

Epidemiological knowledge by genotyping *Chlamydia trachomatis*: an overview of recent achievements

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Characteristics of various methods for typing *C. trachomatis*

Method	Culture Principle	Target	Reference	Detect mixed Culture	Comment
MTPT	Immunisation	Unknown	Wang & Grayston, 1963	NA	Proof of existence of different types.
Micro-Immunofluor.	Immunisation	Unknown	Wang <i>et al.</i> , 1975	NA	Cross-activity between types.
Polyclonal Ab	Serotyping	MOMP	Wang <i>et al.</i> , 1973b	Yes	Cross activity between types.
Monoclonal Ab	Serotyping	MOMP	Newhall <i>et al.</i> , 1986	Yes	Cross activity between types.
RIA	Serotyping	MOMP	Van Der Pol & Jones, 1992	Yes	Cross activity between types.
Dot-ELISA	Serotyping	MOMP	Barnes <i>et al.</i> , 1987	Yes	Cross activity between types.
PCR-RFLP	Genotyping	<i>omp1</i>	Rodriguez <i>et al.</i> , 1991	Yes	13 of 15 serovars identified
PCR-RFLP	Genotyping	<i>omp1</i>	Lan <i>et al.</i> , 1993	Yes	Cervical scrapes used
PFGE	Genotyping	genomic DNA	Birkelund & Stephens, 1992	No	Purification of EBs needed.
RAPD	Random DNA	genomic DNA	Scieux <i>et al.</i> , 1993	NA	Do not reflect serovars
PCR	Genotyping	<i>omp1</i>	Pecharatana <i>et al.</i> , 1993	Yes	Fluorophore marked primers. Only Serotypes A,B,C detected
AFLP	Genotyping	genomic DNA	Morre <i>et al.</i> , 2000	NA	Difficult to standardise
Reverse line blot	Genotyping	<i>omp1</i>	Quint <i>et al.</i> , 2007	Yes	Low technology detection
PCR+sequencing	Genotyping	<i>omp1</i>	Lysen <i>et al.</i> , 2004	No	Serotype-level typing.
Real time PCR	Genotyping	<i>omp1+pmpH</i>	Schaeffer & Henrich, 2008	Yes	L-serovars only.
PCR+sequencing	Genotyping	MLST	Klint <i>et al.</i> , 2007	No	Subtyping serotypes
PCR+sequencing	Genotyping	<i>omp1+VNTR</i>	Pedersen <i>et al.</i> , 2008	No	Subtyping serotypes
Suspension Array	Genotyping	<i>omp1</i>	Huang <i>et al.</i> , 2008	Yes	Serotype-level typing

Genotyping

What can be expected from ompA?

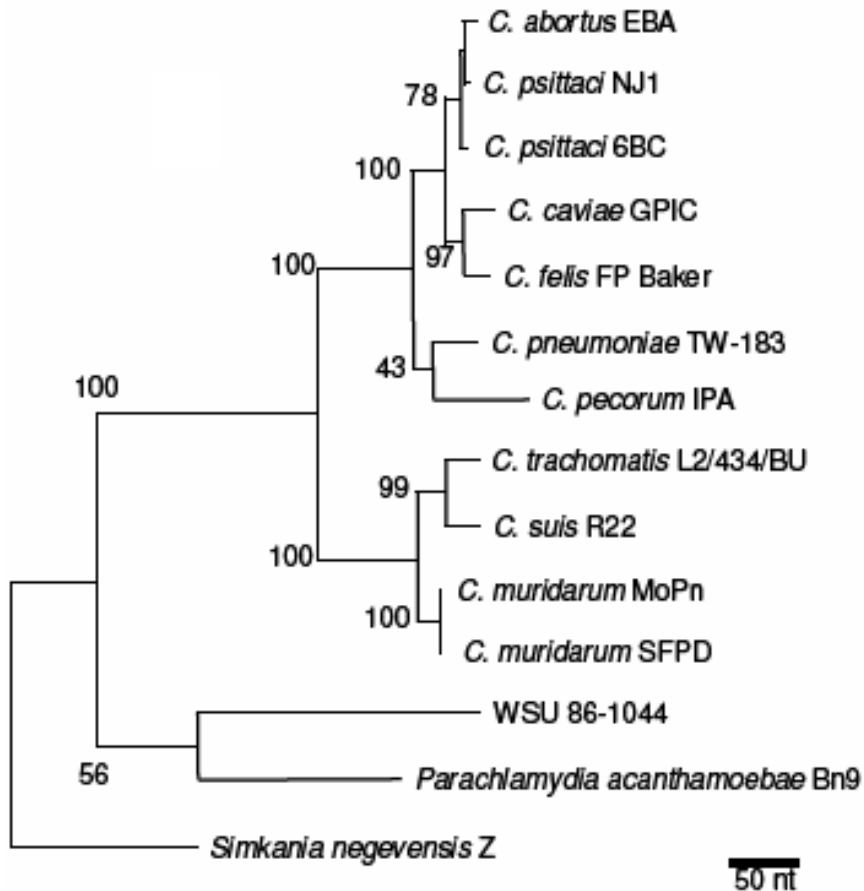
Number of genotype variants of detected cases

