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Annual Report of the Swiss National Reference Center for Meningococci, 2018

Address

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1. Introduction

Invasive strains of *Neisseria meningitidis* are a life-threatening cause of bacterial sepsis and meningitis, mainly in infants, adolescents and young adults. They can cause outbreaks and therefore require a continuous surveillance, especially nowadays with the spread of a hypervirulent serogroup W clone in Europe (Knol et al., 2017; Ladhani et al., 2015). Also, sporadic cases may occur in any age group and every effort must be undertaken to optimize the prevention, diagnosis and treatment of such infections.

In Switzerland, invasive meningococcal diseases have to be reported to the Swiss Federal Office of Public Health (SFOPH), and corresponding isolates should be referred to the Swiss National Reference Center for Meningococci (CNM, Centre National des Méningocoques; http://www.meningo.ch) at the University Hospital in Geneva.
The CNM provides reference testing of invasive *N. meningitidis* isolates in collaboration with the SFOPH, and currently employs serotyping and molecular typing following protocols recommended by the European Meningococcal Disease Society (EMGM) (http://emgm.eu). Based on a combination of serogroup and molecular typing data, each strain is classified and data are integrated into national (SFOPH) and international epidemiological databases (European Meningococcal Epidemiology in Real Time [EMERT] database; http://emgm.eu/emert) in order to monitor and share information about trends in meningococcal populations. This methodology is evolving towards Next Generation Sequencing (NGS) (Mustapha et al., 2016), a method that we used for a selection of cases collected between 2010 and 2016, to determine the clonality of the meningococcal strains of serogroup W finetype (PorA 5,2:FetA 1-1:ST-11). This was executed as a separate subproject supported by the SFOPH (Decision 16.928412). This annual report describes the methods used and results obtained at the CNM in the calendar year 2018.

2. Materials and Methods

The CNM is investigating invasive isolates of *N. meningitidis* as well as native clinical specimens derived from normally sterile body sites.

Isolates are sub-cultured overnight on chocolate agar plates. Confirmation of identification is performed by PCR using the *N. meningitidis*-specific targets *ctrA* (Corless et al., 2001), *sodC* (Dolan Thomas et al., 2011), *tauE, metA*, and *shlA* (Diene et al., 2016).

Serogroups are determined by PCR as well as by commercial agglutination kits: A, B and C (Pastorex Meningitis, Bio-Rad) and W135, X, Y, Z and Z' (Difco *Neisseria Meningitidis* Antisera, Becton Dickinson).
Sequence analysis is performed on each isolate in two variable regions of the gene encoding the antigenic outer membrane protein porin A (*porA*-VR1 and *porA*-VR2) and in one variable region of the *fetA* gene (*fetA*-VR) encoding another outer membrane protein exhibiting sequence data which can be useful for tracing clones emerging or circulating in local populations (World Health Organization Manual – Laboratory Methods for the Diagnosis of Meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [2nd edition]; http://pubmlst.org/neisseria/.

In addition, multilocus sequence typing (MLST) is performed on each isolate according to protocols recommended by the EMGM ((Harrison et al., 2011); http://emgm.eu). This approach is targeting variable regions of seven house-keeping genes (*abcZ*, encoding a putative ABC transporter; *adk*, adenylate kinase; *aroE*, shikimate dehydrogenase; *fumC*, fumurate dehydrogenase; *gdh*, glucose-6-phosphate dehydrogenase; *pdhC*, pyruvate dehydrogenase subunit, and *pgm*, phophoglucomutase). Each isolate is classified according to its multilocus genotype designated as a sequence type (ST), which is the combination of its alleles over the seven genetic loci tested. STs can be further grouped into clonal complexes (CC), which are defined in the *Neisseria* MLST profile database as groups of STs that share at least four of the seven loci in common with a central ST (http://pubmlst.org/neisseria/).

Isolates are then classified based on a combination of serotyping and molecular typing data according to the following scheme:
Serogroup : *porA*-VR1, *porA*-VR2 : *fetA*-VR : MLST (ST or CC)

Isolates are also tested for antimicrobial susceptibility on Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F, bioMérieux) using E-test strips (AB Biodisk, bioMérieux) containing penic illin, ceftriaxone, meropenem, ciprofloxacin, minocycline and rifampicin. Minimum inhibitory concentrations (MICs) are interpreted according to current breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org).

