



Choosing the Right Tool for the Job – Handyman, Expert or DIY?

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Choosing the right tool? first let's define the Job!



Local / hospital
NRC
International



Emergency situation
'One-off' / *ad hoc*
Routine task



Type of organism
Diversity / typeability
Type of sample
Type of setting



Microbiologist
Epidemiologist
Clinician

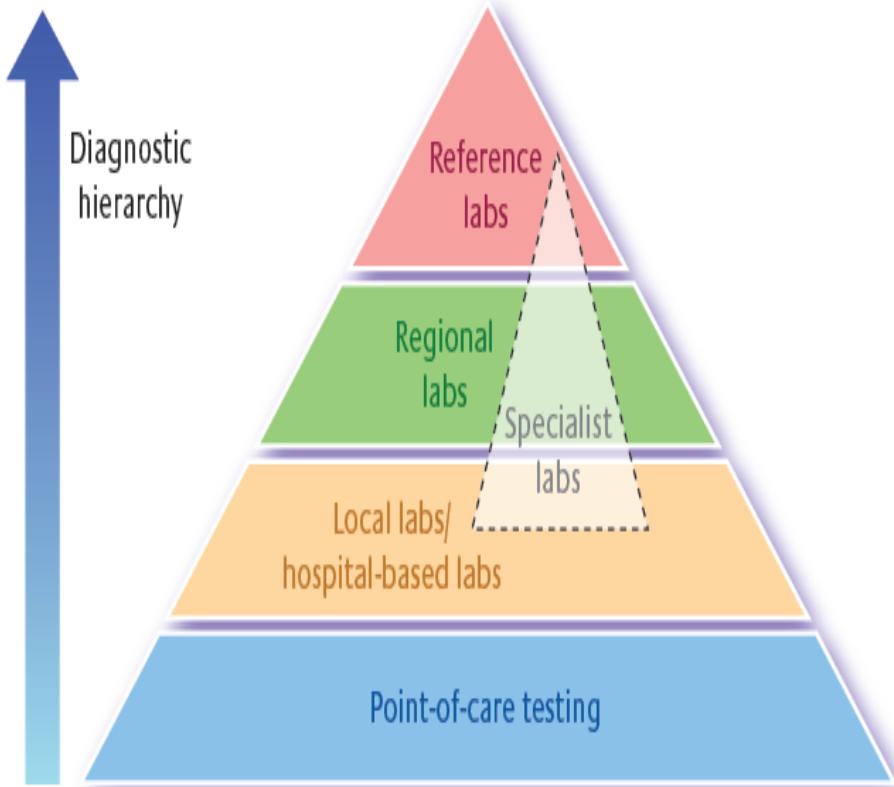


Outbreak investigation
Population study
Research

What makes a Bacterial Typing Tool ‘Right’?

- Typeability & Discriminatory power
- Reproducibility & Stability over time
- Cost effectiveness
- Ease of adoption and use, Speed
- Agreed nomenclature, harmonised results
- Portability and accessibility
- Scalability
- Amenable to QC/QA

Where does typing take place? All over!



Shall we refine the question?

Choosing the Right (Bioinformatics) Tool for the Job?!

What's between typing and mobile phones?



1G
1981

2G
1992

3G
2001

4G
2011

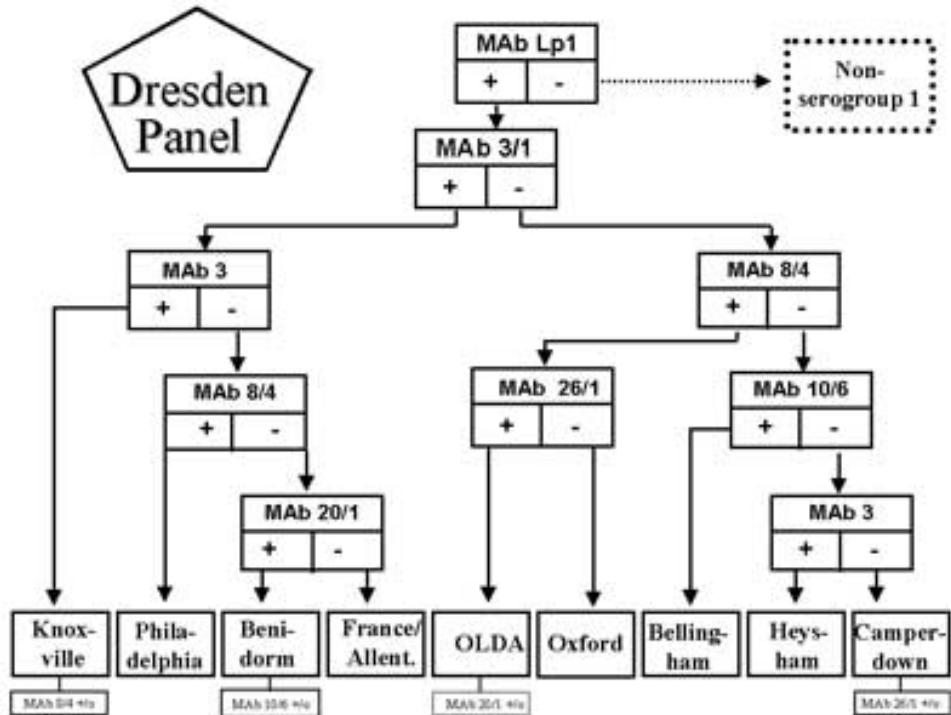
5G
20?

Microbiological challenges - *Legionella pneumophila*

- A significant cause of waterborne infections
- Explosive outbreaks, a ‘celebrity’ disease
- Difficult to control, highly regulated
- Heavy reliance on traditional methods
- Clinical diagnostic challenges
- Cluster investigation challenges
- International networking paramount
- Need to ‘personalise’ risk assessment



Evolution of Lp typing – mAbs



Evolution of Lp typing - RAPD

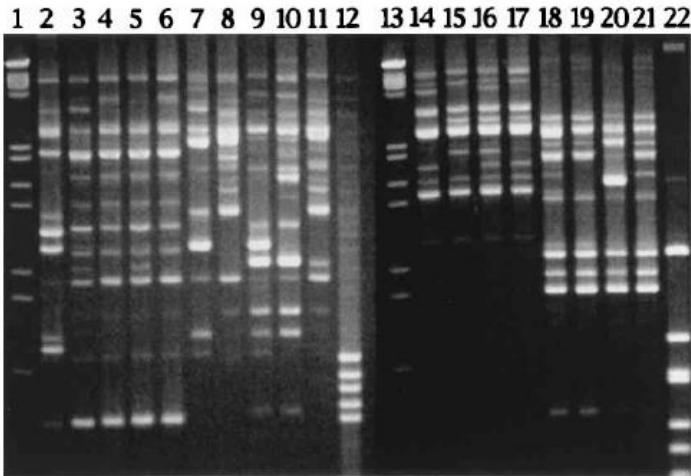


FIG. 3. RAPD fingerprint profile of *L. pneumophila* serogroup 1 reference subtypes. In lanes 2 to 11 a combination of random primers I and L was used. In lanes 14 to 17 primer C was used. In lanes 18 to 21 primer D was used. Lanes: 1 and 13, molecular weight markers (bacteriophage lambda DNA cut with *Eco*RI plus *Hind*III); 2, Allentown; 3, 14, and 18, Oxford; 4, 15, and 19, Camperdown; 5, 16, and 20, Heysham; 6, 17, and 21, OLDA; 7, Benidorm; 8, France; 9, Knoxville; 10, Bellingham; 11, Philadelphia; 12, molecular weight marker (pBR322 DNA cut with *Hae*III); and 22, molecular weight marker (pBR322 DNA cut with *Hinf*I plus *Eco*RI).

