

***Neisseria gonorrhoeae* antimicrobial resistance
surveillance report of Belgium – 2022**



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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 and to adapt treatment guidelines when necessary.

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 molecular detection (isolates obtained from outside the ITM polyclinic) or via the enzymatic/sugar assay API-NH (isolates obtained from patients attending the ITM polyclinic). 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 12.0. **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. A *N. gonorrhoeae* isolate acquired high-level resistance to azithromycin if the MIC is above 256 mg/L.

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 -2022
Tetracycline	Agar dilution method	>1 mg/L	Every three years -2022
Spectinomycin	Agar dilution method	> 64 mg/L	Every three years -2022
Gentamicin	Agar dilution method	No breakpoint defined	Every three years -2022

*MIC of cefixime 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 2022, the NRC-STI received 740 samples originating from all Belgian regions (Flanders, Brussels and Wallonia). However, 6.7% (49/740) of the isolates did not survive the transport, three isolates were contaminated (0.4%) and six isolates (0.8%) were not confirmed to be *N.*

gonorrhoeae (no *Neisseria spp.*) which brings the number of *N. gonorrhoeae* isolates to 682. Annex one lists the number of *N. gonorrhoeae* isolates by the 70 different laboratories. After review, seven isolates were found to be duplicates and were not included in the analysis (n=675). After a decline in the number of isolates identified in 2020 & 2021 due to the Covid-19 pandemic, the number of *N. gonorrhoeae* isolates in 2021 now reaches the number of isolates reported pre-Covid times.

In 2022, Most of the isolates came from Flanders (468/675; 69.3%), followed by Wallonia (132/19.6%) and Brussels (71/675; 10.5%) (**Figure 1**). Four isolates were found in individuals who were not domiciled in Belgium.

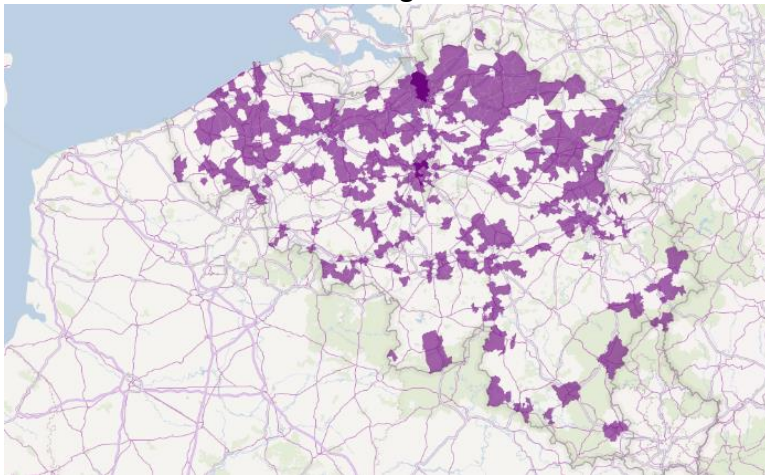


Figure 1: Geographical location of identified gonococcal isolates.

Three quarters of the isolates were found among men (513/675; 76.0%), 23.9% (161/675) among women and one *N. gonorrhoeae* strain was isolated from a transwoman. **Figure 2** shows the number of isolates received per semester from 2013 to 2022 stratified by sexual transmission. The number of female samples increased slightly from 20.8% to 23.7% in 2022.

