

Mehrwert von *Next Generation Sequencing* zur molekularen Charakterisierung von Meningokokken

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und Haemophilus influenzae



Infektiologie Update 2014

24. Jahrestagung der PEG, Weimar

European consensus: antigen sequence typing and MLST nomenclature

B:P1.19,15:F5-1:ST-33(cc32)

serogroup

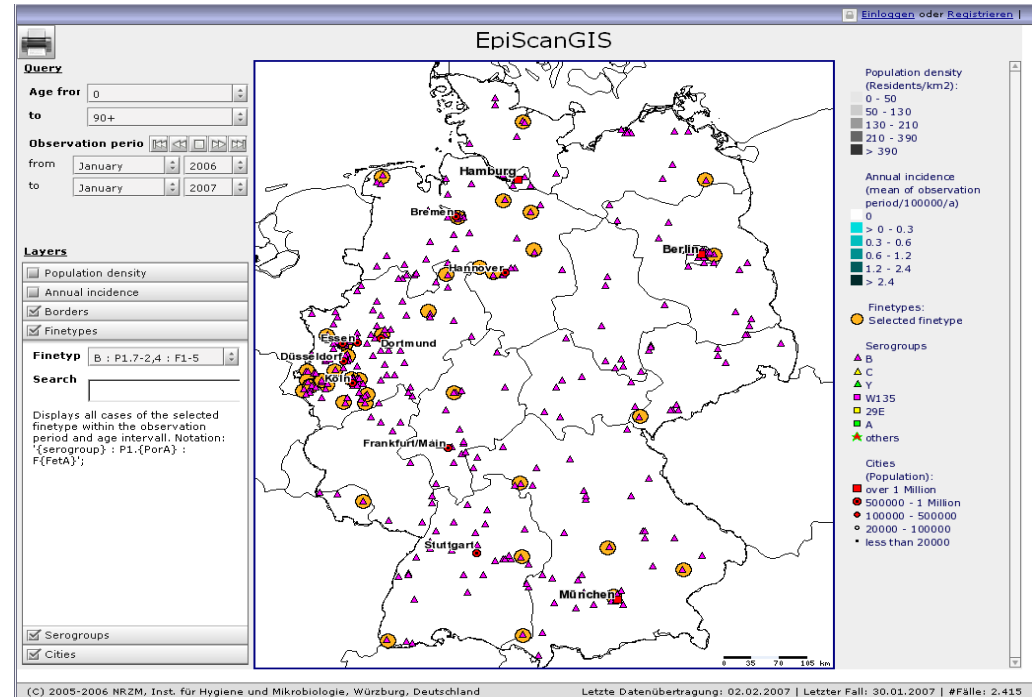
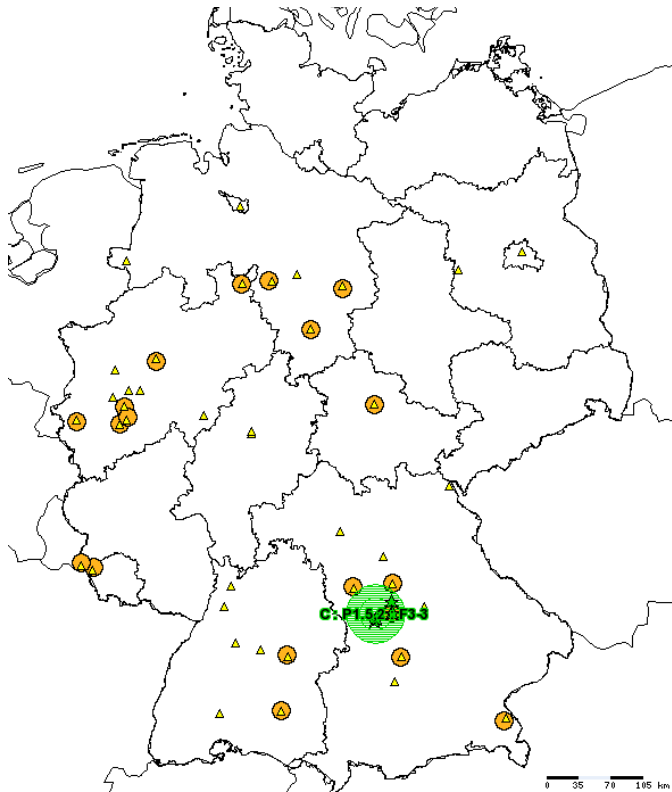
PorA VR1 and 2