Evolution of Lp typing - AFLP

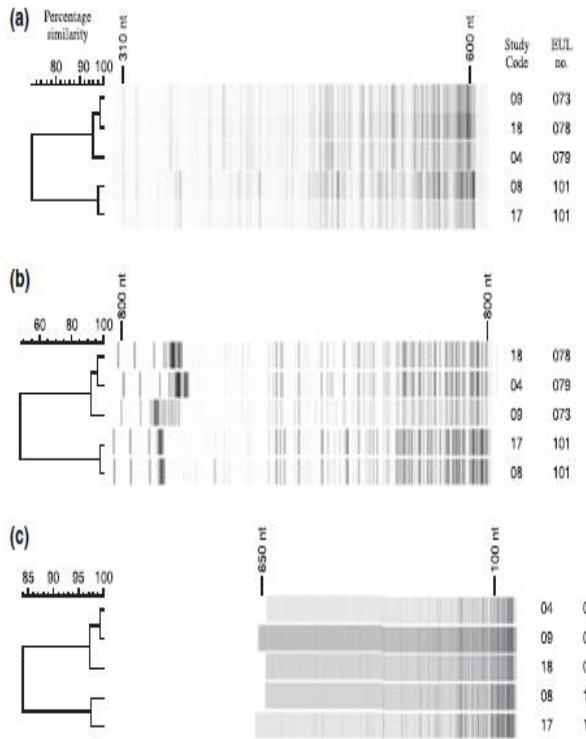


Fig. 1. Examples of normalised patterns obtained following fAFLP analysis of *Legionella pneumophila* sg 1 isolates from the reproducibility and epidemiologically related panels. Study code nos. 8 and 17 are replicates of the same strain. Study codes nos. 4, 9 and 18 are a set of epidemiologically related strains. (a) ALF Express (gel). (b) ABI Prism 3100 (capillary). (c) CEQ 8000 (capillary). nt, nucleotide.

Evolution of Lp typing - PFGE



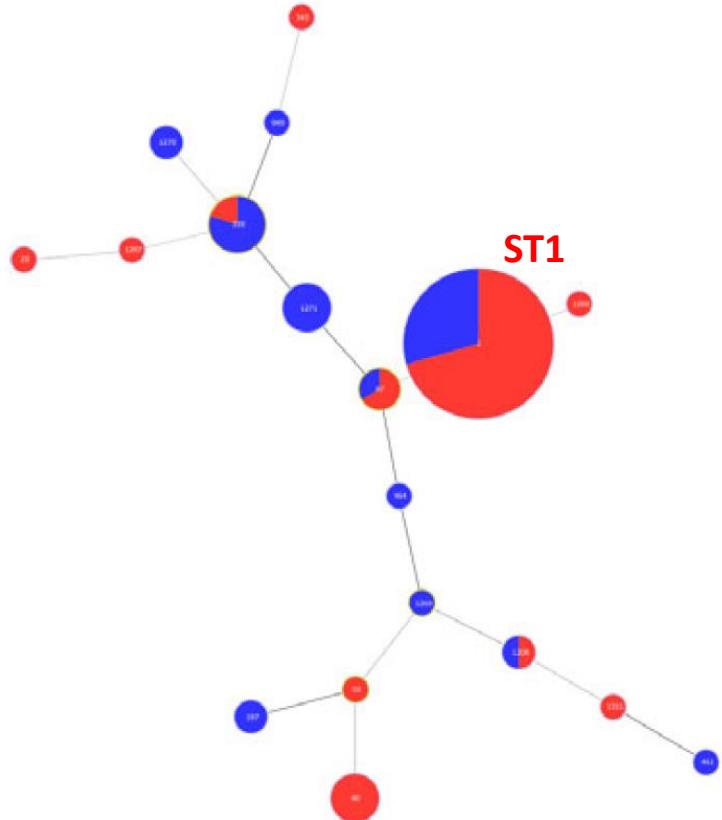
TABLE 4. Molecular typing characters of 11 isolates from five different water systems

Strain	Water system	PFGE type		SBT	Ribotype (RiboPrinter)	RAPD type	MLVA type (Lp1-Lp3-Lp13-Lp17-Lp19- Lp31-Lp33-Lp34-Lp35)
		AsCI	SfI				
JX1	1	PAT1	PST1	1(1-4-3-1-1-1)	EcoRI 413-147-S-1	RT1	MT1 (7-7-10-2-4-9.5-4-2-17)
JX4	1	PAT1	PST1	1(1-4-3-1-1-1)	EcoRI 413-147-S-1	RT1	MT1 (7-7-10-2-4-9.5-4-2-17)
JX5	1	PAT2	PST2	1(1-4-3-1-1-1)	EcoRI 413-147-S-1	RT1	MT1 (7-7-10-2-4-9.5-4-2-17)
Qin1	2	PAT3	PST3	1(1-4-3-1-1-1)	EcoRI 413-147-S-1	RT1	MT1 (7-7-10-2-4-9.5-4-2-17)
Qin5	2	PAT3	PST3	1(1-4-3-1-1-1)	EcoRI 413-147-S-1	RT1	MT1 (7-7-10-2-4-9.5-4-2-17)
GX3-5	3	PAT4	PST4	630(1-4-3-1-1-10)	EcoRI 413-147-S-1	RT1	MT2 (7-7-10-2-4-9.5-4-2-18)
GX4-1	3	PAT4	PST4	630(1-4-3-1-1-10)	EcoRI 413-147-S-1	RT1	MT2 (7-7-10-2-4-9.5-4-2-18)
FS24	4	PAT5	PST5	149(17-10-17-3-2-14-11)	EcoRI 413-147-S-5	RT2	MT3 (8-8-10-2-4-14-2.5-3-6)
FS28	4	PAT5	PST6	155(17-10-17-28-2-14-11)	EcoRI 413-147-S-5	RT2	MT4 (8-8-8-2-5-14-2.5-3-6)
FS25	5	PAT6	PST7	154(11-14-16-16-15-13-2)	EcoRI 413-147-S-6	RT3	MT5 (8-8-3-0-0-16-4-0-8)
FS27	5	PAT6	PST8	154(11-14-16-16-15-13-2)	EcoRI 413-147-S-6	RT3	MT5 (8-8-3-0-0-16-4-0-8)