Native clinical specimens are investigated using PCR to screen for *N. meningitidis* DNA, and if present, to assess the occurrence of the main serogroups by amplifying corresponding genetic targets. Nucleic acid extraction from clinical specimens such as cerebrospinal fluid and EDTA blood is performed using the MagPurix 12 Nucleic Acid Extraction System (Zinexts Life science; Taiwan). DNA is amplified by real-time PCR to screen for the presence of the *N. meningitidis*-specific targets described above. PCR assays targeting the polysialyltransferase (*siaD*) gene are employed to assign *N. meningitidis*-positive specimens to serogroups B, C and Y/W135; assignment to serogroup A is achieved by PCR targeting the *sacC* gene (Mölling et al., 2002). Finally, differentiation between serogroups Y and W135 is done by amplification of the *synF* gene (Y) and *synG* gene (W135) (Fraisier et al., 2009).

3. Strain collection

The CNM stores all the received invasive meningococcal isolates at -80°C. The collection currently includes more than 500 isolates (between 2009 and 2018). Previous strains were also stored but their recovery by culture is not fully guaranteed (n=1'914 isolates between 1989 and 2009).

4. National and International quality assurance

There is currently no international quality assurance pertaining to meningococci. We are actively scouting whether this service would become available.

5. Epidemiological research

The precision of NGS permitted us to identify several independent monoclonal outbreaks related to *N. meningitidis* W135 that occurred between 2010 and 2016 in Switzerland. Our meta-analyses included samples from other previously published works and allowed establishing connections between Swiss MenWs and other European outbreaks (paper in preparation). This project was made possible through a grant from SFOPH (Decision 16.928412).

The manuscript (available upon request) will be submitted to Eurosurveillance (Leo et al., in preparation).

6. Additional meningococcal research

The molecular expertise developed in the framework of the CNM permitted us to decipher a very intriguing case of left-heart failure due to acute aortic valve endocarditis. The combination of qPCR assays for molecular detection and typing, as well as the use of NGS on the valve material permitted to assess the diagnosis of an acute meningococcal endocarditis, due to a serotype B strain, despite the impossibility to cultivate the organism. This exceedingly rare diagnosis, likely underestimated due to rapid pre-emptive therapy, underlines the need to obtain an etiological diagnosis. This work (available upon request) is currently submitted for publication (Choutko et al., under review).

This work was also presented (during lectures) at the following meetings:

07. **J. SCHRENZEL**: Nouveautés et applications diagnostiques en maladies infectieuses. Chair and invited speaker of the SILAMED meeting. Lausanne, November 2018.

06. **J. SCHRENZEL**: Clinical metagenomics. Invited speaker to the ESCMID Postgraduate Technical Workshop Capacity-Building Workshop: Metagenomics in the Diagnostic Laboratory. Groningen, The Netherlands, October 2018.

05. **J. SCHRENZEL**: Clinical metagenomics: beyond clinical microbiology. Invited speaker to the ESGMD Postgraduate Technical Workshop, Lausanne, September 2018.

04. **J. SCHRENZEL:** Microbiome. Invited speaker to 4th Summer School of the European Society of Pharmacogenomics and Personalised Therapy (ESPT), Geneva. September 2018.

03. **J. SCHRENZEL**: Clinical metagenomics: what's new? Invited speaker to the annual meeting of the Swiss Society for Microbiology, Lausanne, August 2018.

02. **J. SCHRENZEL**: Whole-genome sequencing in routine microbiology? Invited speaker to the Personalized Health Technologies and Translational Research Conference 2018. ETH Zürich, June 2018.

01. **J. SCHRENZEL**: Metagenomics for clinical care - ready for prime time? Invited speaker to the 3rd American Society for Microbiology (ASM) Microbe meeting. Atlanta, USA, June 2018.

Finally, a publication was issued last year, to inform clinicians about epidemiological changes in invasive meningococcal infections and their consequences (Emonet et al., 2018).