FetA VR

Sequence type

Clonal complex

Use of genetic typing at the NRZMHi



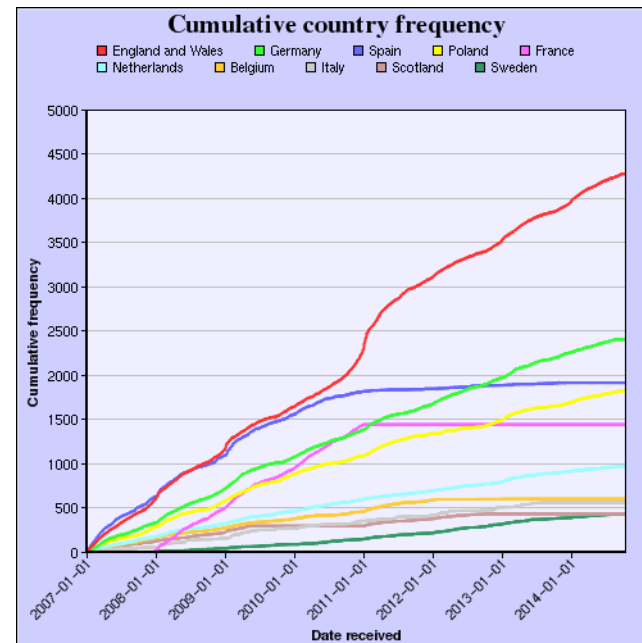
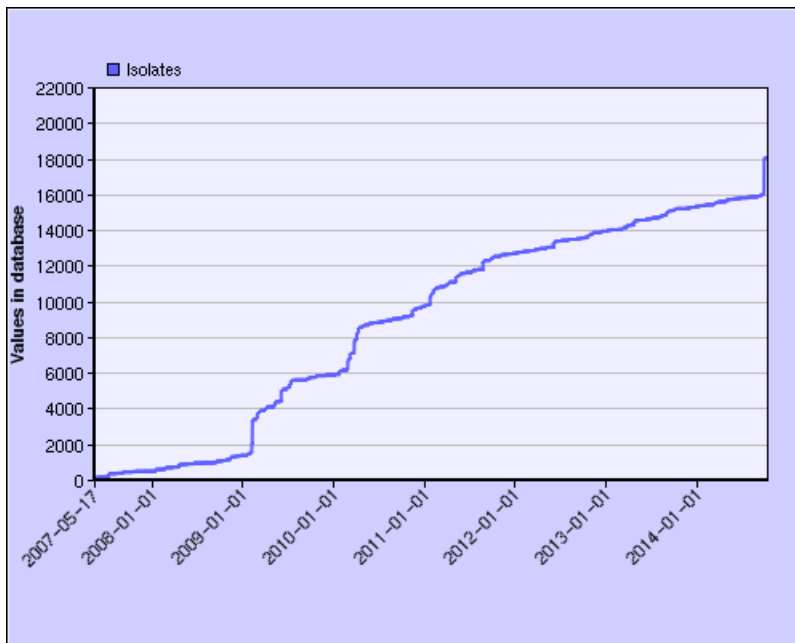
Elias et al. EID 2006
Reinhardt et al. IJHG 2008

ECDC IBD Labnet



- Coordination: M. Frosch
- EQA
- European Meningococcal strain collection
- Agreement on TESSy coding definitions

European Meningococcal Disease Society (EMGM): EMERT database



Multicenter Study for Defining the Breakpoint for Rifampin Resistance in *Neisseria meningitidis* by *rpoB* Sequencing[▽]

