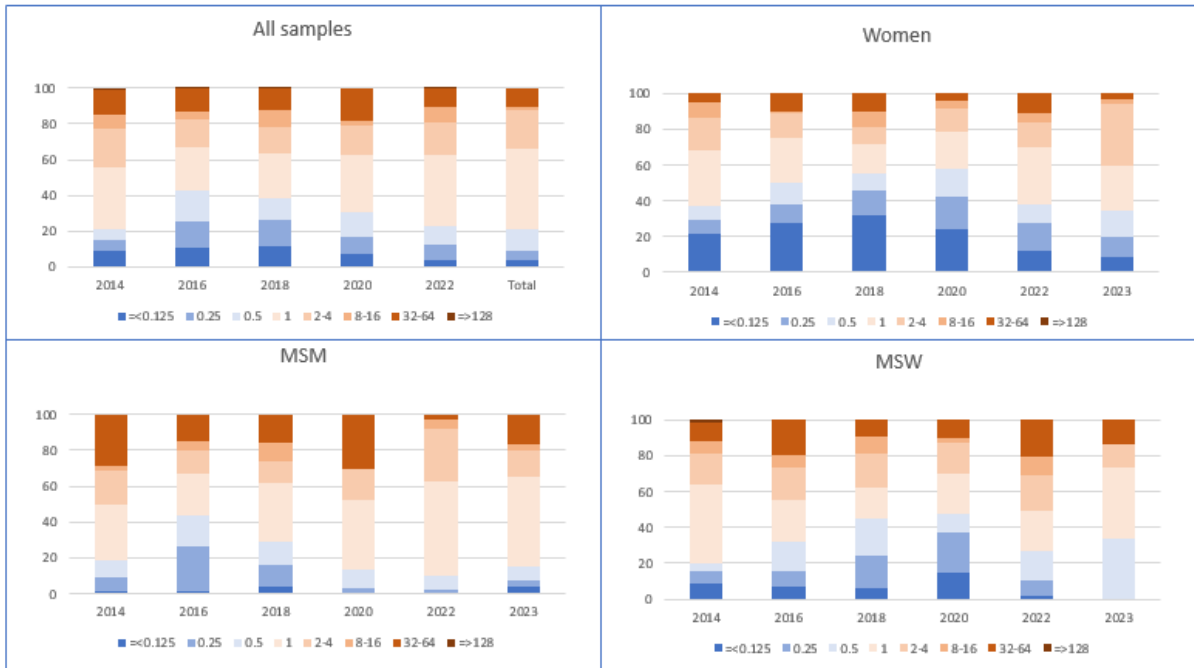


Figure 12: Minimal inhibitory concentration (MIC) distribution (mg/L) for tetracycline over the years for all *N. gonorrhoeae* isolates and stratified per sexual transmission using breakpoint >0.5mg/L. MSM: Men who have sex with men. MSW: Men who have sex with women

3.3 Multidrug resistance of *N. gonorrhoeae*

No isolate was resistant to ceftriaxone, azithromycin and ciprofloxacin in 2023. However, **Figure 13** shows the number of isolates resistant to azithromycin and ciprofloxacin stratified per sexual transmission over the years. Almost 25% is resistant to both antibiotics and the highest proportion of dual resistant isolates to ciprofloxacin and azithromycin were found among MSM.