- 18/172 (10%) Ct cases Brunham 1996
- 15/33 (45%) Ct pos women with LGTI or PID Dean 1995
- Serovar E 11/67 (16%) Ct cases Dean 1997
- 10/31 (32%) Ct cases Nørum Pedersen 2000
- 17/21 (81%) strains from CSW Sturm-Ramirez 2000
- 13/35 (37%) Ct adolescents Stothard 2001

29 variants in 678 (4%) cases - all in the county

Lysen et al 2004

MLST system for the *Chlamydiales*



7 housekeeping genes.
Useful for separation of species and
evolutionary studies

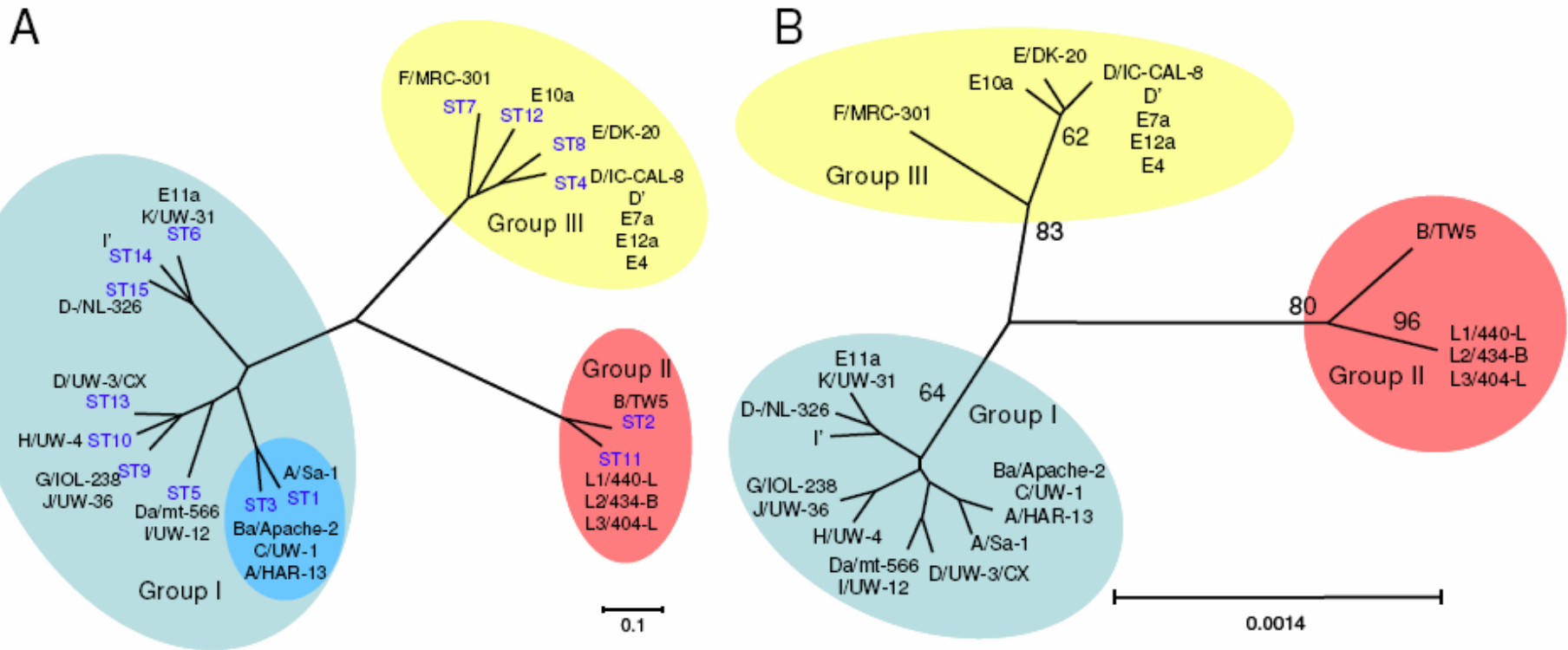
Pannekoek *et al*, *BMC Microbiol* 2008

Dean *et al*, *Emerg Inf Dis* 2009

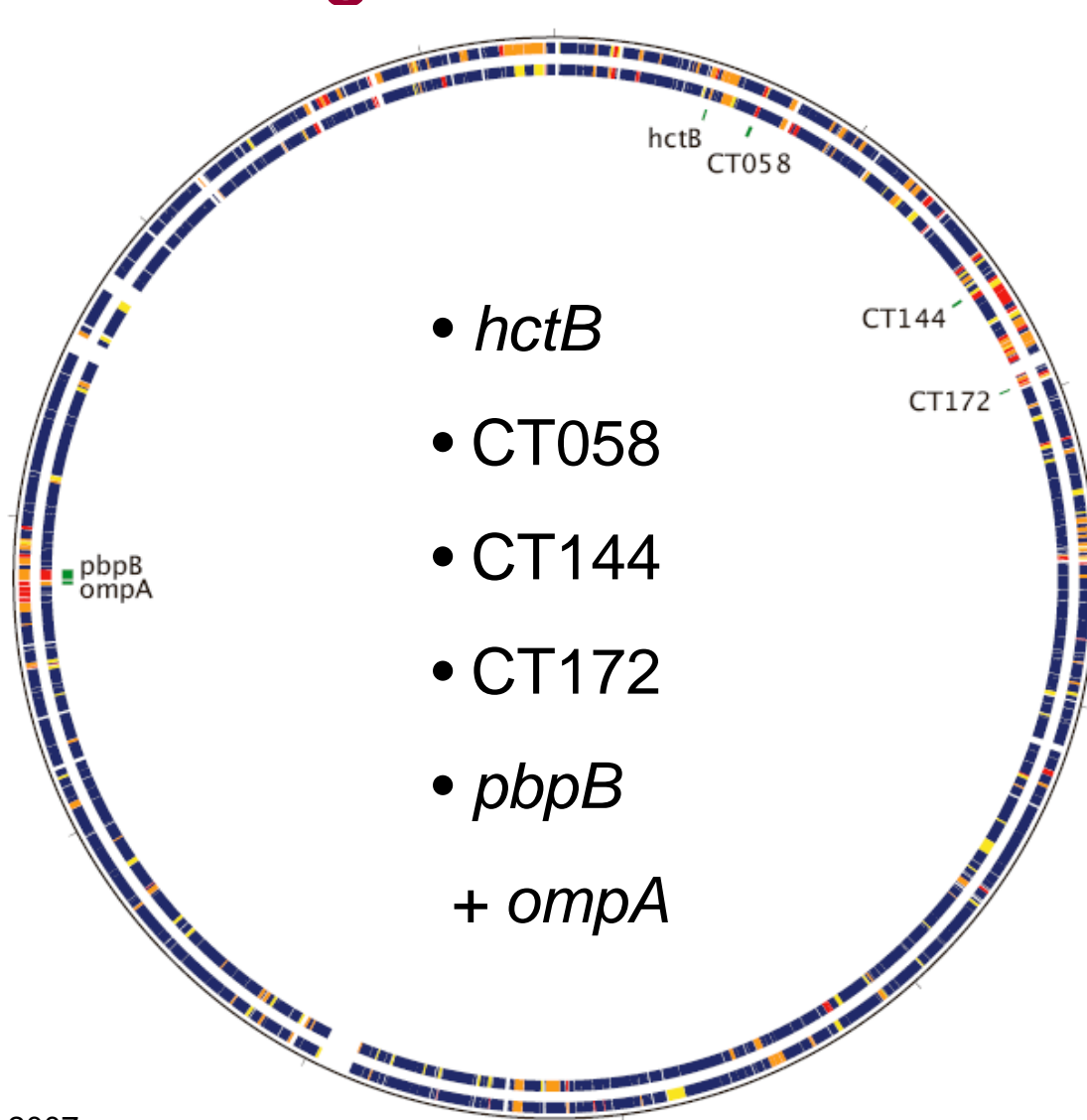
Phylogenetic analyses of concatenated sequences
of 6 housekeeping gene fragments