Evolution of Lp typing – The ‘EWGLI’ SBT



7 genes
flaA, pilE, asd,
mip, mompS,
proA, neuA



Evolution of Lp typing – MLVA-8

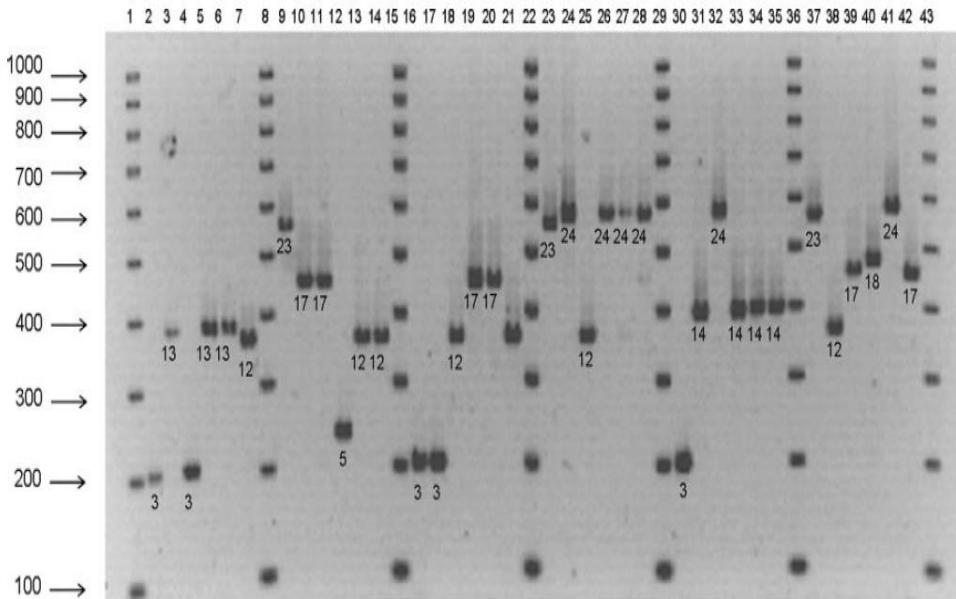


FIG. 2. Analysis of the Lpms35 marker in 30 *L. pneumophila* isolates. Philadelphia-1 (lanes 2, 16, and 30) and Lens (lanes 9, 23, and 37) reference strains were alternately used as internal controls. Lanes and test strains are, respectively: 3, EUL 101; 4, EUL 099; 5, EUL 102; 6, EUL 100; 7, EUL 103; 10, EUL 110; 11, EUL 104; 12, EUL 111; 13, EUL 105; 14, EUL 112; 17, EUL 118; 18, EUL 116; 19, EUL 119; 20, EUL 117; 21, EUL 120; 24, EUL 136; 25, EUL 121; 26, EUL 137; 27, EUL 135; 28, EUL 138; 31, EUL 141; 32, EUL 139; 33, EUL 142; 34, EUL 140; 35, EUL 143; 38, EUL 121; 39, EUL 157; 40, EUL 156; 41, EUL 137; 42, EUL 048. The 100-bp DNA size marker is shown in lanes 1, 8, 15, 22, 29, 36, and 43. The repeat unit size of Lpms35 is 18 bp. The expected sizes for Philadelphia-1 and Lens strains are, respectively, 202 bp (3 repeats, allele 3) and 562 bp (23 repeats, allele 23) (Table 3). The number of repeats is indicated under each band.

Evolution of Lp typing – Spoligotyping



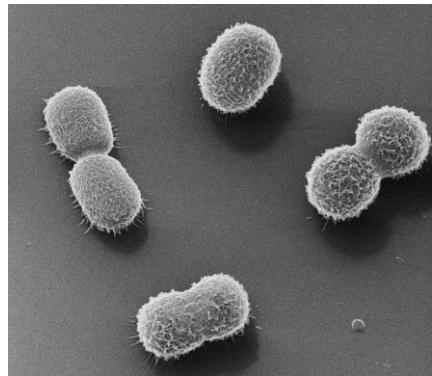
TABLE 2 Distribution of CRISPR arrays and numbers of spoligotypes in different *L. pneumophila* genotypes

Pulsotype and ST	No. of isolates			No. of spoligotypes
	Total	CRISPR positive	Spoligotyping positive	
Paris				
ST1	233	233	233	41
Non-ST1	15	15	14	4
Non-Paris				
ST1	46	45	45	7
Non-ST1	112	11	0	0
All isolates	406	304	292	46 ^a

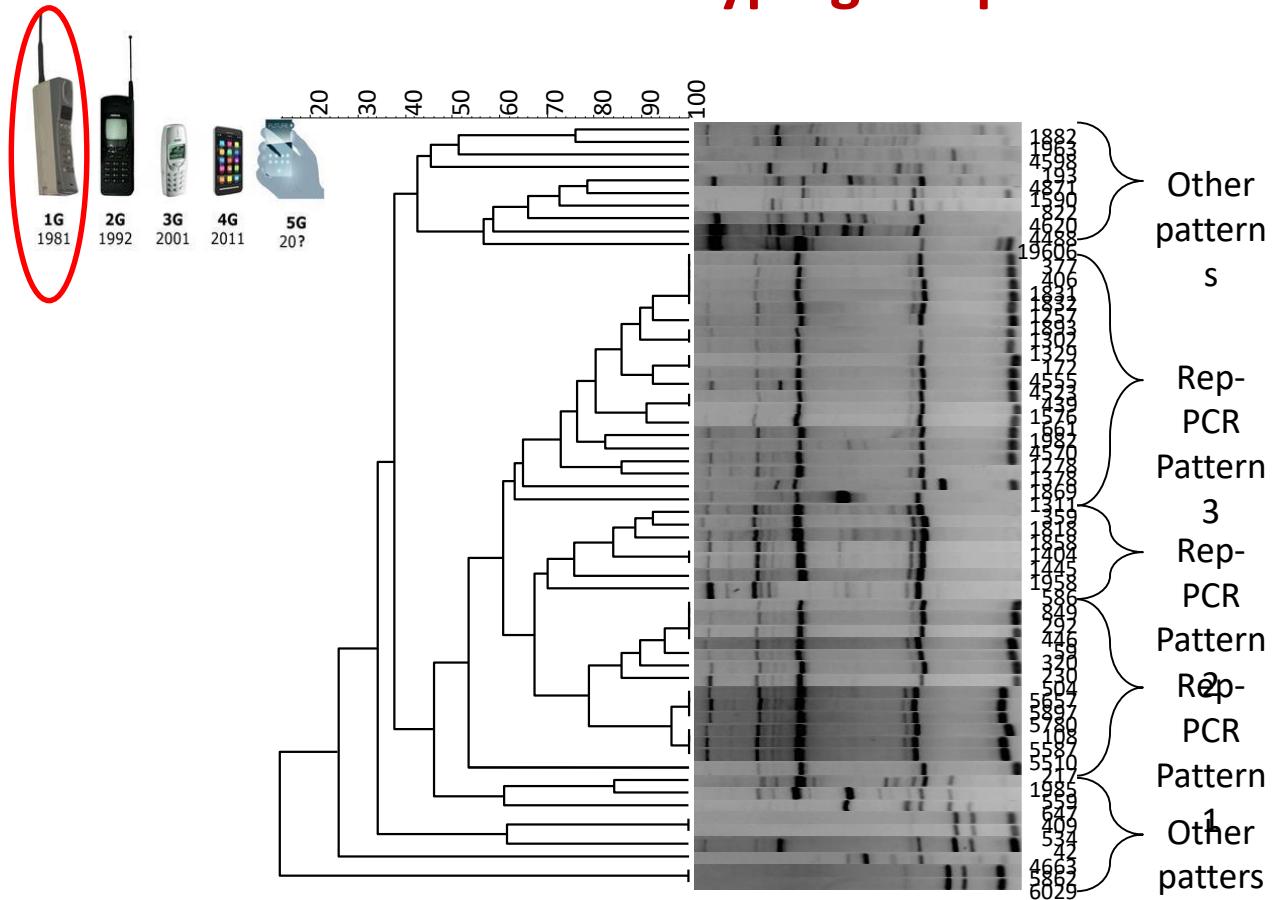
^a Some spoligotypes were shared by isolates from the four different populations.