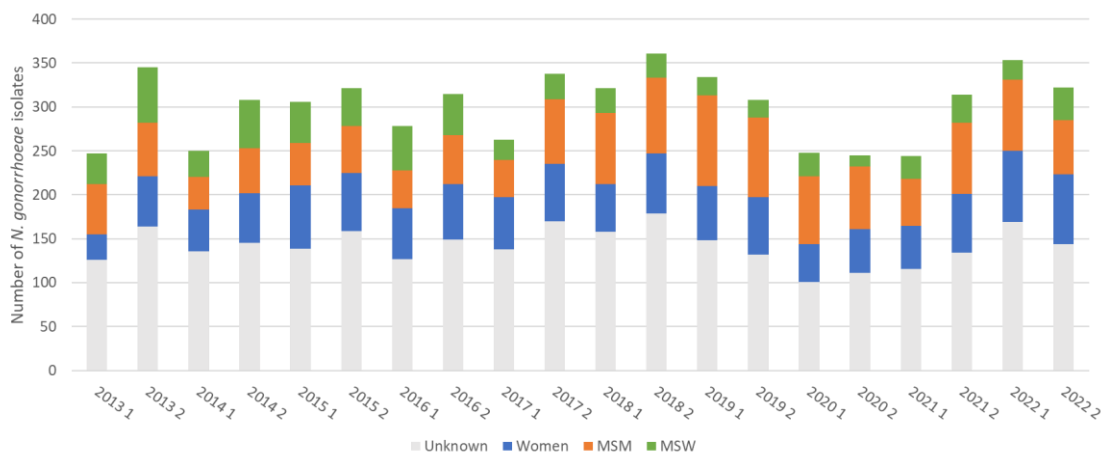


Figure 2: Percentage of *N. gonorrhoeae* isolates received by the NRC-STI from 2013 to 2022 per semester stratified by sexual transmission. MSM: Men who have sex with men and includes all anorectal male samples. MSW: Men who have sex with women. Unknown: Unknown gender or men with unknown sex of sex partners

The infection site of the *N. gonorrhoeae* isolates for 2022 is shown in **Table 1**. Most isolates originated from the urogenital anatomical sites followed by the anorectum.

Table 1: Infection site of the *N. gonorrhoeae* isolates of 2022

Biological origin	Female (n=160)		Male (n=514)		Other (n=1)		Total (n=675)	
	N	%	N	%	N	%	N	%
Urogenital	153	95.6	447	87.0	1	100	600	88.9
Anorectal	1	0.6	48	9.3	0	0.0	49	7.3
Throat	1	0.6	3	0.6	0	0.0	4	0.6
Pooled Sample (urine, anorectal & throat)	0	0.0	7	1.4	0	0.0	7	1.0
Other*	4	2.5	3	0.6	0	0.0	7	1.0
Unknown	1	0.6	7	1.4	0	0.0	8	1.2

*Other includes the following infections sites: ascites fluid (2), eye (4), and joint fluid (1)

3.2 Antimicrobial resistance of *N. gonorrhoeae*

Table 2 lists the MICs of the *N. gonorrhoeae* isolates of 2022 for the following antibiotics: azithromycin, ciprofloxacin, ceftriaxone, cefixime, penicillin, tetracycline and spectinomycin.

Table 2: Minimal Inhibitory Concentration (MIC) and final interpretation of the *N. gonorrhoeae* isolates 2022

	S		MIC limit value	I		MIC limit value	R		MIC limit value
	N	%	mg/L	N	%	mg/L	N	%	mg/L
Azithromycin (n=675)	448	66.4	≤ 1	-	-	-	227	33.6	> 1
Ciprofloxacin (n=675)	295	43.7	≤ 0.032	-	-	0.047- 0.064	380	56.3	> 0.064
Ceftriaxone (n=675)	675	100	≤ 0.125	-	-	-	0	0.0	> 0.125
Cefixime (n=669)	665	99.4	≤ 0.125	-	-	-	4	0.6	> 0.125
Penicillin (n=669)	28	4.2	≤ 0.06	542	81.0	0.125-1	99	14.8	> 1
Tetracycline (n=669)	153	22.9	≤ 0.5	263	39.3	1	253	37.8	> 1
Spectinomycin (n=669)	669	100	≤ 64	-	-	-	0	0.0	> 64

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

In 2022, no ceftriaxone resistant isolate was detected. Only one genital isolate with high-level resistance to azithromycin (>256 mg/L) was found in a woman with urethritis. No other information is known. The isolate was resistant to ciprofloxacin (0.19 mg/L) but sensitive to ceftriaxone.

Figures 3 to 5 show the antimicrobial resistance of *N. gonorrhoeae* to the different antibiotics over the years. Resistance to spectinomycin is not yet detected in Belgium. Resistance to ceftriaxone has been detected in 13 cases since 2013.