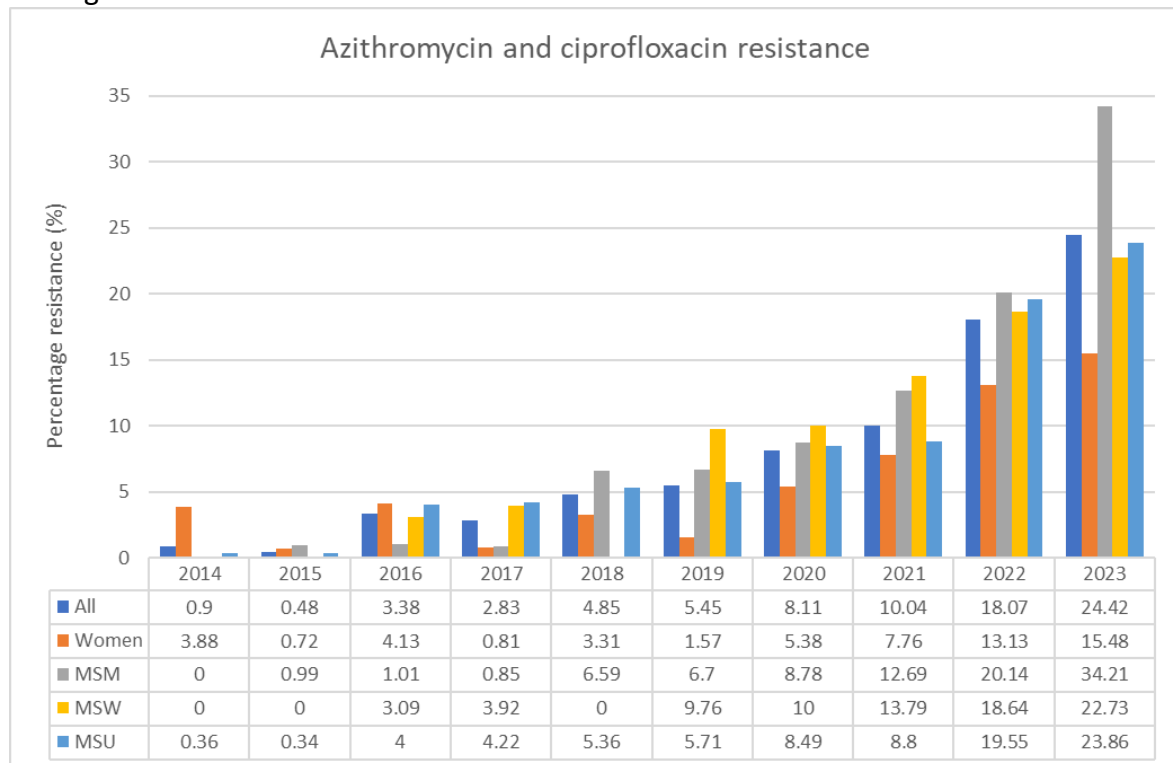


Figure 13: *N. gonorrhoeae* isolates resistant to ciprofloxacin and azithromycin stratified by gender and sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women. MSU: Men with unknown sexual orientation or unknown gender

4. Discussion

In 2023, the NRC-STI confirmed the **highest number of *N. gonorrhoeae* isolates to date**. A rise in *N. gonorrhoeae* cases has also been documented in neighboring countries in 2022, such as The Netherlands.⁵ Notably, an increase in incidence from women and heterosexual men has been observed in several countries. While the number of isolates from women remained relatively stable compared to 2022 (5.0% increase, 168 vs 160), there was a notable 26.1% increase in isolates collected from men (648 vs 514) in our country. Particularly, the most significant increase was observed among heterosexual men (49.2%), although caution is warranted in interpreting this figure due to missing data on sexual orientation for most of the isolates which limits the number of isolates collected from heterosexual men to 88. Among men who have sex with men (MSM), the percentage increase was 31.9%. Intriguingly, the rise in *N. gonorrhoeae* isolates was confined to Flanders. A plausible hypothesis for this trend could be that there may be more awareness regarding the necessity of conducting cultures for antimicrobial resistance surveillance of *N. gonorrhoeae* in Flanders, although no additional public actions were taken to increase this awareness nor was there a change in testing practice.

In addition to the absolute increase in cases, there has **been a rise in confirmed cases of Disseminated Gonococcal Infection**. DGI, a complication of untreated gonorrhoea, is rarely reported and can lead to serious health consequences such as arthritis, bacteremia, endocarditis, or meningitis. The increase in DGI cases could be an indicator of a concerning uptrend in *N. gonorrhoeae* infections in Belgium. Among the DGI cases, 5 were identified in women and 9 in men, including one MSM and were isolated in the Brussels (6/14) and Flanders (8/14) in different laboratories.

Further analysis will involve whole genome sequencing of these isolates to examine the genetic diversity of these isolates and the association of DGI with possible virulence factors. Additionally, raising awareness of DGI among clinicians, including those working in non-sexual health settings will be imperative.

Unfortunately, **the trend of increasing antimicrobial resistance persists for azithromycin and ciprofloxacin**. Nearly two-thirds of the isolates exhibit resistance to ciprofloxacin, while one-third demonstrate resistance to azithromycin. Moreover, almost one in four isolates show dual resistance to both antibiotics. Resistance rates are highest among MSM, with one in three being resistant to both antibiotics. Importantly, **the increase in azithromycin resistance was the highest among women and is now also reaching 31.0%**. Azithromycin is currently replaced as the first line treatment for chlamydia infections by doxycycline, however, it is still one of the five most used antibiotics in an ambulant setting in Belgium.⁶

The MIC distribution indicates a replacement of highly susceptible isolates with low-level resistant ones for azithromycin, while high-level resistance to azithromycin remains rare.

Encouragingly, **only one isolate was resistant to ceftriaxone** and the patient responded well to treatment with ceftriaxone 1g IM and azithromycin 1g PO. The MIC distribution over time does not indicate a shift to less susceptible strains. Importantly, within the European region, seven extensively drug resistant *N. gonorrhoeae* cases have been reported, including two detected in France between 2022 and 2023.⁷ This underscores the necessity of ongoing monitoring of phenotypic antimicrobial resistance and **underlines the recommendation to culture *N. gonorrhoeae* cases detected by molecular assays**.

The escalating resistance trend is alarming, particularly in the context of antibiotic use for preventing STIs such as **doxycycline Post-Exposure Prophylaxis (doxy-PEP)**. Given the implementation of doxy-PEP among MSM using PrEP to prevent HIV in many countries, antimicrobial resistance surveillance activities now include yearly tetracycline antimicrobial susceptibility testing on a subset of up to 200 isolates. Resistance to tetracycline stands at 78.7%, whereby doxy-PEP is likely ineffective to prevent gonorrhoea infections. Moreover, of the doxycycline resistant isolates, almost one in four had an MIC of over 4 mg/L. These findings suggest that doxy-PEP may not be effective against gonorrhoea and could potentially drive the selection of highly resistant strains. Moreover, tetracycline resistance associated mutations and genes are linked to mutations causing resistance to other classes of antibiotics.⁸ As such, the implementation of doxy-PEP will merit close monitoring for individual and population-level antimicrobial resistance in different bacterial species including *N. gonorrhoeae* as recommended by ECDC.⁹

5. Acknowledgements

We would like to thank Amaryl Lecompte for her constructive feedback.