Microbiological challenges - *Acinetobacter spp.*

- A significant cause of healthcare-associated infections worldwide
- High morbidity and mortality
- Difficult-to-treat – MDR / XDR / PR
- Various syndromes; unpredictable clinical course
- Traditional micro methods – limited
- Complex hospital epidemiology
- Suboptimal typing schemes



Evolution of Ab typing – rep-PCR



Evolution of Ab typing - AFLP

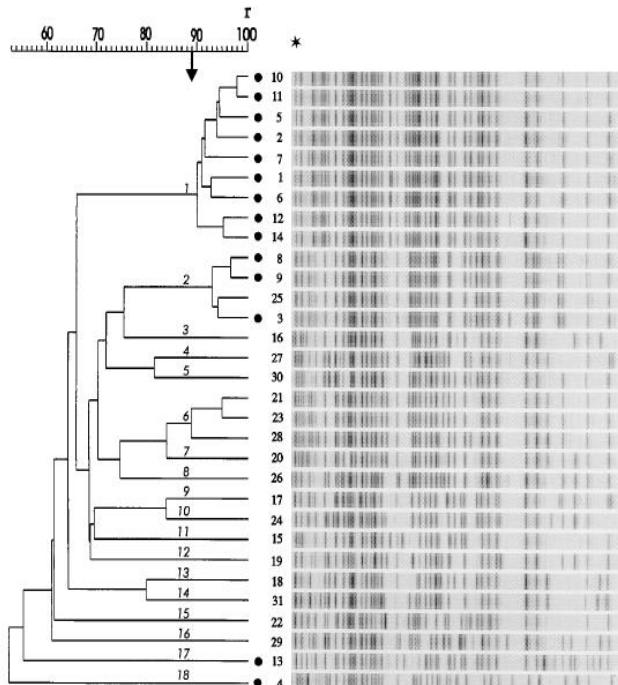


FIG. 1. Digitized AFLP patterns and dendrogram of grouping according to similarity of outbreak (●) and nonoutbreak *A. baumannii* strains. Strains are designated with the serial numbers given in Table 1. Clustering was done with GelCompar by using UPGMA linkage of correlation coefficients. The arrow denotes the cutting level for separation clusters and single strains. The asterisk indicates the top of the gel.

Evolution of Ab typing - PFGE

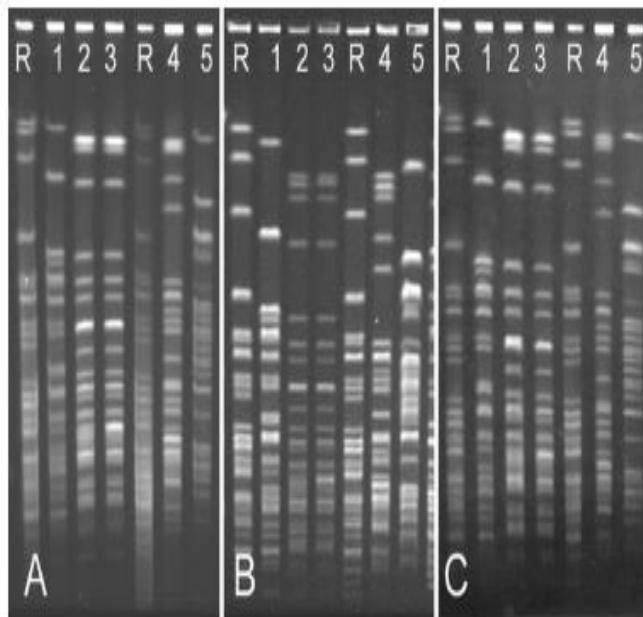


FIG. 2. PFGE profiles of representative *A. baumannii* isolates (lanes 1 to 5) digested with Apal and obtained by using a highly standardized protocol for PFGE, as performed in laboratories A to C (panels A to C, respectively). Lanes R, *A. baumannii* COL 20820, which was the external reference standard. See Table 1 for details about the strains.

Evolution of Ab typing - MLVA

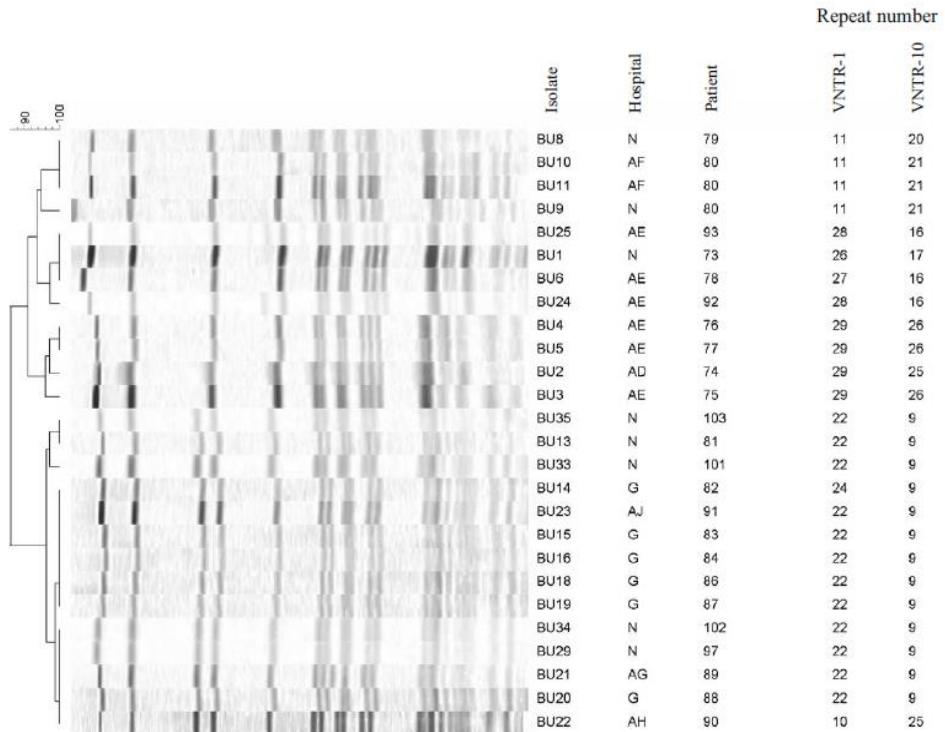


Fig. 1 Pulsed-field gel electrophoresis profiles of *Apal*-digested genomic DNA of representatives of the Burns Unit strain, together with their repeat numbers at VNTR-1 and VNTR-10. Isolates with

highly similar profiles could be distinguished on the basis of their repeat numbers at these loci. All isolates clustered within 86% similarity

Evolution of Ab typing – MLST (Oxford)

