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Molecular typing of *Mycoplasma pneumoniae* and epidemiology of infections

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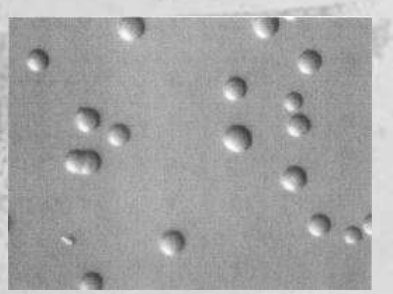
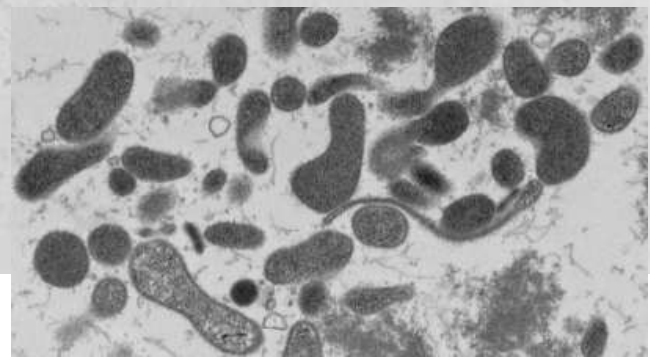
Mycoplasmas

- *Mycoplasmas* represent the smallest self-replicating organisms
- Small genome and limited biosynthetic capabilities
- Parasitic existence in association with eukaryotic cells of their host
- > 17 documented species in humans





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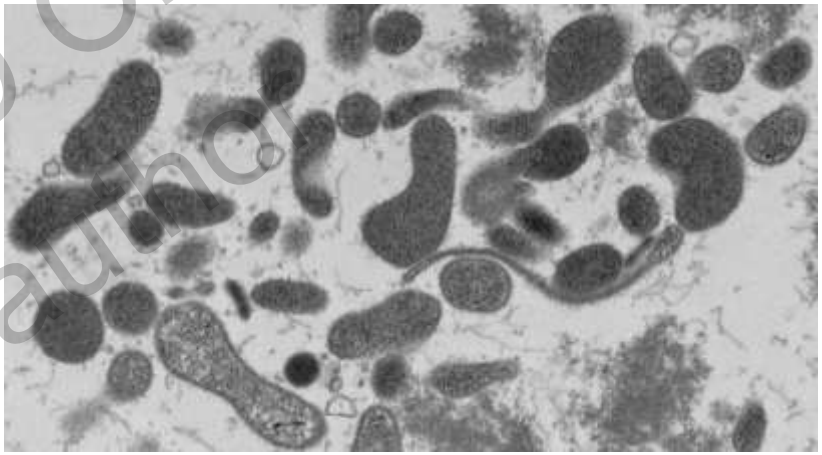
Pathogenesis

Mycoplasma pneumoniae

- Community-acquired pneumonia
- Encephalitis
- Dermatological disorders e.g. Stevens-Johnson syndrome
- Septic arthritis
- Haemolytic anaemia



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3 main typing methods described

P1 typing – in use in a few laboratories internationally

MLVA – increasingly used

Multi-locus typing (MLST) – one publication, new scheme in development.

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Current typing methods

P1-adhesin protein based typing

Analysis of the P1 gene, encoding a major adhesion, is the most common genotyping method.

PCR & sequencing of repetitive elements RepMp2/3 and RepMp4

3 subtypes and 4 variants (V1, V2a, V2b, V2c) = 7 total

Speculation that a shift in P1 adhesin type may be the cause of epidemics has been proposed.

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Current typing methods

MLVA multiple-locus variable-number tandem-repeat (VNTR) analysis

Variation in copy number of tandem repeats at 5 different loci. Initially 265 isolates grouped into 26 MLVA types.

- Lack of international consistency
- Varying methods and nomenclature used
- No EQA
- ESGMI study to validate internationally
- Multiple MLVA types are present during epidemic peaks
- Single MLVA types have been documented in localised clusters of infection

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International validation of MLVA

ESGMI study – 6 international laboratories (China, England, France, Germany, Netherlands, USA)

Panel of *M. pneumoniae* for MLVA typing and interpretation

24 clinical isolates were included, as well as the reference strain M129 (ATCC 29342).

The commercial quantitative type strain NCTC10119 FH (Minerva Biolabs) used to determine sensitivity of MLVA at four dilutions (1000, 100, 10 and 1 copy/µL).