Muhamed-Kheir Taha,^{1*} Sara Thulin Hedberg,² Marek Szatanik,¹ Eva Hong,¹ Corinne Ruckly,¹ Raquel Abad,³ Sophie Bertrand,⁴ Françoise Carion,⁴ Heike Claus,⁵ Alejandra Corso,⁶ Rocío Enríquez,³ Sigrid Heuberger,⁸ Waleria Hryniewicz,¹³ Keith A. Jolley,⁹ Paula Kriz,¹⁰ Marta Mollerach,⁷ Martin Musilek,¹⁰ Arianna Neri,¹¹ Per Olcén,² Marina Pana,¹² Anna Skoczynska,¹³ Cecilia Sorhouet Pereira,^{6,7} Paola Stefanelli,¹¹ Georgina Tzanakaki,¹⁴ Magnus Unemo,² Julio A. Vázquez,³ Ulrich Vogel,⁵ and Izabela Wasko¹³

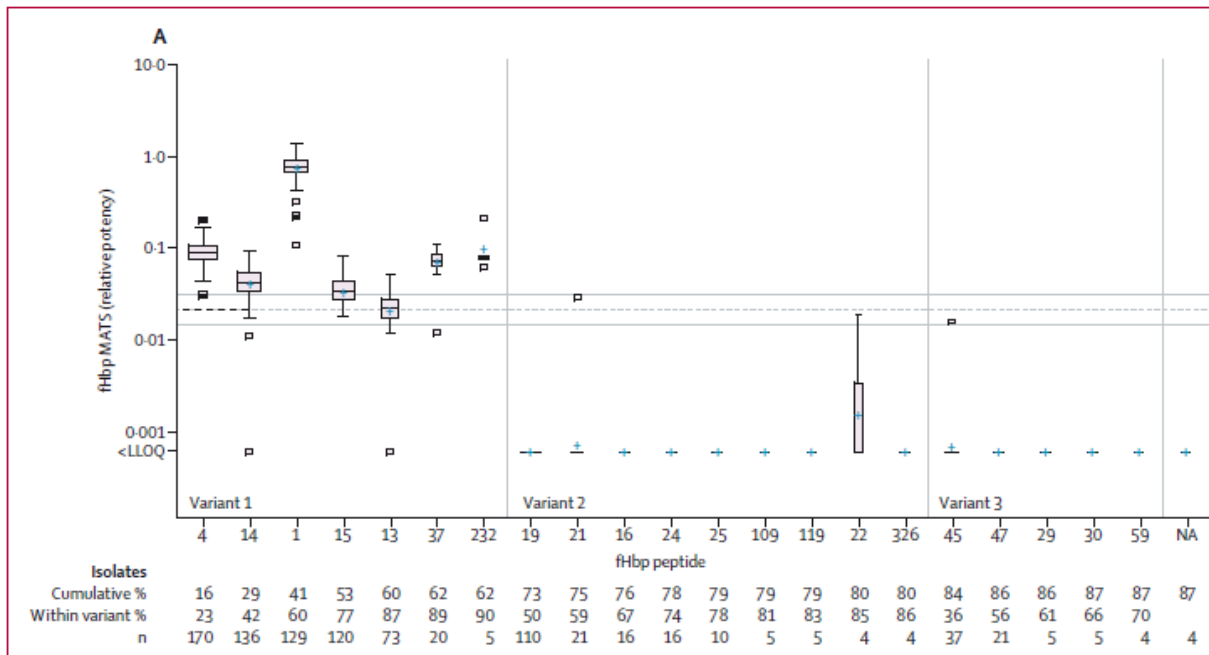
Target Gene Sequencing To Characterize the Penicillin G Susceptibility of *Neisseria meningitidis*[▽]