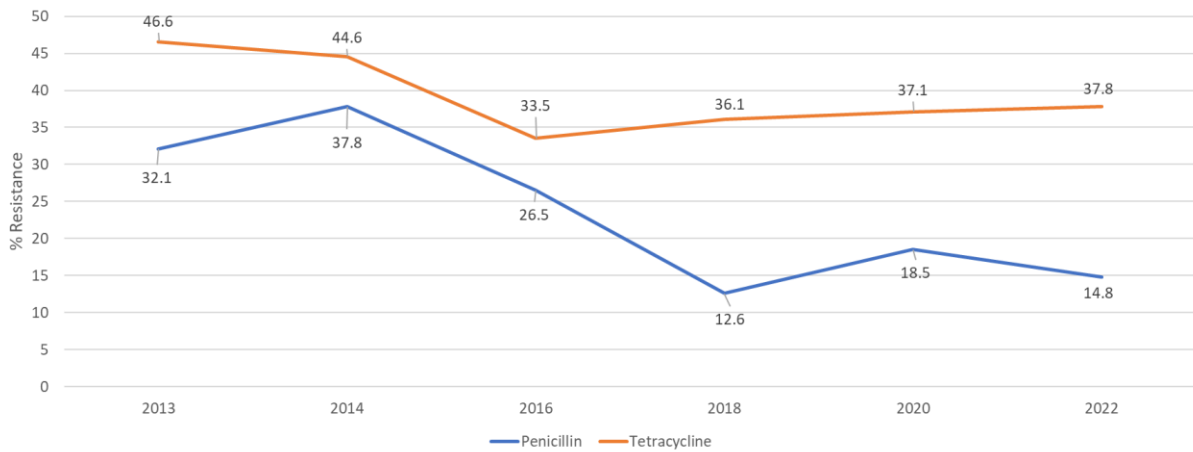


Figure 3: Antimicrobial resistance (%) of *Neisseria gonorrhoeae* to penicillin and tetracycline from 2013 to 2022 in Belgium.

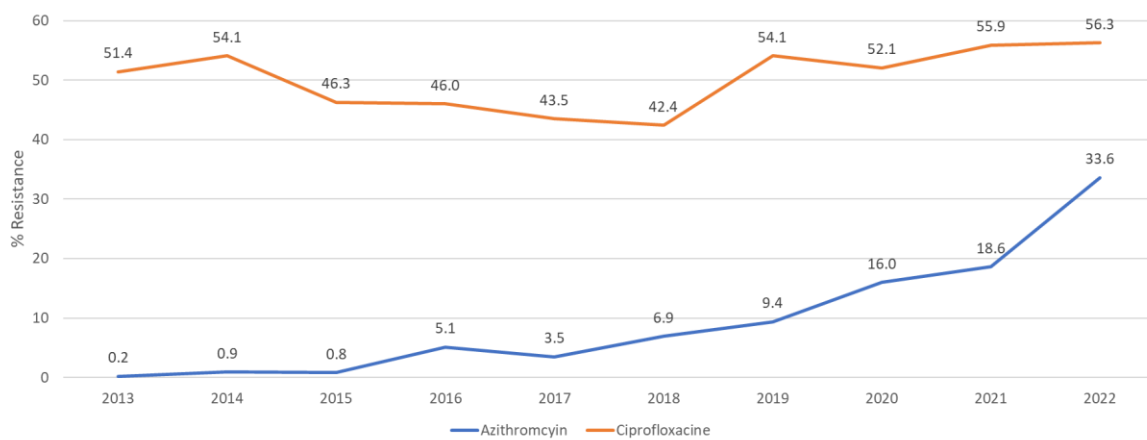


Figure 4: Antimicrobial resistance (%) of *Neisseria gonorrhoeae* to azithromycin and ciprofloxacin from 2013 to 2022 in Belgium.

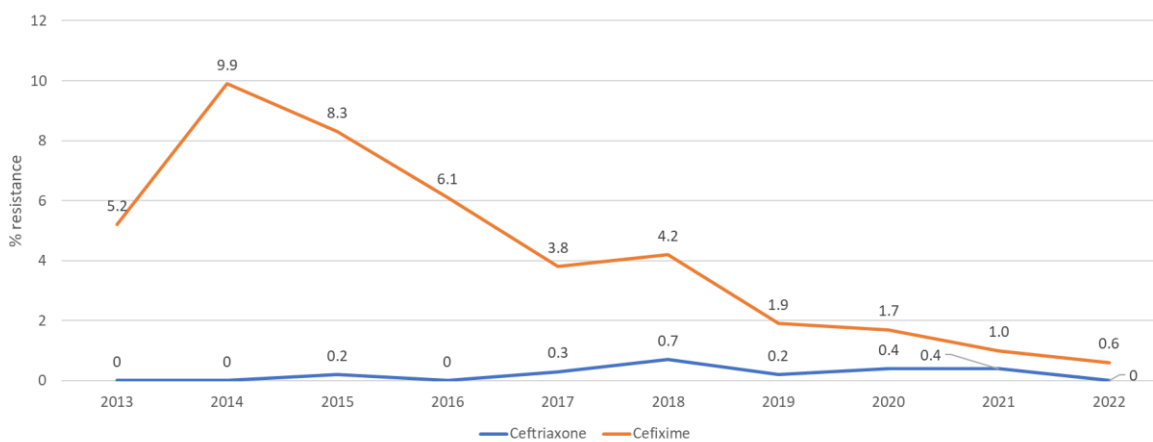


Figure 5: Antimicrobial resistance (%) of *Neisseria gonorrhoeae* to extended spectrum cephalosporines cefixime and ceftriaxone from 2013 to 2022 in Belgium.