Application of MLST on *C. trachomatis*

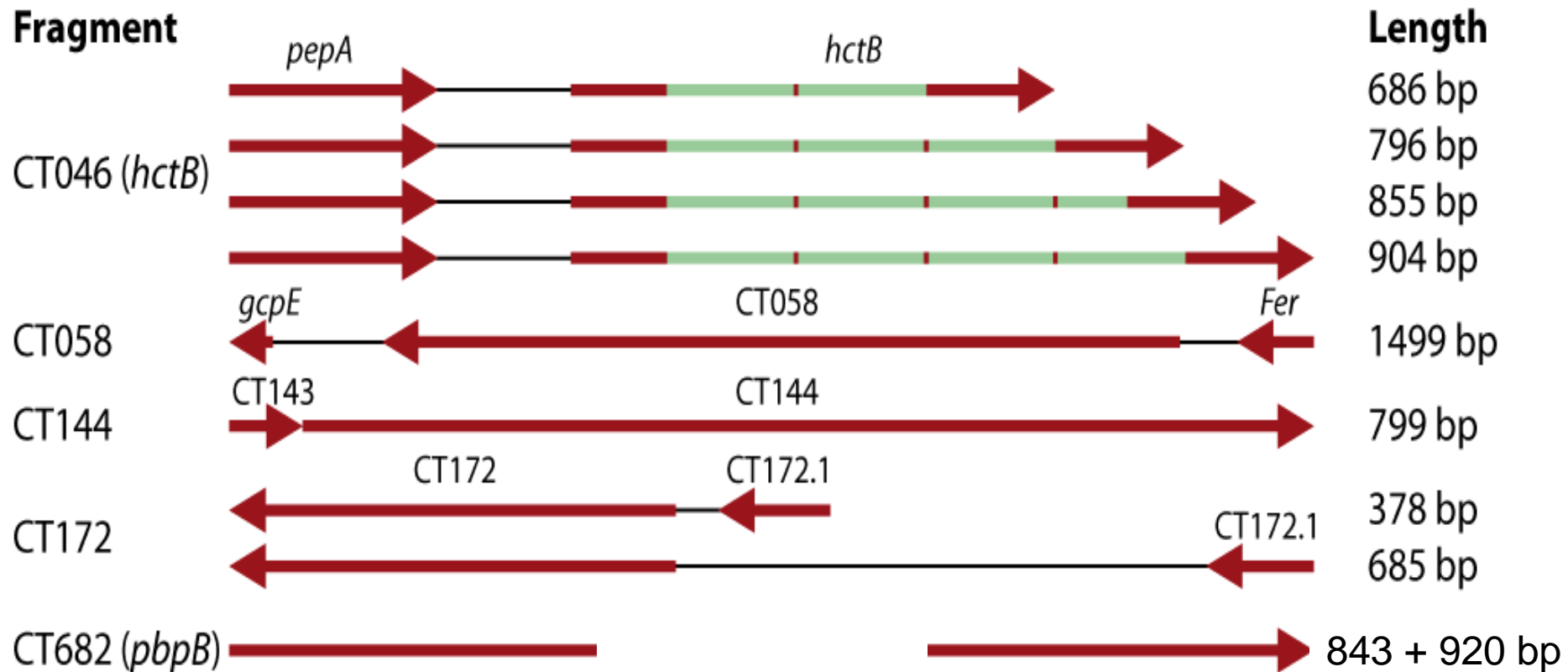
Limited resolution within *C. trachomatis*:
25 *ompA* genotypes → 15 MLST sequence types