Muhamed-Kheir Taha,^{1*} Julio A. Vázquez,^{2*} Eva Hong,¹ Desiree E. Bennett,³ Sophie Bertrand,⁴ Suzana Bukovski,⁵ Mary T. Cafferkey,³ Françoise Carion,⁴ Jens Jørgen Christensen,⁶ Mathew Diggle,⁷ Giles Edwards,⁷ Rocío Enríquez,² Cecilia Fazio,⁸ Matthias Frosch,⁹ Sigrid Heuberger,¹⁰ Steen Hoffmann,⁶ Keith A. Jolley,¹¹ Marcin Kadlubowski,¹² Amel Kechrid,¹³ Konstantinos Kesanopoulos,¹⁴ Paula Kriz,¹⁵ Lotte Lambertsen,⁶ Ileana Levenet,¹⁶ Martin Musilek,¹⁵ Metka Paragi,¹⁷ Aouatef Sagner,¹³ Anna Skoczynska,^{12,1} Paola Stefanelli,⁸ Sara Thulin,¹⁸ Georgina Tzanakaki,¹⁴ Magnus Unemo,¹⁸ Ulrich Vogel,⁹ and Maria Leticia Zarantonelli¹

Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment



Ulrich Vogel, Muhamed-Kheir Taha, Julio A Vazquez, Jamie Findlow, Heike Claus, Paola Stefanelli, Dominique A Caugant, Paula Kriz, Raquel Abad, Stefania Bambini, Anna Carannante, Ala Eddine Deghmane, Cecilia Fazio, Matthias Frosch, Giacomo Frosi, Stefanie Gilchrist, Marzia M Giuliani, Eva Hong, Morgan Ledroit, Pietro G Lovaglio, Jay Lucidarme, Martin Musilek, Alessandro Muzzi, Jan Oksnes, Fabio Rigat, Luca Orlandi, Maria Stella, Danielle Thompson, Mariagrazia Pizza, Rino Rappuoli, Davide Serruto, Maurizio Comanducci, Giuseppe Boccadifuoco, John J Donnelly, Duccio Medini, Ray Borrow



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C. diphtheriae
C. jejuni
C. upsaliensis
Chlamydiales
Cronobacter
H. parasuis
H. pylori
Neisseria
P. acnes
P. aeruginosa
P. multocida (RIRDC)
S. agalactiae
S. oralis
S. uberis
S. zooepidemicus

Bacterial Isolate Genome Sequence Database (BIGSdb)

Written by [Keith Jolley](#), © 2010 University of Oxford

[Jolley & Maiden 2010, BMC Bioinformatics 11:595](#)

BIGSdb is software designed to store and analyse sequence data for bacterial isolates. Any number of sequences can be linked to isolate records - these can be small contigs assembled from dideoxy sequencing through to whole genomes (complete or multiple contigs generated from parallel sequencing technologies such as 454 or Illumina Solexa).

BIGSdb extends the principle of [MLST](#) to genomic data, where large numbers of loci can be defined, with alleles assigned by reference to sequence definition databases (which can also be set up with BIGSdb). Loci can also be grouped into schemes so that types can be defined by combinations of allelic profiles, a concept analogous to MLST.

All the functionality of [mlstdbnet](#) and [agdbnet](#) will be incorporated into BIGSdb and this software will be used to eventually host all the databases on this site. It is currently being used to host the [Neisseria](#) and [Campylobacter](#) sites.

The software has been released under the [GNU General Public Licence version 3](#).

Download from 

- [Installation guide](#)
- [Database setup and administration](#)
- [User guide](#)
- [Data curation](#)

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Details

The BIGSdb software is written in Perl, also utilizing some client-side Javascript. It runs on Linux using the [Apache](#) web server and [PostgreSQL](#) database.

BIGSdb is open-source software, published under the [GNU General Public Licence version 3](#).