Resistance of *N. gonorrhoeae* to ciprofloxacin is above 50% and remains stable over time. More than one in three (34%) of the isolates is resistant to azithromycin. Stratifying per sexual transmission, the increase in resistance to azithromycin is found to be the highest among MSM however the steady increase of azithromycin resistance we observed among MSM, is now also

spreading to other populations, albeit with delays. Almost one in four of the female isolates is also resistant to azithromycin in 2022 (Figure 6). Figure 7 provides the distribution of the MICs for azithromycin stratified per sexual transmission and shows clearly that isolates with very low MICs are replaced by isolates with higher MICs in all populations, mostly pronounced in MSM.

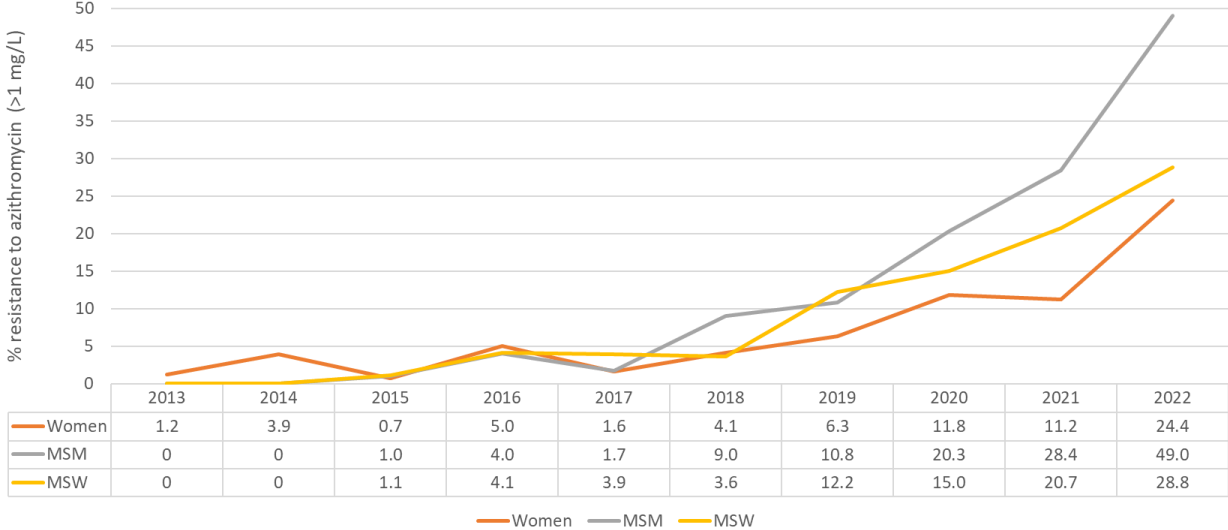


Figure 6: Resistance to azithromycin of *N. gonorrhoeae* stratified by gender and sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