Multilocus sequence typing (MLST) high resolution



Characterization of target regions



Summarized sequence variation in different strains

Strain	<i>pbpB</i>	<i>hctB</i>	CT058	CT144	CT172	<i>ompA</i>	Allele	Sequence type (ST)
E1	1	1	1	1	2	1	1,1,1,1,2,1	1
E2	1	1	1	1	2	1	1,1,1,1,2,1	1
E3	2	2	1	1	2	1	2,2,1,1,2,1	2
E4	3	1	1	1	2	1	3,1,1,1,2,1	3
E5	1	1	2	1	2	2	1,1,2,1,2,2	4
E7	1	1	3	1	2	1	1,1,3,1,2,1	5
E8	1	1	1	1	2	1	1,1,1,1,2,1	1
E9	1	1	4	1	2	2	1,1,4,1,2,2	6
E10	1	1	5	1	1	1	1,1,5,1,1,1	7
G1	4	3	6	3	3	4	4, 3, 6, 3, 3, 4	8
G2	5	5	7	2	3	5	5, 5, 7, 2, 3, 5	9
G3	6	3	7	3	1	6	6, 3, 7, 3, 1, 6	10



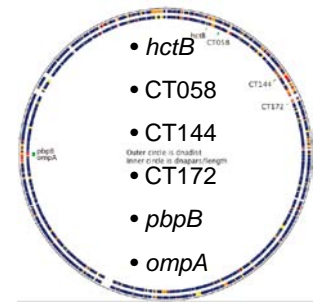
Genetic variation within serotypes: No. of variants for each target region

Serotype (n)	Number of variants		
	MLST	MLST + <i>ompA</i>	<i>ompA</i>
D (14)	11	11	5
E (12)	7	8	2
G (11)	9	9	4
K (10)	5	5	1
Total (47)	32	33	12

Isolates randomly selected from diagnosed cases in Malmö during six months

Between 50 and 82% of tested strains within serovars had unique sequence patterns