6. References

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7. Annexes

7.1 Number of *N. gonorrhoeae* isolates received per laboratory

Laboratory	Number of isolates
11601 A.Z. KLINA	4
11603 UZ ANTWERPEN	4
11622 AZ VOORKEMPEN	1
11642 AZ MONICA	3
11653 AML ANTWERPEN	20
11677 G.Z.A. CAMPUS ST. AUGUSTINUS	21
11704 ITG	176
12609 A.Z. SINT-MAARTEN	1
12616 SOMEDI - HEIST-OP-DEN-BERG	6
12620 IMELDA - BONHEIDEN	2
12647 AKL - LIER	8
13608 AZ ST.-DIMPNA	1
13618 AZ TURNHOUT CAMPUSSINT-JOZEF	1
13656 CMA - HERENTALS	131
23604 A.Z. JAN PORTAELS	2
23666 LABO MEDISCHE ONTLEDINGEN - GROOT-BIJGAARDEN	1
24650 MCH LEUVEN	8
24700 UZ KUL CAMPUS GASTHUISBERG	5
25607 LIMS - OTTIGNIES	3
25611 CLINIQUE ST-PIERRE - LABORATOIRE DE BIOLOGIE CLINIQUE	2
26678 LHUB - ULB	4
26692 CHU Brugmann - BRUSSEL	5
26726 CEBIODI SITE DU JARDIN BOTANIQUE	11
26750 CLINIQUES UNIVERSITAIRES SAINT-LUC	1
27729 ULB INSTITUT DE BIOLOGIE CLINIQUE	6
28604 EUROPA ZH - ELISABETH	4
28612 LABORATOIRE CHIREC - SITE DELTA	2
28667 HOPITAUX IRIS SUD - SITE ETTERBEEK / IXELLES LABORATOIRE DE BIOLOGIE CLINIQUE	1
28967 LHUB-ULB - HOPITAL ERASME - site Anderlecht	4
29636 UZ BRUSSEL	5
31639 AML West (Ardoeie)	46
33606 REGIONAAL ZIEKENHUIS - JAN YPERMAN	6
34605 EUROFINS LABO VAN POUCKE	1
34612 AZ GROENINGE	3
34616 MEDISCH LABO BRUYLAND	1
36609 AZ DELTA - ROESELARE	5
41658 O.L.V. ZIEKENHUIS - AALST	4
42609 MEDINA DENDERMONDE	114
42615 AZ SINT-BLASIUS	2

Laboratory	Number of isolates
43620 AZ ALMA CAMPUS EEKLO	2
44610 LABORATORIUM NUYTINCK	3
44624 MEDILAB - GENT	4
44673 A.Z. MARIA MIDDELARES GENT	9
44696 A.Z. SINT-LUCAS GENT	16
44700 UZ GENT	4
44711 CERBA HEALTHCARE BELGIUM BVBA - CRI	6
46615 VITAZ - ST.-NIKLAAS	7
52610 SYNLAB HEPPIGNIES	5
52626 C.H.U. DE CHARLEROI - HOPITAL CIVIL MARIE CURIE	6
53606 C.H.U. AMBROISE PARÉ	1
53639 EPICURA - HORNU	6
54602 CENTRE HOSPITALIER DE MOUSCRON	2
55604 CENTRE HOSPITALIER DE TIVOLI	4
55614 CHR HAUTE SENNE SITE C.H. DE SOIGNIES	2
55624 CENTRE HOSPITALIER DE JOLIMONT- SITE DE JOLIMONT	2
56603 CENTRE DE SANTÉ DES FAGNES	1
57606 CHWAPI LABORATOIRE SITE NOTRE DAME	3
61602 CENTRE HOSPITALIER RÉGIONAL DE HUY	2
62668 CLINIQUE MONT-LEGIA	31
62700 HÔPITAL UNIVERSITAIRE DU SART TILMAN	5
63608 CENTRE HOSPITALIER REGIONAL DE VERVIERS	1
71604 LABORATORIUM KLINISCHE ONTLEDINGEN - L.K.O. - L.M.C.	10
71615 JESSAZIEKENHUIS - CAMPUS VIRGA JESSE	5
71620 SINT-TRUDO	1
71622 ZIEKENHUIS OOST-LIMBURG	13
71725 KLINISCH LABO RIGO	10
72602 MARIA ZIEKENHUIS NOORD-LIMBURG	1
91605 CHU - UCL - NAMUR	7
92611 APP CHR SAMBRE ET MEUSE - SITE MEUSE	3
92613 CLINIQUE MATERNITÉ STE-ELISABETH - NAMUR	6
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