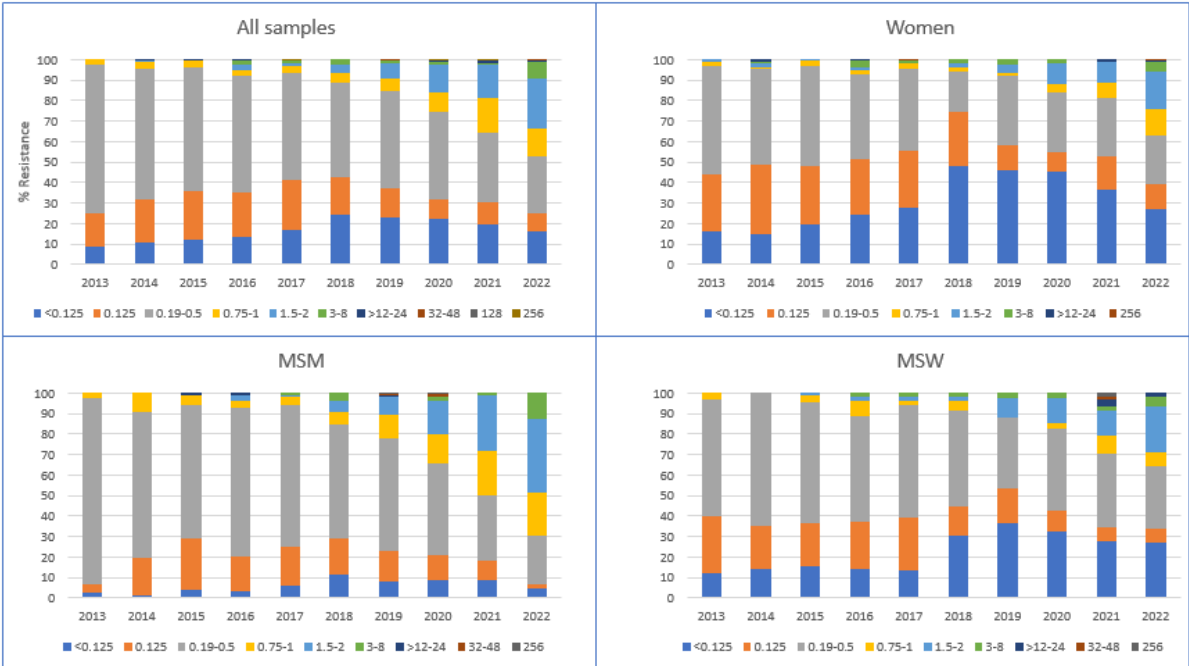


Figure 7: Minimal inhibitory concentration (MIC) distribution (mg/L) for azithromycin over the years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

In the case of ciprofloxacin, resistance was also lower among women compared to male samples but as seen in Figure 8, ciprofloxacin resistance fluctuates within populations around 40-50%. The MIC distribution over the years for antimicrobials ciprofloxacin and ceftriaxone can be found in Annex 2 and 3.

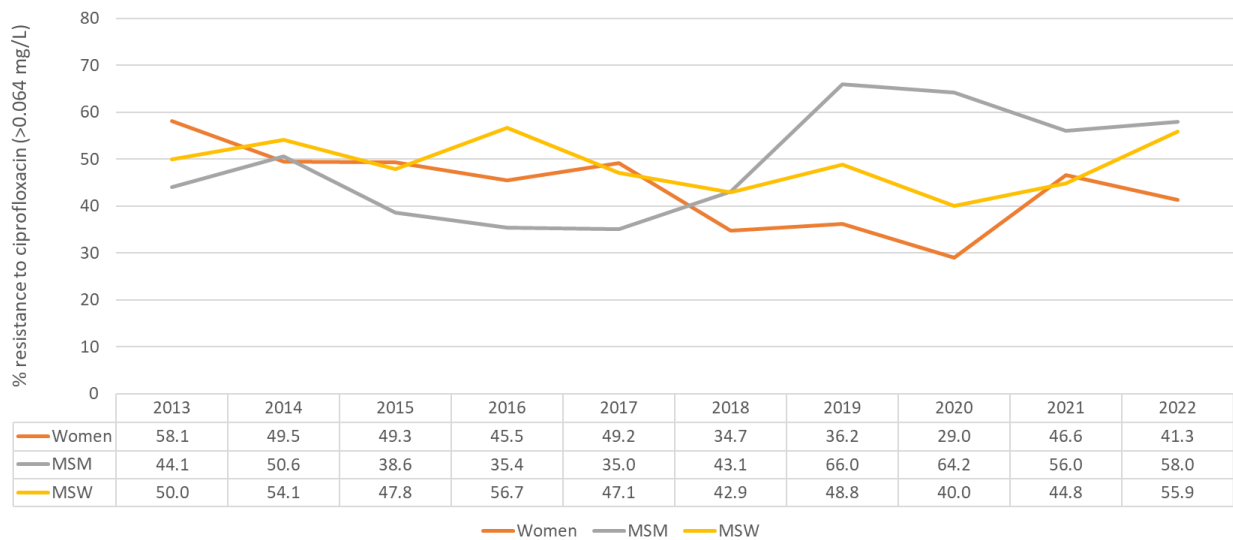


Figure 8: Resistance to ciprofloxacin of *N. gonorrhoeae* stratified by gender and sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

There is no breakpoint defined for gentamicin in EUCAST. The MIC distribution of gentamicin over the years can be found in Annex 4. A shift to higher MIC over years is also noted, however, the proportion of samples with MIC $\geq 16 \mu\text{g/mL}$ remained stable compared with the previous tested year (2019 - 26.8% vs 2022 - 24.8%). Gentamicin is currently not used as treatment in Belgium.

4. Discussion

Whilst the antimicrobial resistance of *N. gonorrhoeae* to ciprofloxacin remains stable over time, we report that one in three of the isolates is resistant to azithromycin. Although the increase was first noted among MSM, we now document an increase in all other populations (MSW and women) and almost one in four female samples were found to be resistant to azithromycin. However, the resistance is mainly caused by isolates with low-level resistance (MIC: 1-8 mg/L) and it remains unclear if these isolates presented with clinical resistance. The shift to higher azithromycin MICs has also been noted by our neighbouring countries such as the Netherlands (personal communication, data unpublished). A Belgian genomic surveillance study has been set-up to explain the noticeable increase in gonococcal azithromycin resistance in all populations and results will be published in 2023.