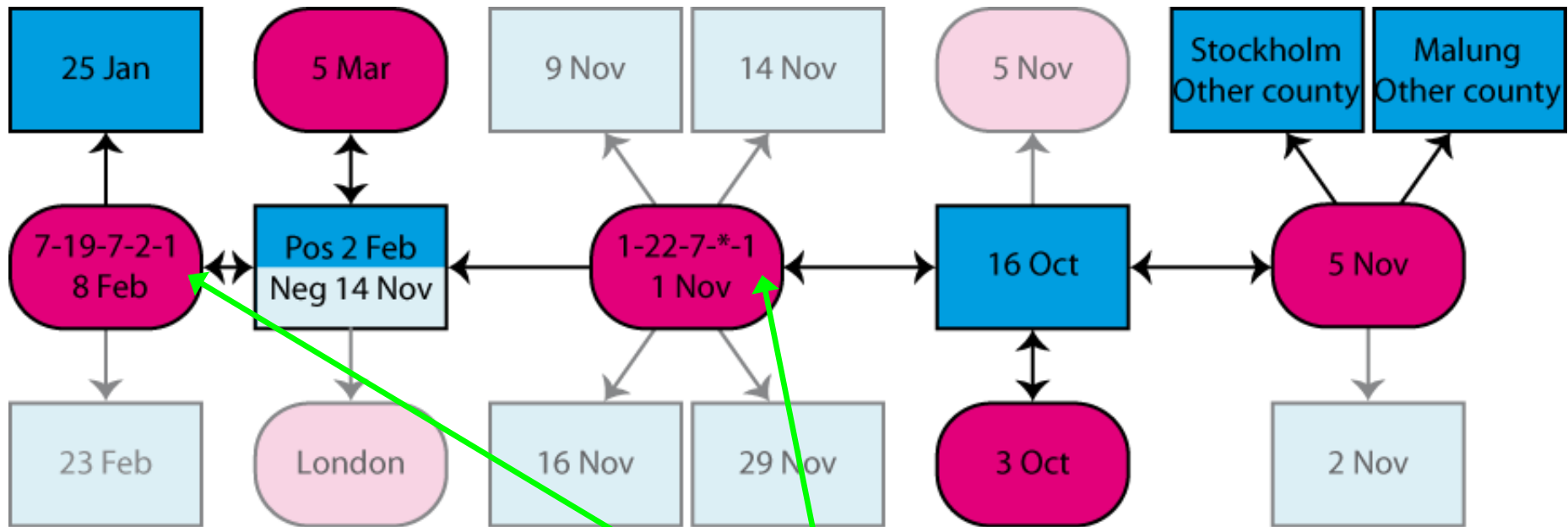
MLST applications



1. Contact tracing
2. Is transmission of nvCT clonal?
3. Variation of LGV – clonal outbreak?
4. Genotype association to symptoms?
5. Trachoma epidemiology
6. Variation in local populations