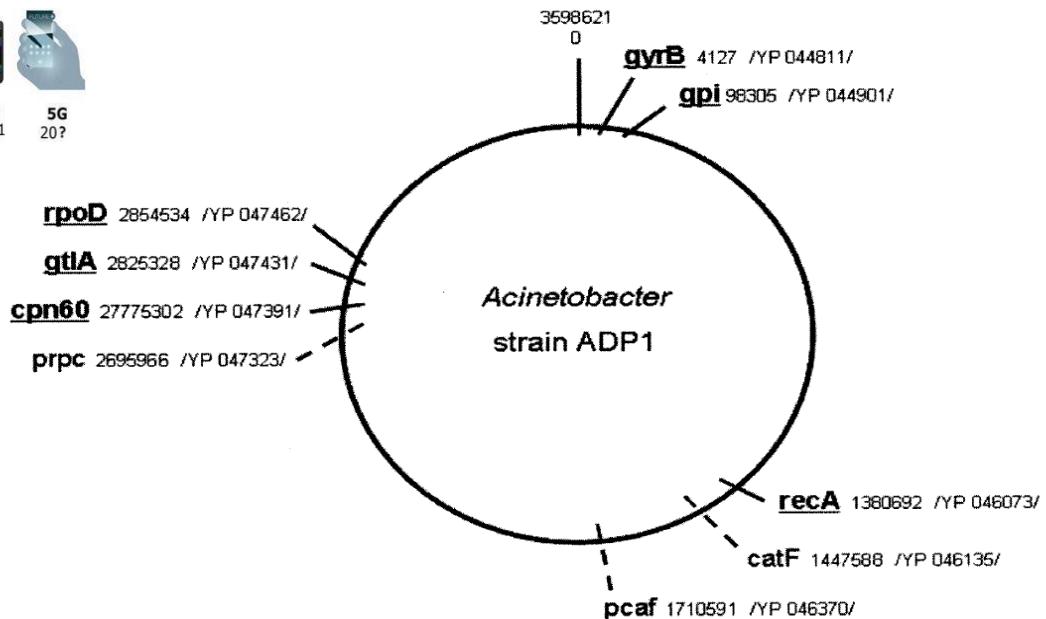
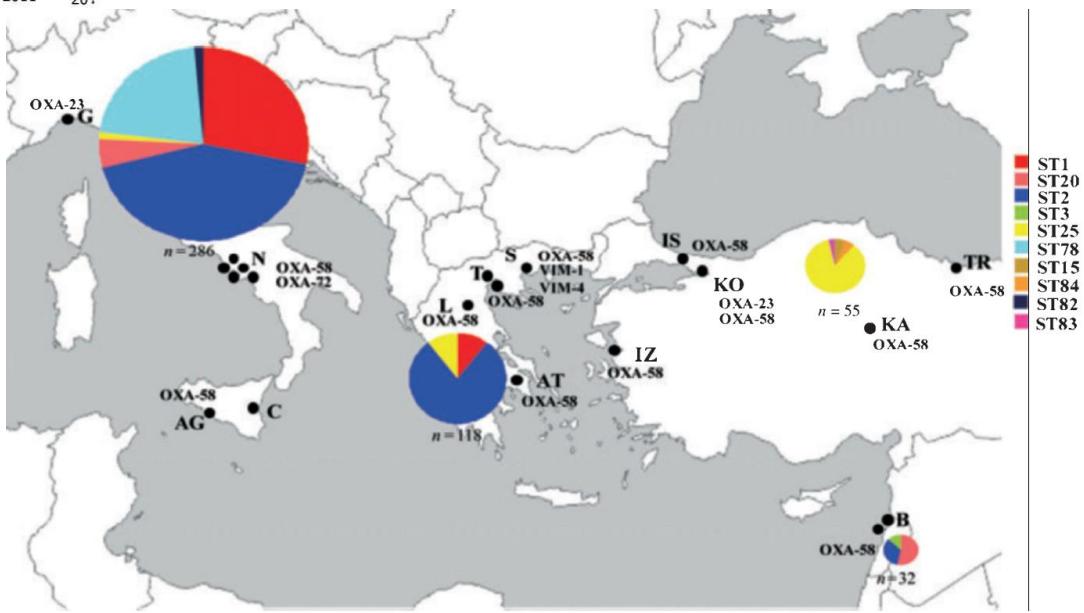


FIG. 2. Relative location of the selected housekeeping genes used in the present study on a schematic map of the genome of *Acinetobacter* strain ADP1 (NC_005966.1). The *A. baumannii* *cpn60* gene is homologous to chaperone Hsp60 from ADP1. Genes used in the present MLST scheme are underlined. The coordinates are given in bases, and the protein accession numbers are indicated.

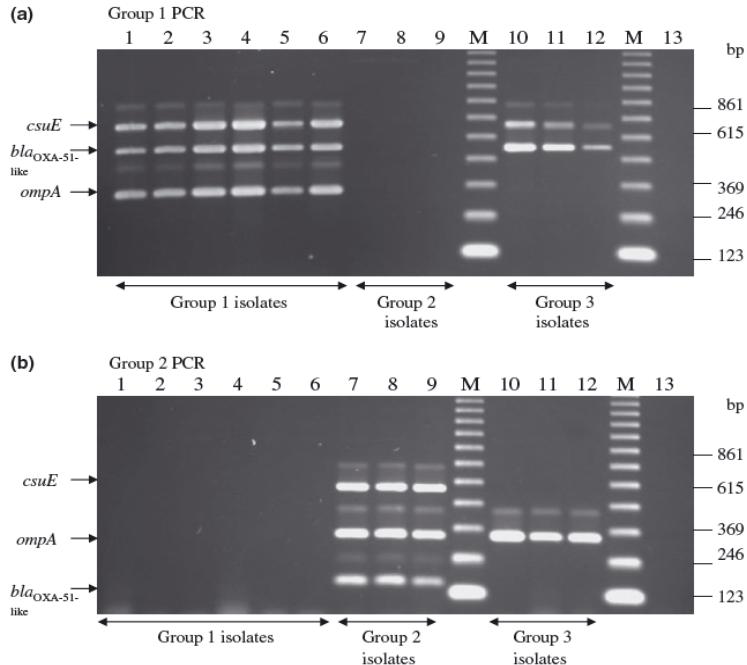
Evolution of Ab typing – MLST (Pasteur)



Evolution of Ab typing – 3LT (Poor man's MLST)