Documentation

[Download and installation guide](#)
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Ion Torrent PGM study

- Explore the feasibility and accuracy of Ion Torrent Personal Genome Machine™ (PGM™) sequencing for genomic typing of meningococci
- Approach:
 - Comparison of known isolates from an outbreak
 - Analysis of previously established typing results (genetic and phenotypic)
 - Extended typing was evaluated under the assumption that strains are clonal
 - Validation of sequencing by analysis of a type strain

Sequencing (Dag Harmsen and colleagues in Münster and Bielefeld)

- PGM™ (Life Technologies, Germany).
- Libraries were generated using 1 µg of the genomic DNA and the Ion Xpress™ Plus Fragment Library Kit comprising the Ion Shear™ chemistry
- Clonal amplification on Ion Spheres™ particles during the emulsion PCR according to the Ion Xpress™ Template 200 Kit manual.
- Ion 316™ chip
- 105 sequencing cycles
- Average reading length of 200 nucleotides.
- Genome projects have been registered as NCBI Bioprojects PRJNA78229, PRJNA78227, and PRJNA78225.
- MIRA (v. 3.4.0) was used for *de novo* assembly of all four genomes
- Draft genomes were uploaded to the BIGSdb website (<http://pubmlst.org/software/database/bigsdb/>)

Strains

- Serogroup B strains DE9622, DE9686, and DE9938
- 2003 and 2004, Düren, Aachen and Heinsberg
- Sequence type (ST)-42 (ST-41/44 clonal complex [cc])
- Highly similar to the New Zealand outbreak strain (**Elias et al. EID 2010**)
- MC58 (serogroup B, ST-74, ST-32 cc), reference strain

Category	Locus	DE9622	DE9938	DE9686	MC58 (outgroup)
MLST (ref. 28)	<i>abcZ</i>	10	10	10	4
	<i>adk</i>	6	6	6	10
	<i>aroE</i>	9	9	9	5
	<i>fumC</i>	5	5	5	4
	<i>gdh</i>	9	9	9	5
	<i>pdhC</i>	6	6	6	3
	<i>pgm</i>	9	9	9	2
	ST	42	42	42	74
	cc	ST-41/44cc	ST-41/44cc	ST-41/44cc	ST-32cc
	eMLST (ref. 9)	<i>aspA</i>	8	8	8
<i>carB</i>		32	9	9	3
<i>dhpS</i>		11	11	11	5
<i>glnA</i>		3	3	3	8
<i>gpm</i>		7	7	7	4
<i>mtgA</i>		7	7	7	5
<i>pilA</i>		5	5	5	3
<i>pip</i>		4	4	4	2
<i>ppk</i>		3	3	3	9
<i>pykA</i>		9	9	9	6
<i>rpiA</i>		1	1	1	5
<i>serC</i>		4	4	4	3
<i>talA</i>		7	7	7	3
AST (ref. 22, 44)		PorA VR1/VR2	7-2,4	7-2,4	7-2,4
	<i>porA</i> partial	39	39	39	12
	<i>porA</i> full length	26	26	26	2
	FetA	1-5	1-5	1-5	1-5
	<i>porB</i> partial	3-1	3-1	3-1	3-24
	<i>porB</i> full length	42	42	42	10
4CMenB (ref. 6)	<i>fhhp</i>	14	14	14	1
	FHBP	14	14	14	1
AR (ref. 40, 41)	<i>penA</i>	1	1	1	3
	<i>rpoB</i>	18	18	18	2

NOTE: MLST, multilocus sequence typing; ST, sequence type; cc, clonal complex; eMLST, extended MLST; AST, antigen sequence typing; VR, variable region; 4CMenB, Novartis investigational MenB vaccine; AR, antimicrobial resistance