Results fragment size, calculated MLVA repeat number and MLVA profile.

No guidelines were given to the participating laboratories

Table 1. Target sequences, repeat size and fragment size (bp) of *M. pneumoniae* MLVA loci MPN1, MPN13, MPN14, MPN15 and MPN16 according to number of repeats.

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		MPN1	MPN13	MPN14	MPN15	MPN16
Repeat sequence based on M129 TRF (2,3,5)[a]		CCGAGCTAAGCG	TATTAATAACTATTCT	TGGACAAAATGGAAGT AAAAA	TTGTCCATTTTTTCTT CCATC	ATTTTTTAAAAGTTTT TATTTATCCGTTTTGA CAACTGCTTTTTGTT
Repeat size (bp)		12	16	21	21	47
Repeat number	0	287	364	294	108	259
	1	299	380	315	129	306
	2	311	396	336	150	353
	3	323	412	357	171	400
	4	335	428	378	192	447
	5	347	444	399	213	494
	6	359	460	420	234	541
	7	371	476	441	255	588
	8	383	492	462	276	635
	9	395	508	483	297	682
M129 fragment size		333	415	399	241	353
M129 repeat number		3.8 → 4	3.2 → 4	5	6.3 → 7	2

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International recommendations

Predicted fragment sizes and repeat numbers should be assigned using the information provided in Table.

Sequence of repeat fragments listed in Table should be considered as the sequence of interest.

If tandem repeat finder software is used (http://tandem.bu.edu/trf/trf.html) [17] to determine repeat numbers, the following settings should be used: match, mismatch, indel (2,3,5).

Repeat number should be expressed as whole integers and partial sequences should be **rounded up** to the next integer number

The MPN1 target should be removed from future analyses due to its instability

Naming system recommended: MLVA 1,2,3,4, where each digit corresponds to repeat numbers at loci MPN13, MPN14, MPN15 and MPN16, respectively.

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Current typing methods

MLST Multi-locus sequence typing

- *Nesseria meningitidis*
- *Staphylococcus aureus*
- *Legionella*
- *M. bovis*
- *M. agalactiae*
- *M. hyorhinis*
- *M. hyopneumoniae*

Genetic variation multiple housekeeping genes (~ 7)

Differences are assigned as distinct alleles.

Combination of the alleles at each loci define the allelic profile (sequence type)

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Current typing methods

MLST Multi-locus sequence typing

Previously attempted *M. pneumoniae* - little polymorphism found and not considered useful

Development new scheme – 8 loci

ppa, pgm, gyrB, atpA, gmk, glyA, arcC & adk.

51 strains tested, devised on genomic data and extended with PCR derived sequence data

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	reca	ppa	pgm	gyrb	gmk	glya	efp	atpa	arcc	adk
Bacillus cereus					✓					
Chlamydia trachomatis						✓				
Campylobacter jejuni			✓			✓				
Escherichia coli	✓			✓						✓
Enterococcus faecium								✓		✓
Haemophilus influenzae	✓									✓
Helicobacter pylori		✓					✓	✓		
Moraxella catarrhalis		✓					✓			✓
Neisseria meningitidis			✓							✓
Staphylococcus aureus					✓				✓	
Staphylococcus epidermidis									✓	
Streptococcus suis	✓									
Vibrio vulnificus				✓						
Y. pseudotuberculosis										✓