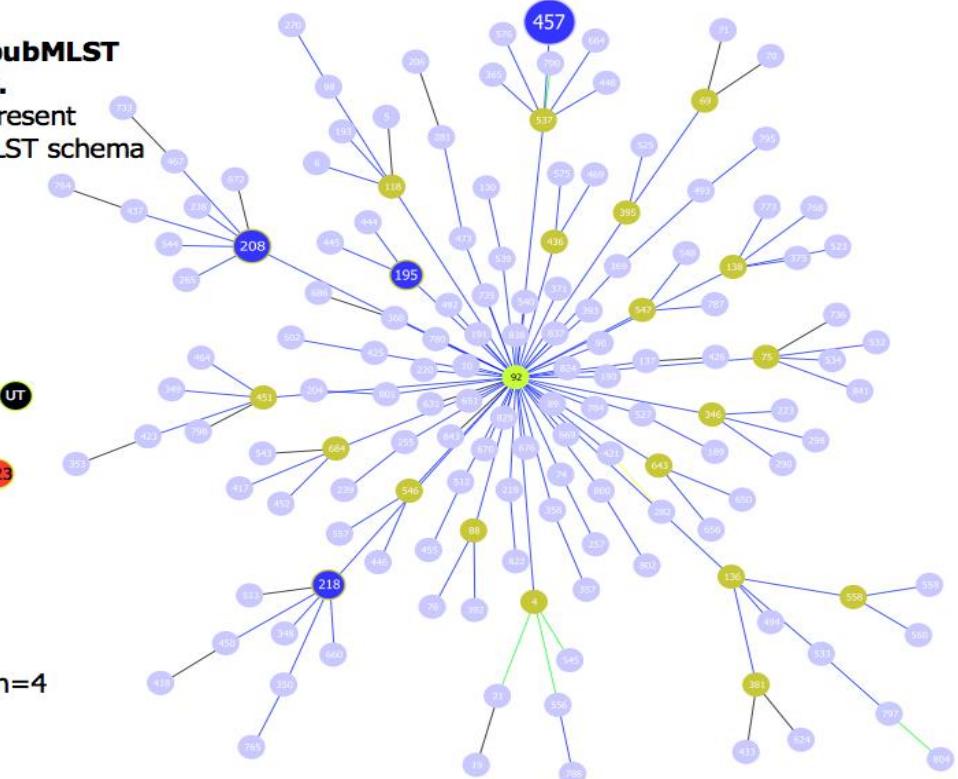
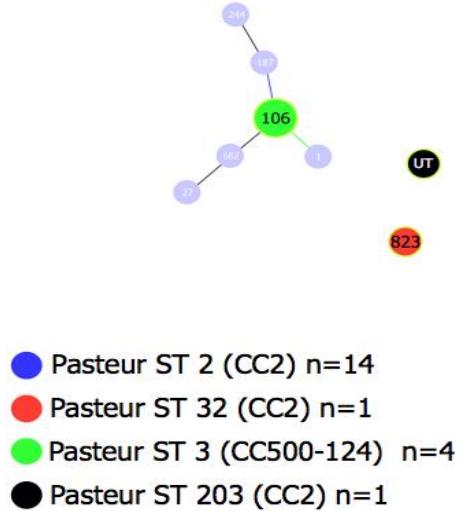
Fig. 4. Examples of multiplex PCRs designed to selectively amplify *ompA*, *csuE* and *bla_{OXA-51-like}* alleles of isolates belonging to (a) Group 1 and (b) Group 2. Genotypes and country of isolation were: lanes 1–6 (Group 1), Strain A (Israel), SE clone, 24AC-1, NW strain (UK), clone II (Spain), T strain (UK); lanes 7–9 (Group 2), AYE VEB-1 (France), OXA-23 clone 2, W strain (UK); lanes 10–12 (Group 3), Midlands 2 (UK), clone I, clone I (Spain). Strains in lanes 1, 3, 4, 5, 6, 7, 9, 10, 11 and 12 were Israel 1, 24, 33, 5/28, 4A, AYE VEB-1, 1, 4C, 5/5 and 9/46, respectively. Lanes labelled M contain a 123-bp size ladder; a negative (water) control is contained in lane 13.



Evolution of Ab typing – MLST comparison

goeBURST representation of pubMLST CC92, CC106 and 2 singletons.

Colors identified in the legend represent the ST classification by Pasteur MLST schema for the 20 strains in the study



Evolution of Ab typing – ‘Tower of Babylon’

Table 2

Clonal lineages of multidrug-resistant *Acinetobacter baumannii* and their worldwide distribution.

AFLP	Pasteur's MLST ^a	PubMLST ^a	3LST	DiversiLab™	References
I	CC1 (14)	CC109 (18)	SG2	WW1	[6,10,11,13,19,22,43]
II	CC2 (13)	CC92 (45)	SG1	WW2	[6,10,11,13,19,22,43]
III	CC3 (4)	CC110 (8)	SG3	WW3	[1,10–13,19,22,43]
	ST25		SG4	WW7	[14,19,23,43]
Cluster A	CC15 (5)	CC103 (6)	SG5	WW4	[6,10,13,14,19,22,40,43]
	ST78		SG6	WW6	[18,19,24,43]
Cluster B	CC10 (3)		Allelic profile 3/2/8 ^b	WW8	[6,13,14,19,43]
Cluster 6	CC32 (4)				[6,13]
Cluster C	ST52				[6,13]
	CC79 (11)	CC113 (18)			[41,42]

AFLP, amplified fragment length polymorphism; MLST, multilocus sequence typing; 3LST, trilocus sequence-based typing; CC, clonal complex; ST, sequence type; SG, sequence group; WW, worldwide clone.

^a CCs are numbered according to the most prevalent clone. STs are indicated when singletons. The numbers of STs identified for each CC by eBURST analysis of MLST databases are indicated in parenthesis.

^b No SG was assigned to strains with allelic profile 3/2/8 because they were microepidemic strains [23].

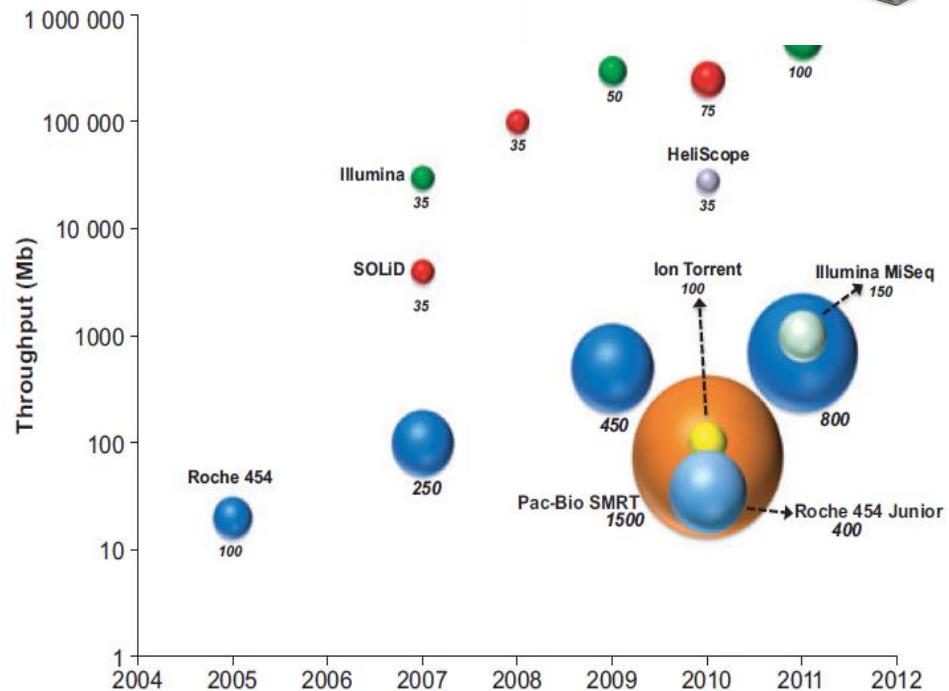
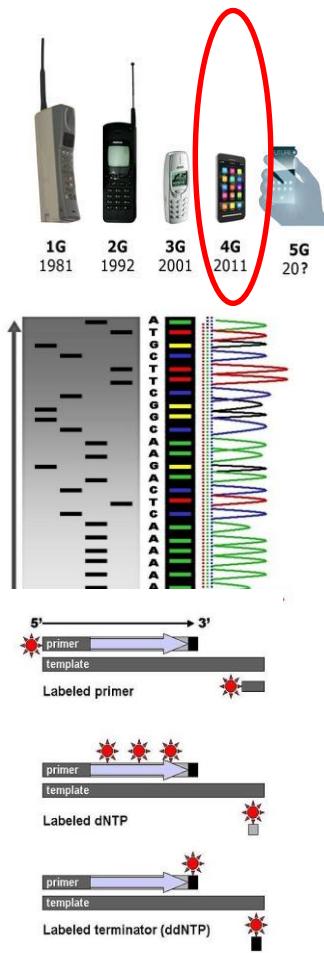
One ring to rule them all



The One Ring is a gold band with a single line of Elvish script engraved on its inner surface. The script is written in a cursive, flowing font, typical of the Tengwar used by the Elves in Middle-earth. The ring is set against a dark background, making the glowing yellow-gold of the metal and the bright script stand out.