INFECTIOUS DISEASES

Bacterial Meningitis Finds New Niche in Gay Communities

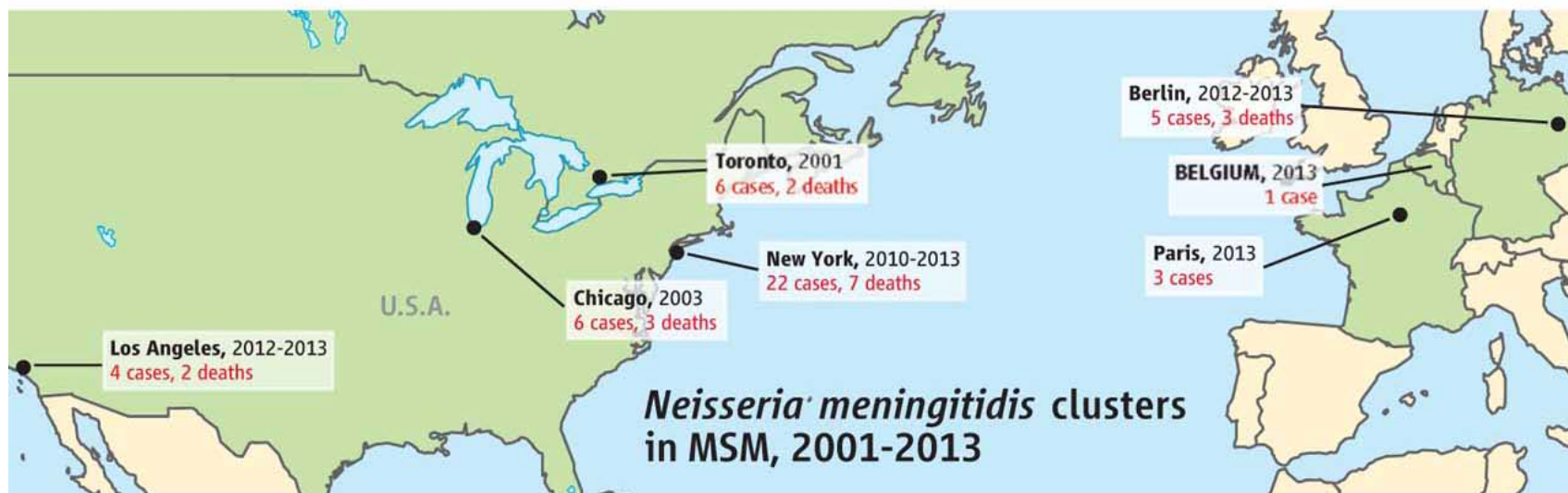
BERLIN—For two young Berliners, what began as a fun night on the town in early May ended in tragedy. The 24-year-olds went home together after an evening out with friends at one of the many gay clubs here. The next day, one of them complained of fever and nausea.

scientists are trying to figure out the reasons for the emerging pattern.

N. meningitidis infects people around the globe. Ten percent of us may be walking around with it at any given time; in most cases, the bacterium resides in the mucosa of

Amanda Cohn, an epidemiologist at the U.S. Centers for Disease Control and Prevention in Atlanta. “We have clusters in groups that have strong social networks, and the MSM community is very close,” she says.

But Don Weiss, a researcher at the New



Genomic analysis reveals that sexual transmission may contribute to an outbreak of meningococcal disease among MSM (Manuscript in preparation)

Muhamed-Kheir Taha¹, Martin Lappann², Frederic Veyrier¹, Heike Claus², Dag Harmsen⁵, Karola Prior⁵, Ala-Eddine Deghmane¹, Andreas Otto⁴, Isabelle Parent du Châtelet¹, Wiebke Hellenbrand³, Dörte Becher⁴, Ulrich Vogel²

¹Institut Pasteur, ²University of Würzburg, ³Robert Koch-Institute,
⁴University of Greifswald, ⁵University of Münster

Originaldaten entfernt da unveröffentlicht

- Die Daten zeigten in Bezug auf NGS, dass die Genomsequenzierung erlaubt, eng verwandte Stämme, hier aus dem ST-11 cc, phylogenetisch sinnvoll und robust zu gruppieren und Hypothesen abzuleiten.
- Des Weiteren wurde die NGS genutzt, um funktionelle Daten aus Proteomanalysen und biochemischen Tests genetisch zu validieren.

Summary

- NGS is the future of meningococcal typing and will replace Sanger sequencing
- BIGSdb provides a platform for retrieval of allele assignments
- Ion Torrent performance was satisfying on the small set of strains with no need for further editing
- MiSeq provided excellent genome sequences for closely related strains of the ST-11 / ET-15 clade
- Each reference lab will need individual decisions about in- or outsourcing of sequencing capacities