1. Contact tracing

All chlamydia cases identical *ompA*, serotype E

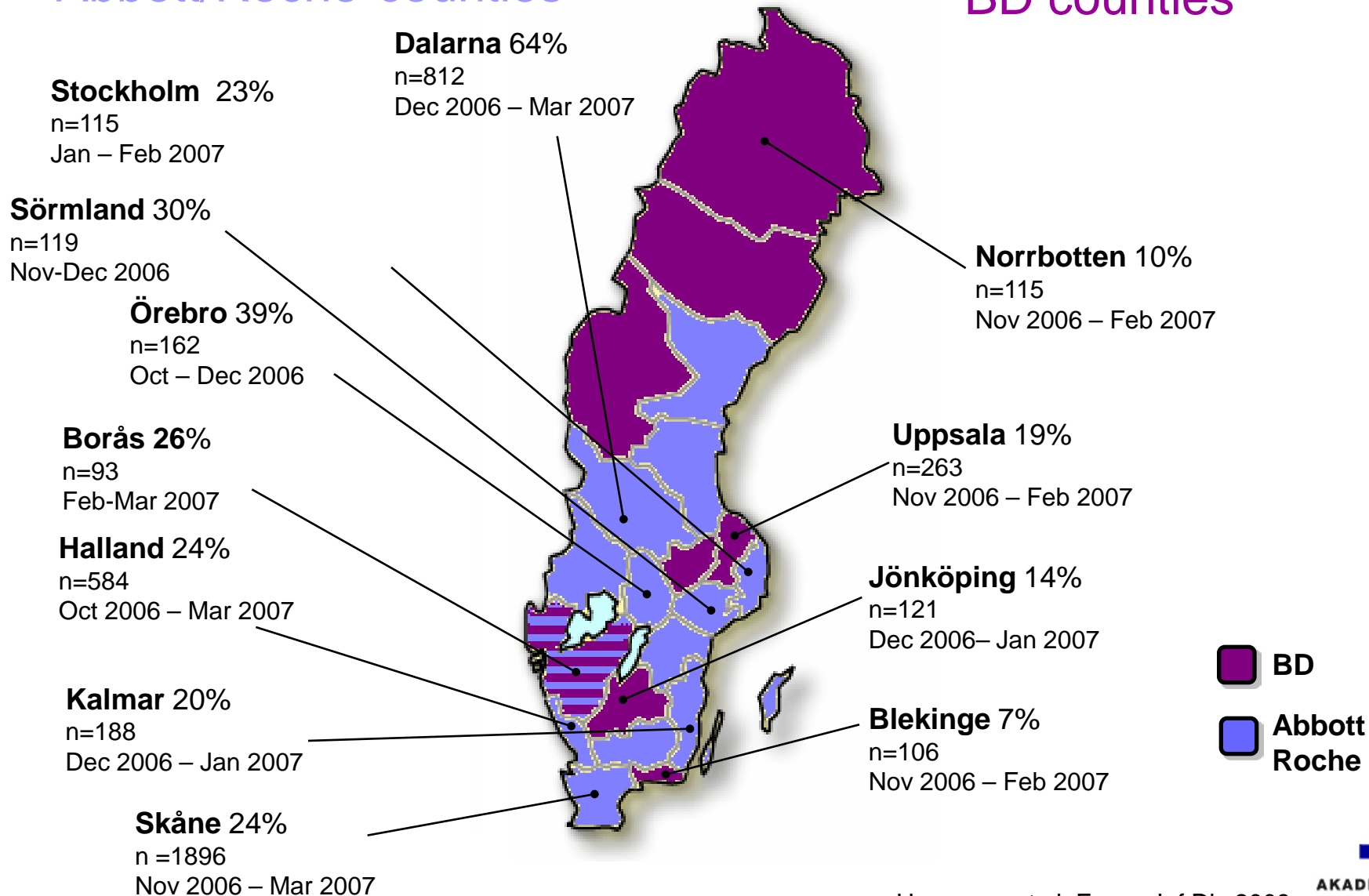


MLST differentiates in two types

2. New variant CT in Sweden

Abbott/Roche counties

BD counties



Genotypes of 67 consecutive serotype E specimens in 2006, Örebro

Number of specimens	MLST profile					
	<i>hctB</i>	CT058	CT144	CT172	<i>pbpB</i>	<i>ompA</i>
nvCT n = 41	21	19	1	2	1	6
wtCT n = 4	1	19	7	2	1	6
wtCT n = 4	5	19	6	2	2	6
wtCT n = 3	7	19	14	2	1	6
wtCT n = 3	24	19	7	2	1	6
wtCT n = 2	7	19	7	2	36	6
wtCT n = 2	23	19	14	2	1	6
wtCT n = 1	1	2	6	2	2	6
wtCT n = 1	1	19	7	2	35	6
wtCT n = 1	7	19	7	2	1	6
wtCT n = 1	22	19	7	2	1	6
wtCT n = 1	23	19	17	2	1	6
wtCT n = 1	23	19	17	2	38	6
wtCT n = 1	24	19	1	17	2	6
wtCT n = 1	25	19	7	2	1	6

- One *ompA* genotype
- 15 MLST genotypes
- nvCT unique

nvCT unique MLST profile

- 48 nvCT specimens from Skåne, Örebro, Uppsala, Norrbotten, Norway, Ireland and France.
- 41 nvCT 2006 Örebro
- 13 nvCT 2009 Uppsala and Örebro

Conclusion: One clone

3. LGV

- First in the Netherlands 2003
- In the MSM population
- Not only typical symptoms - also asymptomatic cases

How many out there?