One ring to rule them all

Evolution of typing - NGS



NGS - a Disruptive Technology

Quality, speed, accessibility and costs
of extraction and sequencing are rapidly
improving BUT

Sample
Processing

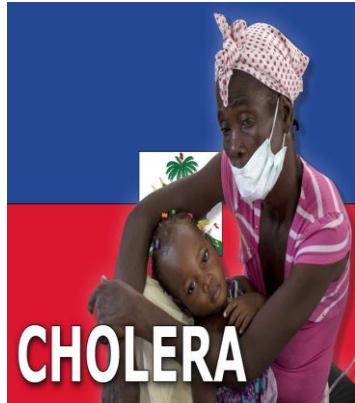
NGS
Platforms

Bioinformatics, IT
infrastructure



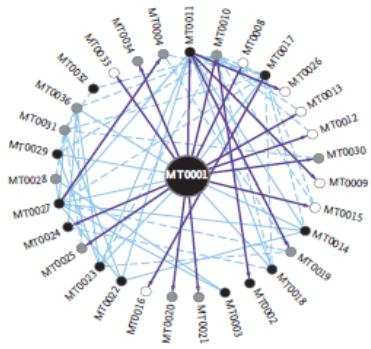
Bioinformatics is
emerging as the
major bottleneck

NGS Application in Public Health Micro

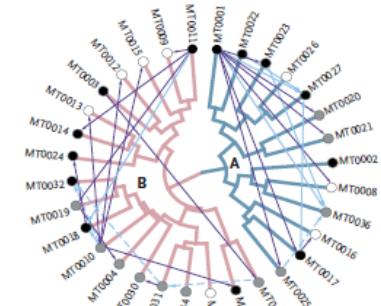


Importation following a disaster

A MIRU.VNTR and Social-Network Analysis



B Whole-Genome Sequencing and Social-Network Analysis



Cracking a TB outbreak in Canada

2011 O104 STEC outbreak



Contribution of *Eurosurveillance* to the body of knowledge on WGS typing

Automated extraction of typing information for bacterial pathogens from whole genome sequence data:
Neisseria meningitidis as an exemplar

C A Jolley¹, M C Maiden (martin.maiden@zoo.ox.ac.uk)¹

Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011

C W Knetsch¹, T R Connor², A Mutreja³, S M van Dorp¹, I M Sanders¹, H P Browne², D Harris³, L Lipman⁴, E C Keessen⁴, J Corver (j.corver@lumc.nl)¹, E J Kuijper¹, T D Lawley³

A multi-country *Salmonella Enteritidis* phage type 14b outbreak associated with eggs from a German producer: 'near real-time' application of whole genome sequencing and food chain investigations, United Kingdom, May to September 2014

T Inns (thomas.inns@phe.gov.uk)¹, C Lane², T Peters², T Dallman², C Chatt³, N McFarland⁴, P Crook⁵, T Bishop⁶, J Edge⁶, J Hawker³, R Elson², K Neal², G K Adak², P Cleary¹, on behalf of the Outbreak Control Team⁷

Monitoring meticillin resistant *Staphylococcus aureus* and its spread in Copenhagen, Denmark, 2013, through routine whole genome sequencing

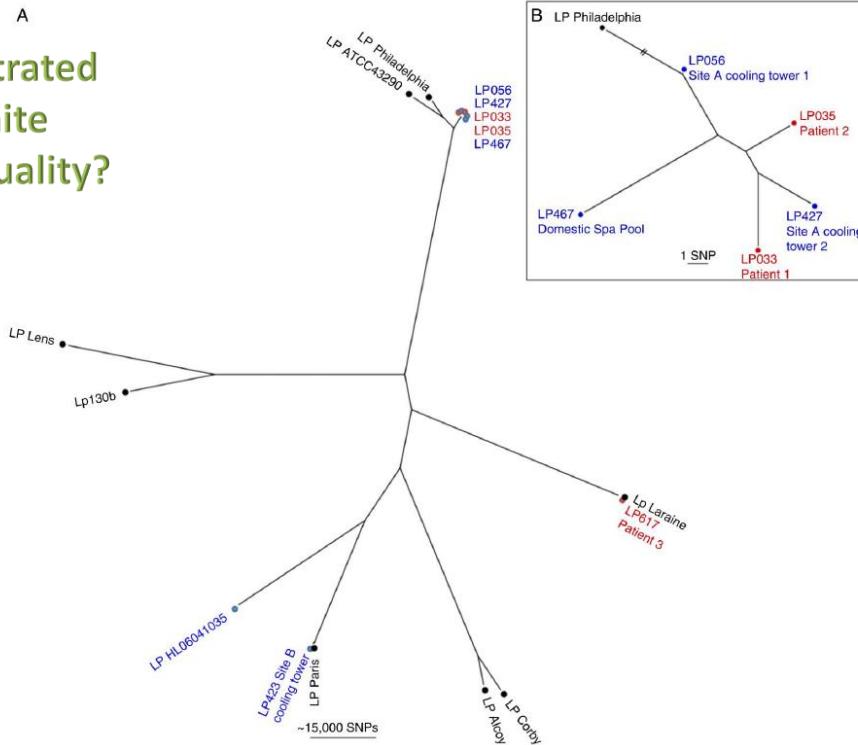
M D Bartels (mette.damkjaer@dadlnet.dk)^{1,2}, H Larner-Svensson^{2,3}, H Meinicke³, K Kristoffersen³, K Schønning^{1,4}, J B Nielsen^{1,3}, S M Rohde², L B Christensen², A W Skibsted³, J O Jarløv⁵, H K Johansen⁶, L P Andersen⁷, I S Petersen⁸, D W Crook⁵, R Bowden⁹, K Boye¹, P Worning¹, H Westh^{1,3,4}

Epidemiological investigation of *Pseudomonas aeruginosa* isolates from a six-year-long hospital outbreak using high-throughput whole genome sequencing

L A Snyder^{1,2}, N J Loman¹, L A Faraj³, K Levi⁴, G Weinstock⁵, T C Boswell⁴, M J Pallen (m.pallen@warwick.ac.uk)⁶, D A Al-Adeen³

NGS Application for LD Investigation - England

A
Feasibility demonstrated
Source not definite
Hampered by seq quality?