Since last year, the Belgian guidelines to treat *N. gonorrhoeae* altered due to the upsurge of azithromycin resistance we reported last year and now recommend to treat uncomplicated gonorrhoea infection with ceftriaxone 1g intramuscular (BAPCOC 2022 - <https://www.bcfi.be/nl/chapters/12?frag=8000010>).

In snapshot years, resistance to other antibiotics such as penicillin, spectinomycin and tetracycline is measured. Doxycycline, a second-generation tetracycline, is frequently used to treat other STIs such as *Chlamydia trachomatis* and the usage of doxycycline to prevent STIs (doxy-PEP) including *N. gonorrhoeae* is currently investigated internationally.^{4,5} Here, we show that 38% of the isolates were resistant to tetracycline. In 2023, new breakpoints (EUCAST guidelines V13.0) for tetracycline will be introduced (Resistant if MIC > 0.5 mg/L) which would imply that 62.2% of the isolates are resistant to tetracycline. Further research is therefore

needed to investigate what the impact of the usage of doxy-PEP could be on *N. gonorrhoeae* resistance patterns.

Luckily, resistance to ceftriaxone remains rare in European countries, as in Belgium. In 2022, no ceftriaxone resistant *N. gonorrhoeae* has been identified. Continued national surveillance of antimicrobial resistance of *N. gonorrhoeae* and other STIs is imperative.

5. Acknowledgements

We would like to thank Chris Kenyon, Amaryl Lecompte and Wim Vanden Berghe for their valuable input. Moreover, we would like to thank Tania Crucitti who was the former head of the NRC-STI until 2018.

6. References

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7. Annexes

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

Laboratories	Number of isolates sent
11601 AZ Klina Brasschaat	1
11603 UZ Antwerpen Edegem	1
11642 AZ Monica Middelaes Deurne	1
11645 AZ Monica Eeuwfeestkliniek Antwerpen	1
11653 AML Antwerpen	29
11677 St Augustinus / St Vincentius / St Jozef Wilrijk	15
11704 ITG Antwerpen	113
12616 Somedi Heist-Op-Den-Berg	3
12647 AKL Lier	3
13602 Heilig Hartziekenhuis Mol	1
13608 AZ St. Dimpna Geel	1
13618 AZ St Jozef Turnhout	1
13656 CMA Herentals	112
23604 AZ Jan Portaels Vilvoorde	1
24650 MCH Leuven	10
25611 Clin St Pierre Ottignies	1
26631 CHU Brugmann Brussel	5
26677 Labo Porte de Hal CHU St Pierre Brussel	9
26726 Cebiodi St Jean Brussel	16
27647 Clin. De l'Europe Site Europe St. Michel Brussel	2
27729 ULB Inst Clin Biol Brussel	1
28604 Europa ziekenhuis St Elisabeth Brussel	1
28611 Chirec Nouvelle Clin. De la Basilique Lab Biol Clin Brussel	1
28612 Chirec Edith Cavell Brussel	4
28615 ULB Hop Erasme CUB Brussel	2
29636 UZ Brussel	12
31639 D. van Waes Medisch Lab St Andries Brugge	2
33606 Jan Yperman ZH Ieper	18
34605 Labo van Poucke Kortrijk	1
35603 AZ Damiaan St Jozef Oostende	1
36609 AZ Delta Roeselare-Menen	6
36654 Klin. Labo Termote - Declerck Ardoois	17
41658 OLV ZH Aalst	4
42609 Medina Dendermonde	63
43609 Labo Gabriel Peter Eeklo	1
44610 Labo Nuytinck Anacura Evergem	1
44624 Medilab Bvba Labo Gent	2
44633 AZ Maria Middelaes St. Jozef Gentbrugge	1
44644 AZ Jan Palfijn Site Watersportbaan Klin. Labo Gent	2
44673 AZ Maria Middelaes Gent	5
44692 Medisch Labo Medina Aalter	2
44696 AZ St Lucas Gent	19