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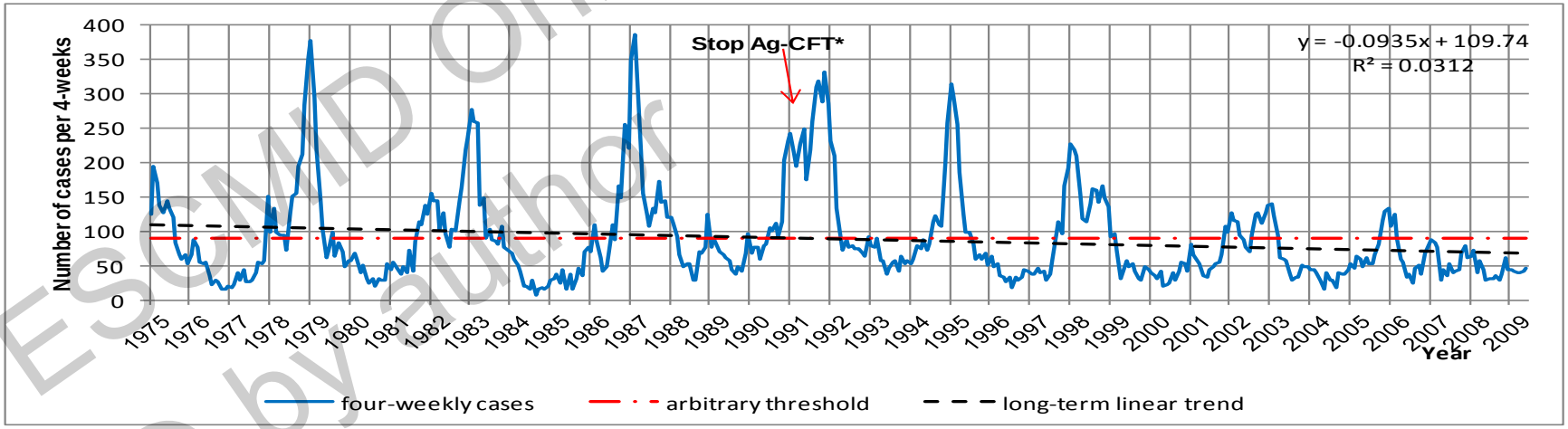
Epidemiology

Typing of clinical isolates is of practical importance for understanding of the epidemiology of infections & for analysis of endemic outbreaks.

***M. pneumoniae*:** in England and Wales endemic periods last on average 18 months at ~ 4 yearly intervals. Low-level sporadic infection occurs with seasonal peaks from December-February.



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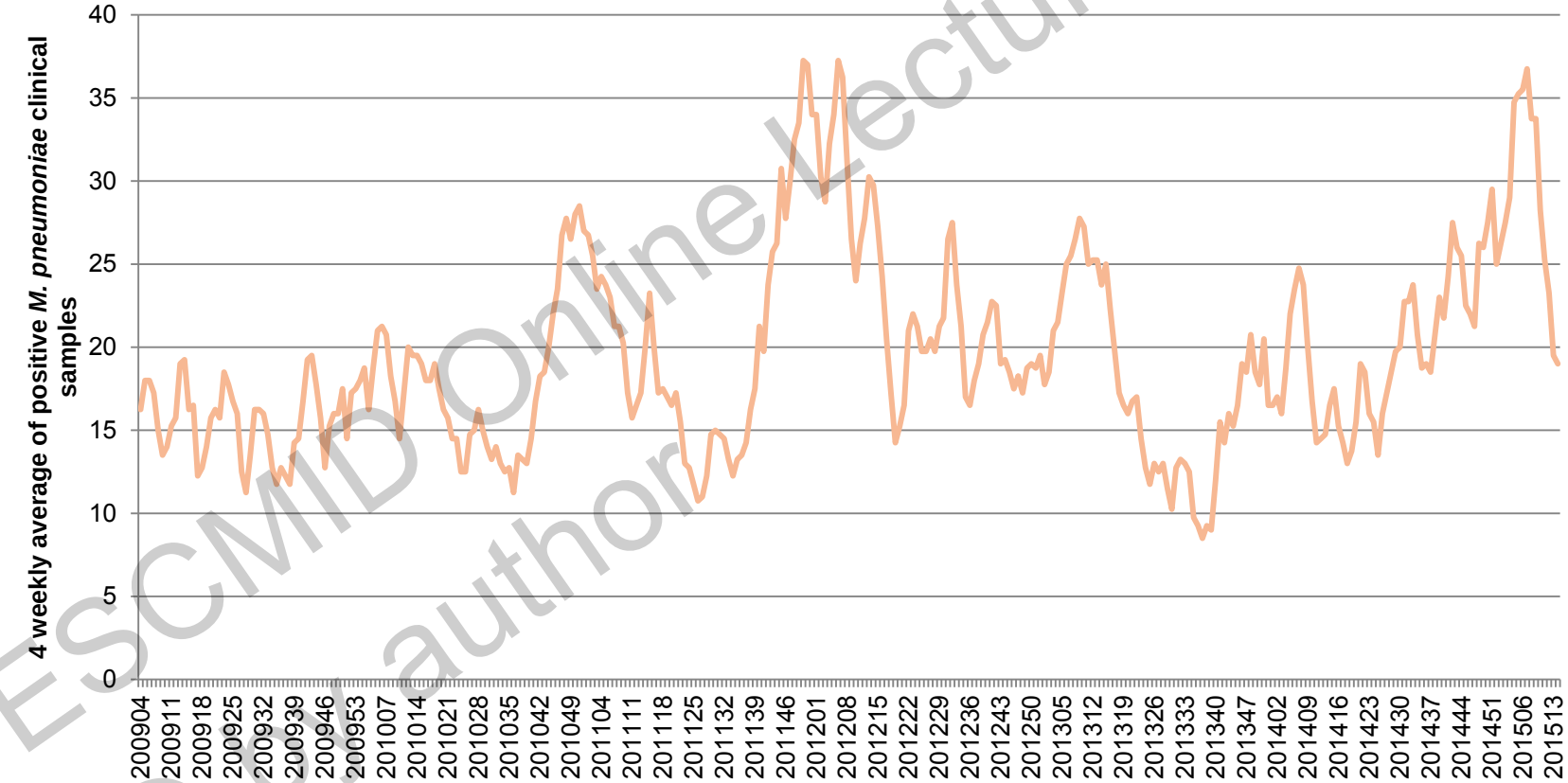
Current trend in England and Wales