7. Advisory service and Networking

7.1 Advisory service

Molecular testing: We systematically conduct molecular assays to determine the serotype directly from clinical invasive *N. meningitidis* specimens (or suspicion thereof). As mentioned above, it is likely that the true incidence of invasive *N. meningitidis* infection is missed by rapid empiric therapy (precluding successful cultivation), nor to mention the new clinical presentations related to W135 such as pneumoniae (typically undetected and not referred to the CNM unless presenting with a bacteraemia and thus fulfilling the current definition of invasive infection). Our current molecular approach covers the most frequent serotypes and a result can usually be communicated to the clinicians.

7.2 Networking

We have established contact with the Italian reference center for meningococci to analyse further our peculiar W135 epidemics, in conjunction with their national epidemiology.

7.3 Website

The dedicated website (www.meningo.ch) was fully rebuilt in 2018, and is available in French, German, Italian and English.

8. Results

During the calendar year 2018, the CNM has received a total of 57 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (n=48) and cerebrospinal fluids (n=8) as well as from a kidney, during autopsy (n=1).

The strains received were isolated from 25 female and 32 male patients and represented 90% (57/63) of all cases of invasive meningococcal diseases reported to Swiss public health authorities in 2018 (SFOPH; Figure 1).

There was no real pattern of serogroup (based on agglutination or PCR) associated to the patient's age, except for seniors that were mostly affected by serogroup W (40% - 23/57) and serogroup Y (21% - 12/57) (Figure 2). In contrast, babies and children were mostly affected by serogroup B (26% - 15/57) and serogroup C (11% - 6/57) (Figure 3). Among the 57 strains sent to CNM, one strain was nontypeable (2% - 1/57).

All main serogroups (B, C, Y and W) were recovered in all regions of Switzerland except for the Italian speaking area where only serogroups Y and C were detected. Serogroup B and W were essentially found in the German and French speaking part of Switzerland, respectively. Figure 4 shows the geographical distribution of the serogroups.

Molecular characterization using MLST revealed that ST-11 was the most prevalent sequence type present in Switzerland in 2018, with 91% of the serogroup W strains and 83% of serogroup C strains harbouring a sequence type 11 profile. ST-23 is the second most frequent and represents almost half of serogroup Y strains (Table 1). When looking in more details, all serogroup W strains were also of the same finetype (PorA 5,2:FetA 1-1:ST-11) inside the ST-11.

Applying EUCAST breakpoints, all invasive *N. meningitidis* strains tested were found to be susceptible to ceftriaxone, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 63% of these isolates were considered fully susceptible to penicillin (13 points more susceptible, as compared to 2017)(Table 2). Penicillin non-susceptible strains were not associated to a specific serogroup.

Summary of key observations

- Serogroup W was the most frequently determined in invasive strains of meningococcus (40%), followed by serogroup B (26%) and serogroup Y (21%). The remaining cases were associated with serogroup C (11%) and nontypeable strain (2%).
- Predominant MLST profiles were ST-11 and ST-23.
- All but 2 of our serogroup W strains were of the exact same finetype: PorA 5, 2:FetA 1-1:ST-11, suggesting the possibility of a clonal distribution. This observation warrants further NGS-based investigation, as suggested by our 2010-2016 analysis. The objective would be to assess whether the increasing incidence of such strains results from an ongoing monoclonal outbreak (analysis is ongoing).
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%. However, susceptibility to penicillin, according to EUCAST breakpoints, was only 63%.

9. Discussion

In 2018, a total of 63 cases of invasive meningococcal diseases were reported to the SFOPH. According the the SFOPH, the incidence is higher as compared to 2017 (0.74 in 2018 and 0.65 in 2017 for 100'000 inhabitants) and also slightly increasing since 2014. However, average incidence for the last 10 years is 0.65 with a standard deviation of 0.15, suggesting that the incidence has remained stable over the last decade. The main change in meningococcal epidemiology in Switzerland is the development of serogroup W135 (40%) hypervirulent strain that is mostly of clonal origin, some of them linked with the strain described in the UK (Ladhani et al., 2015) and spreading in other European countries as described in the Netherlands by Knol and colleagues in 2017 (Knol et al., 2017). Importantly, no such expansion has been observed for any other meningococcal subpopulation since the emergence of serougroup C between 1994-1996 (Gray et al., 2006).