Laboratories	Number of isolates sent
44700 UZ Gent	8
44711 Bvba Cri Zwijnaarde	7
46615 AZ Nikolaas Campus SM Labo Klin Biol St Niklaas	7
52610 SYNLAB Heppignies	19
52626 CHU Charleroi	8
53605 RHMS Epicura Baudour	1
53611 CHR Site de Boussu Labo Biol Clin Boussu	1
53639 CH Hornu	8
55604 CHU Tivoli La Louviere	2
55614 CHR de la Haute Senne Site CH Soignies	3
56603 Centre de Sante des Fagnes Chimay	2
57606 AI du Tournaisis ASBL AIT Labo de Biol Clin Tournai	6
61602 CHR Huy	3
62668 CHC MontLégia Liège	44
62700 CHU Sart Tilman Liege	3
62730 CH Regional de la Citadelle Labo Liege	5
62960 CHU Sart Tilman Liege	1
63603 Labo dr. J. Collard SPRL Verviers	1
71604 LKO-LMC St Truiden	15
71606 C.A.Z. Midden Limburg Campus Salvator Labo Hasselt	1
71615 Virga Jesse ZH Hasselt	4
71622 ZH Oost-Limburg St Jan Genk	9
71725 Klinisch Labo Rigo Genk	1
81602 Clin. Sud Luxembourg Site St Joseph Aarlon	2
84609 Centre Hosp de l'Ardenne Libramont	5
91605 CHU de Mont-Godinne Yvoir	3
92611 CHR Namur	5
92618 CHR Val de Sambre Auvelais	1
92629 Clin Saint Luc Bouge	15
Grand Total	682

7.2 MIC distribution for ciprofloxacin over the years stratified per sexual transmission

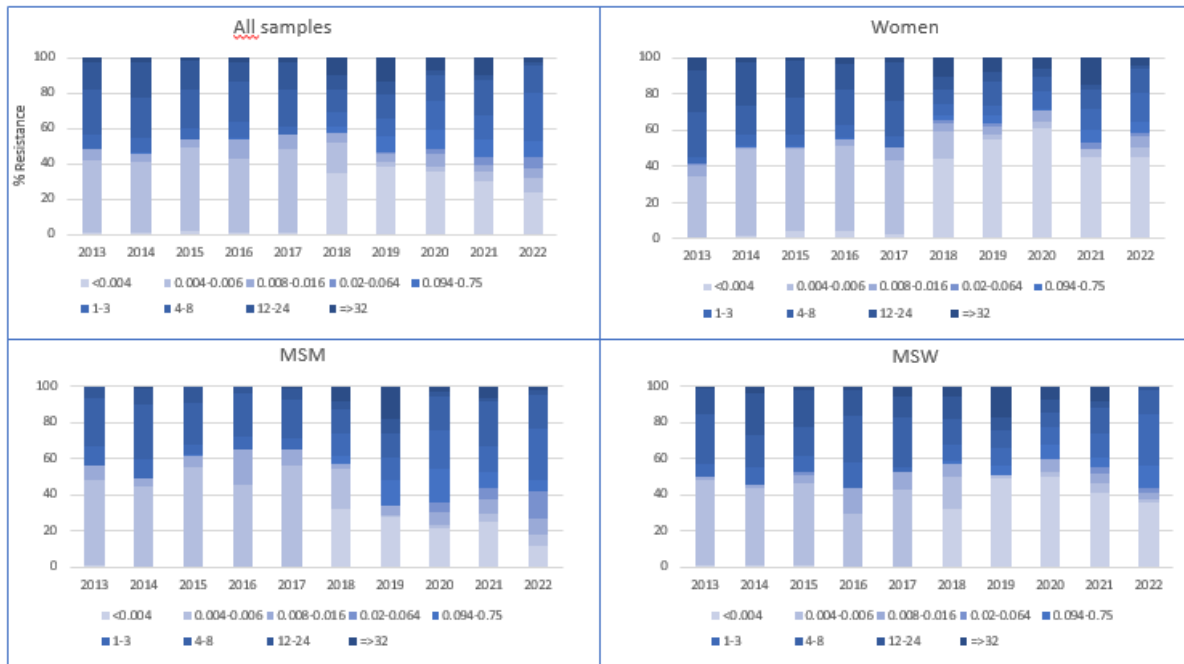


Figure 9: Minimal inhibitory concentration (MIC) distribution (mg/L) for ciprofloxacin over the years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