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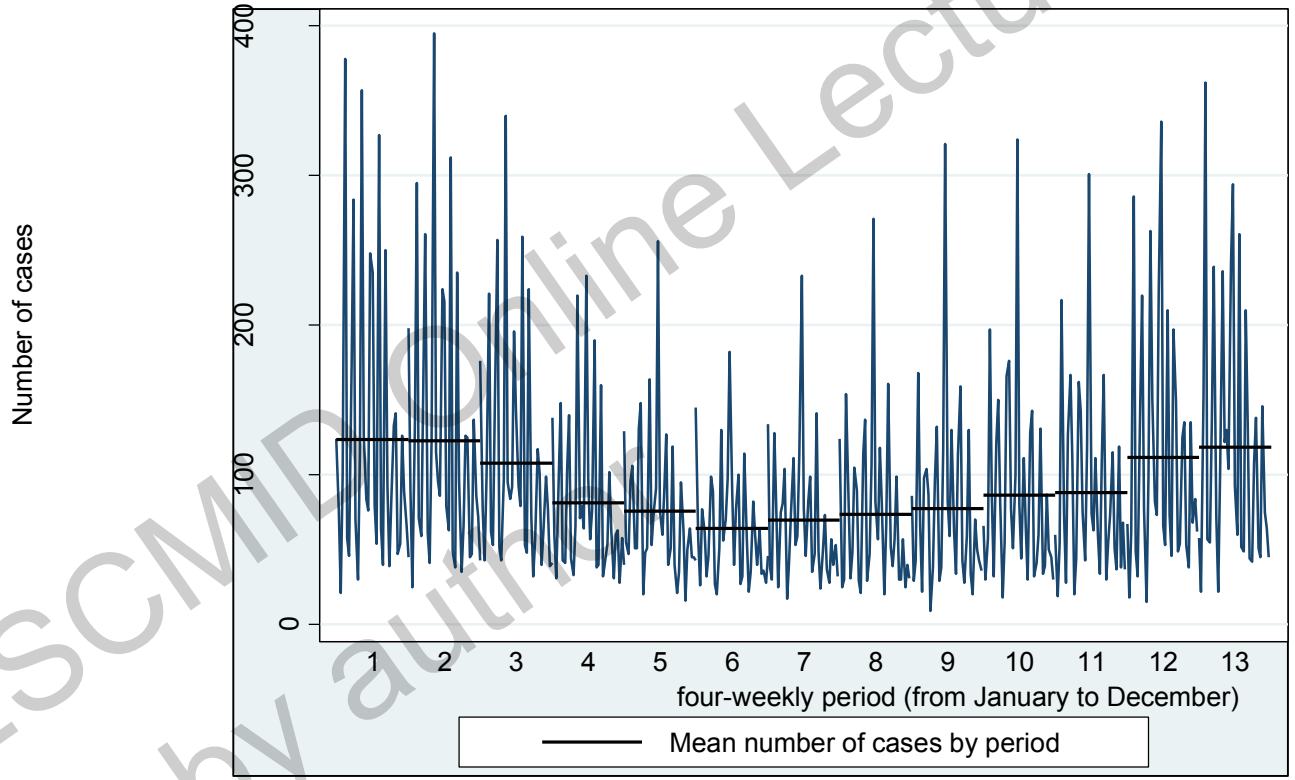
Seasonality – peak winter