This particular strain of meningococcus (W135) is associated with unusual clinical pictures (especially pneumonia, more often bacteriemic or with purpura fulminans), and an unusual target population (more often seen in patients over 50 years old). Therefore, Swiss recommendations for vaccination against meningococcal disease have been and will be further adapted, with the use of the quadrivalent MenACWY (capsular antigen) conjugate vaccine (See SFOPH website for last updated recommendations).

Finally, our surveillance of antimicrobial susceptibilities of *N. meningitidis* strains involved in invasive diseases in Switzerland speaks against the use of penicillin as first line empirical treatment of meningococcal disease. Ceftriaxone remains the drug of choice in these situations.

10. Acknowledgements

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Figures

Figure 1. Annual number of cases of invasive meningococcal diseases reported to the Swiss Federal Office of Public Health (SFOPH) and number of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci (SNRCM) from 2009 to 2018.

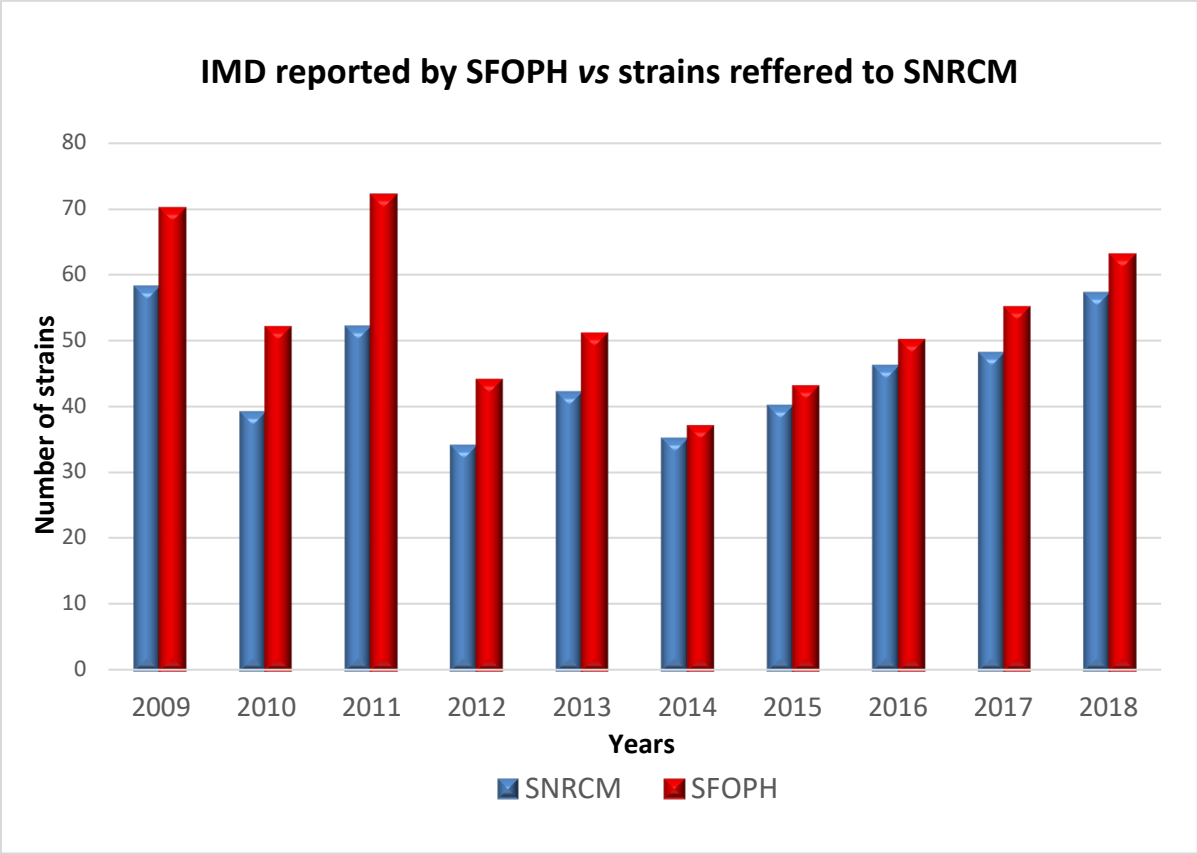

Figure 2. Number of isolates in 2018, by serogroups and age category.