7.3 MIC distribution for ceftriaxone over the years stratified per sexual transmission

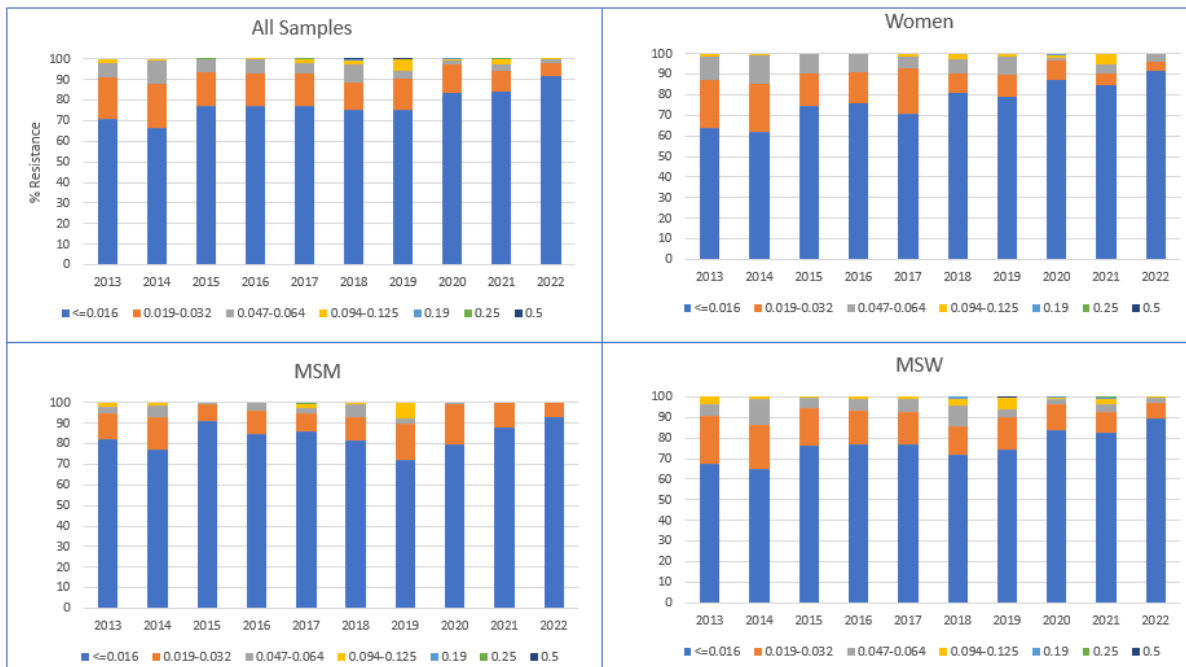


Figure 10: Minimal inhibitory concentration (MIC) distribution (mg/L) for ceftriaxone over the years for all *N. gonorrhoeae* isolates and stratified per sexual transmission. MSM: Men who have sex with men. MSW: Men who have sex with women

7.4 MIC distribution for gentamicin over the years

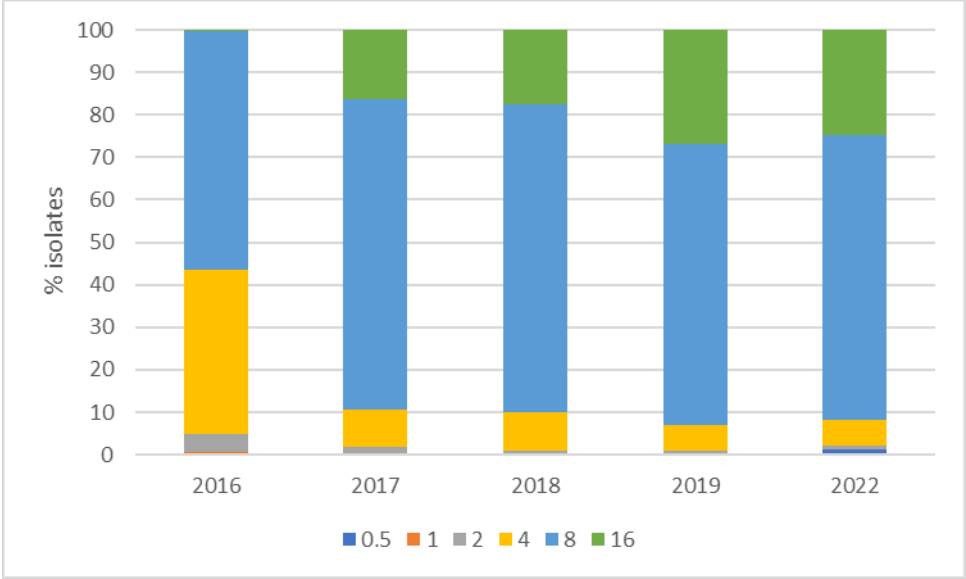


Figure 11: Minimal inhibitory concentration (MIC) distribution (mg/L) for gentamicin over the years for all *N. gonorrhoeae* isolates. Only snapshot years are now tested (every three years)