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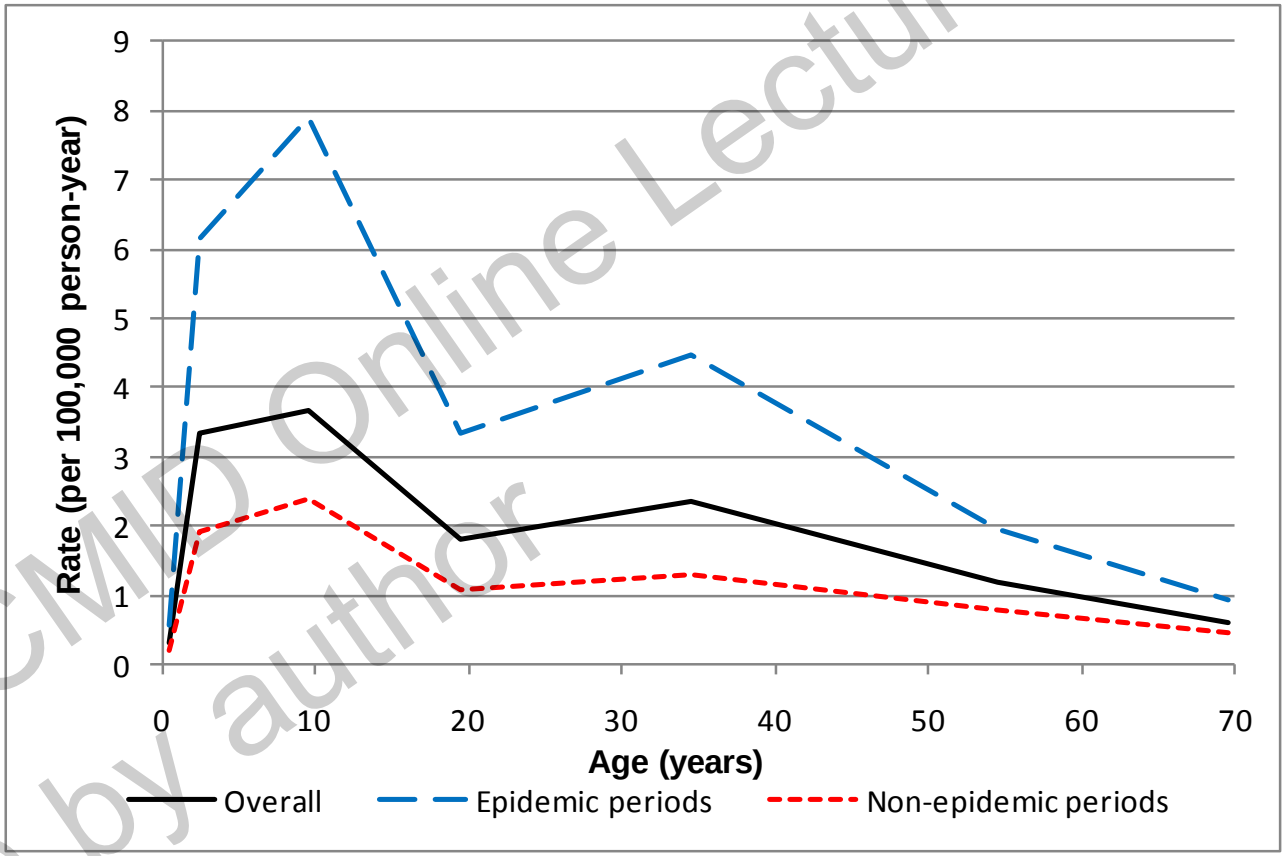
Age - highest in children



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Epidemiology

Epidemic cycles are intrinsic to MPN natural transmission dynamics in the population – herd immunity

Epidemics due in part to interaction between seasonal variation in transmissibility (winter peaks) and chance variations in environmental factors

Confirmation of P1/MLVA/MLST shift in epidemics is required with larger isolate number

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Conclusions

- MLVA international recommendations presented – currently submitted
- MLST scheme in development higher discriminatory power than the established MLVA method.
  - Need to test direct on clinical specimens
- Currently using genomic data to ascertain reflection of phylogeny.
- Increasing co-operative international surveillance required to monitor and define nature of epidemics and resistance

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Acknowledgements

ESGMI international team

Rebecca Brown

Patrick Nguipdop-Djomo

Brad Spiller

Ulf Schaefer & Antony Underwood

Sarah Philips