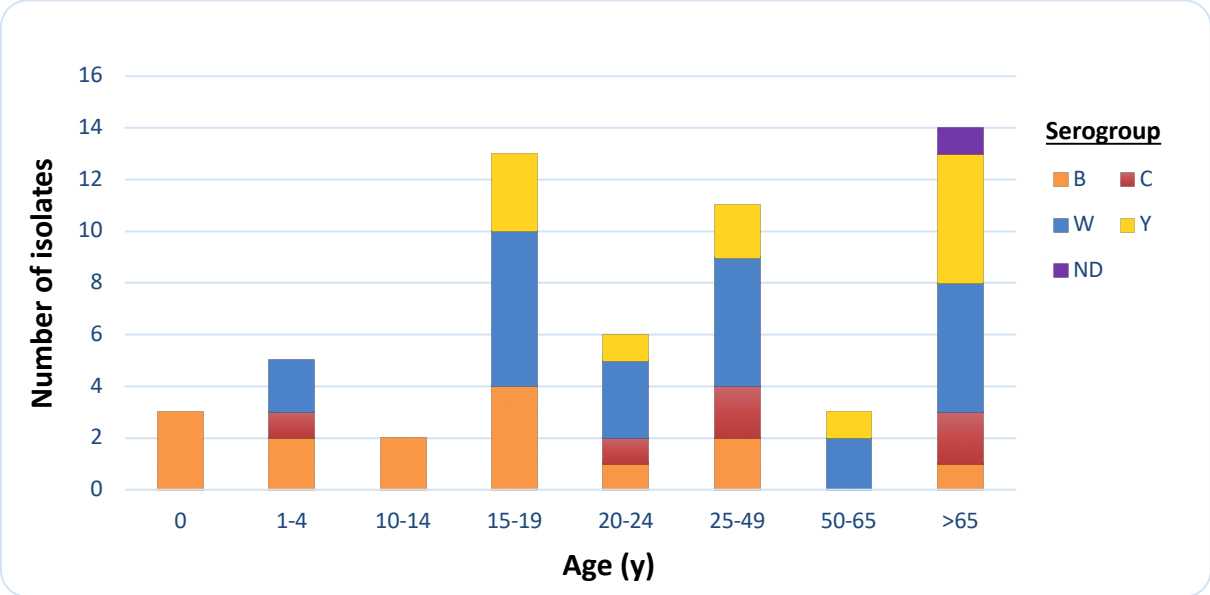

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Figure 3. Annual number of strains representing main serogroups B, C, X, Y and W135 of invasive *N. meningitidis* as determined at the Swiss National Reference Center for Meningococci from 2009 to 2018