MLST profiles 50 contemporary European LGV specimens (8 countries)

Number of specimens	MLST profile					
	<i>hctB</i>	CT058	CT144	CT172	<i>pbpB</i>	<i>ompA</i>
n = 49	27	13	17	13	29	28
n = 1	27	13	17	13	29	39

- Clonal spread L2b

MLST profiles of LGV specimens from USA

Number of specimens	MLST profile					
	<i>hctB</i>	CT058	CT144	CT172	<i>pbpB</i>	<i>ompA</i>
Baltimore2007						
n = 5	27	13	17	13	29	28

- Same strain in Europe and contemporary strains in USA

4. Trachoma in The Gambia

Intra-individual variation for children present at both baseline and follow-up

- 8 present at both time-points
- 7 of these treated
- 4 of these have full sequencing
 - For 2, MLST variants are the same: persistence vs. new infection?
 - For 2, MLST variants are different: new infection

5. MLST analysis of Ctr cases from 3 "local" youth populations in Norway

2100 youths 16-19 years

248 Ctr cases MLST analysis

80 Finnmark

80 Tromsö

88 Trondheim

100% success fr 248 urine specimens

Aim: To characterize chlamydia infections in Finnmark and compare with Tromsö and Trondheim



6. Association to symptoms?

70 Dutch urogenital *C. trachomatis* strains from women

Categories: Asymptomatic, Symptomatic, Lower abdominal pain

Outcome: 46 MLST types

No correlation MLST profiles and symptomatology

MLST database for *C. trachomatis*

<http://mlstdb.bmc.uu.se/>



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[Home](#) [Profiles](#) [Specimens](#) [PubMLST.org](#) [Software](#) [User guide](#)

Welcome to our *Chlamydia trachomatis* multi locus sequence typing (MLST) database!

This high resolution genotyping system has been developed for epidemiological studies of *C. trachomatis* infections. It is based on five target regions and in addition contains information about the commonly used *ompA* gene. Our MLST system has a threefold higher resolution than ordinary *ompA* genotyping [Klint *et al.*, 2007]

The system has been applied to hundreds of specimens collected from a variety of countries and patient populations, including the three different types of *C. trachomatis* infections: Trachoma, urogenital chlamydia and lymphogranuloma venereum (LGV).

If you are interested in more background information about the specimens please contact [Björn Herrmann](#).

- [Profiles database](#)
- [Specimens database](#)

This Multilocus Sequence Typing (MLST) database is engineered/hosted by the [Computing Department](#) at BMC, Uppsala Biomedical Centre, Uppsala University.

The MLST database comprises:

- Profiles database 178
- Specimens database 721



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MLSTs



High resolution
Several applications

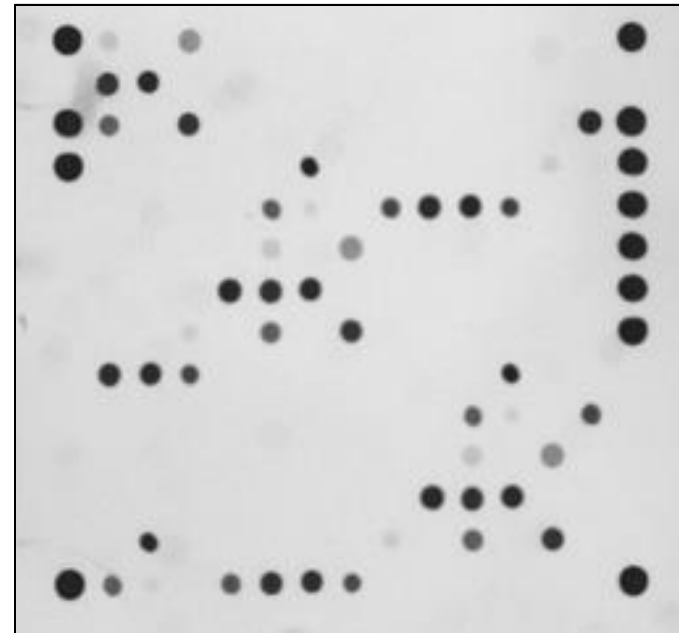


Laborious
Time consuming
Expensive



High resolution DNA microarray

- Based on the MLST system by Klint *et al.*
- Circumvent expensive DNA sequencing
- 2.4 times higher resolution than *ompA* sequencing (n=100 cases)



The role of CT-genotyping?

- Increase scientific knowledge
- Useful tool in clinical epidemiology
- Still not ready for broad scale implementation
- Continuous development of methods