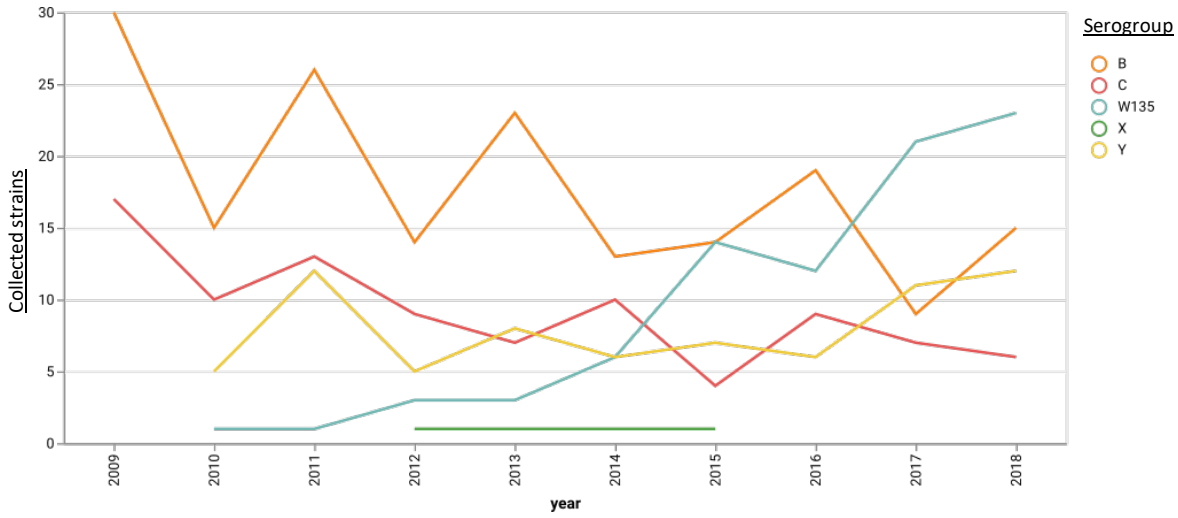
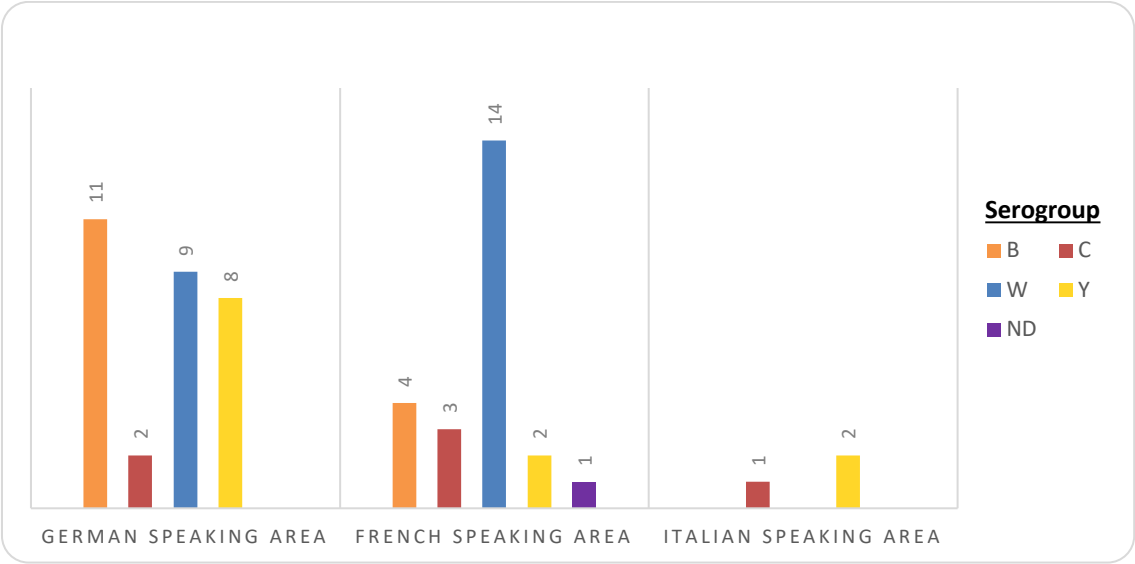


Figure 4. Distribution of serogroups by geographical regions in 2018



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Tables

Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2018.

Serogroups[a]	Sequence type (MLST)
B	13 different ST + 2 ND
C	5 **ST11** (83%) + 1 ST5752
W135	21 **ST11** (91%) + 2 ST9316
Y	5 **ST23** (42%) + 3 ST1655 (25%) + 3 other ST + 1 ND

[a] One strain could not be assigned to a serogroup

Table 2. Antimicrobial susceptibility testing of 57 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2018.

	Minimum inhibitory concentration (MIC)[a]			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	MIC50	MIC90		
Penicillin	0.0032-0.75	0.064	0.38	0.06	62.5
Ceftriaxone	<0.002-0.004	0.002	0.002	0.125	100
Meropenem	0.003-0.047	0.008	0.047	0.25	100
Ciprofloxacin	0.002-0.008	0.004	0.006	0.03	100
Minocycline	0.003-0.38	0.19	0.38	1	100
Rifampicin	0.002-0.125	0.012	0.094	0.25	100

[a] One strain could not be tested for antibiotic susceptibility