NGS Application for LD Investigation – Australia

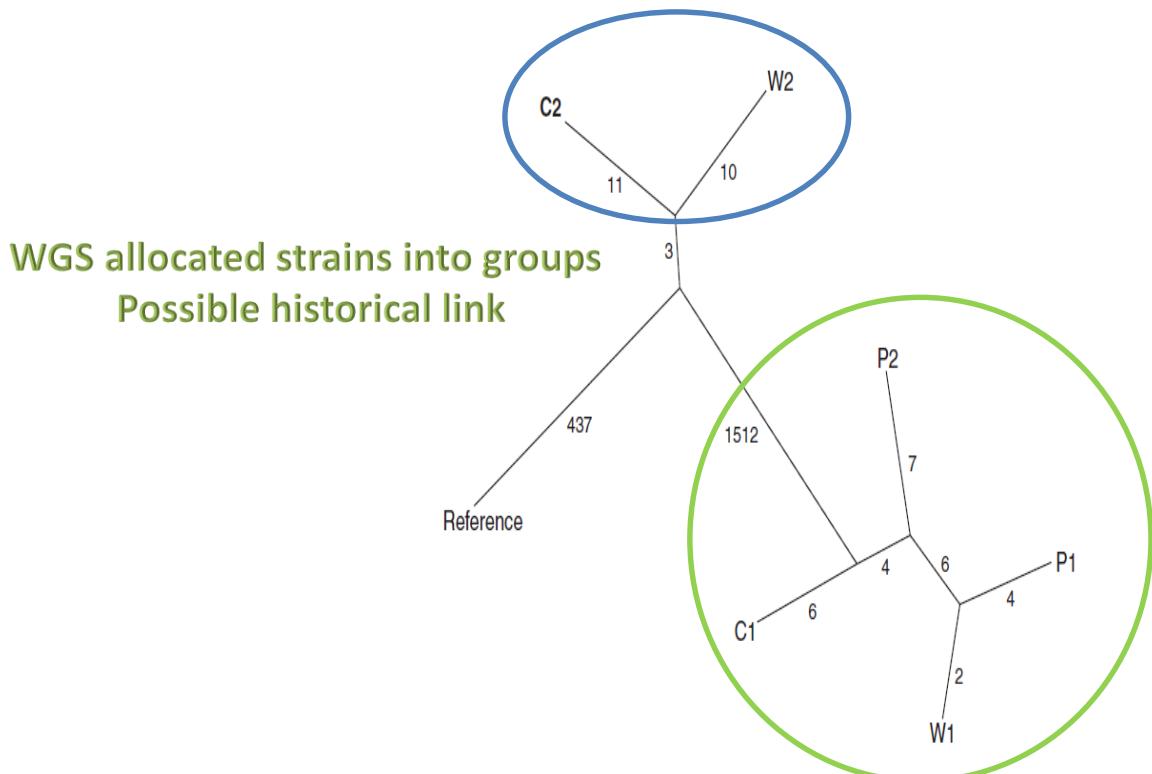


Fig. 1. Maximum-likelihood tree of *L. pneumophila* isolate single nucleotide polymorphisms (SNPs). Branch numbers indicate the number of SNPs.

NGS Application for LD Investigation - Canada

WGS confirmed PFGE and SBT results
All pointing out a likely source

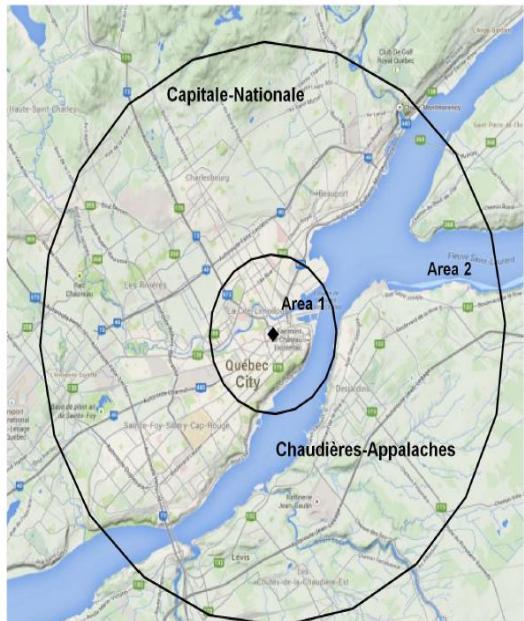
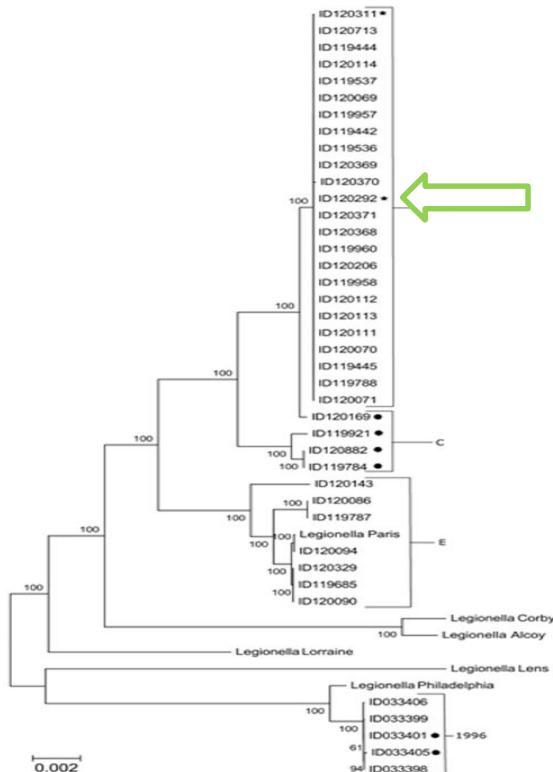
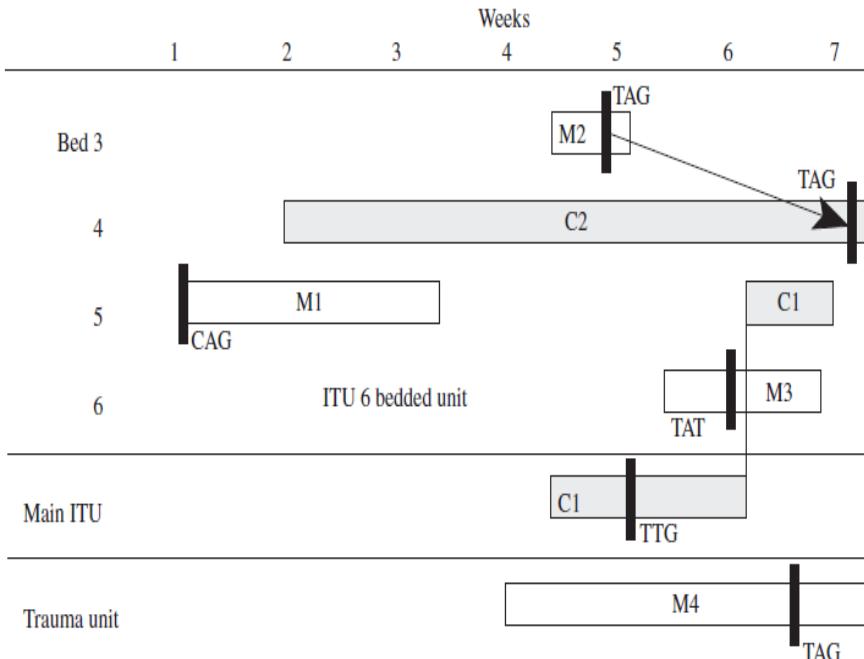


Figure 5. Mapping of the legionellosis cases around the outbreak source. Ninety six percent (96%) of the cases were within 3 km (area 1) of the cooling tower responsible for the outbreak (identified by the black dot). The remaining cases were within 11 km of the outbreak source (area 2).
doi:10.1371/journal.pone.0103852.g005



NGS Application for Ab investigation



WGS confirmed the chain of nosocomial transmission amongst ICU patients despite identical PFGE and MLST

Table II

Single nucleotide polymorphism (SNP) loci which vary between outbreak isolates

	SNP loci		
	1	2	3
Locus tag	AB57_2551	AB57_2001	AB57_1823
SNP coordinate	2645863	2093446	1906419
Predicted product	Two-component heavy metal response regulator	Hypothetical protein	Transcriptional regulator, AraC family
Predicted SNP effect	Synonymous	Non-synonymous (E to V)	Premature termination at codon 203
Alleles			
AB0057	C	A	G
M1	C	A	G
M2	T ^a	A	G
M3	T ^a	A	T ^a
M4	T ^a	A	G
C1	T ^a	T ^a	G
C2	T ^a	A	G

The corresponding annotation for each locus is shown for the ancestral strain AB0057.

^a Alleles demonstrate variation from the ancestral state.

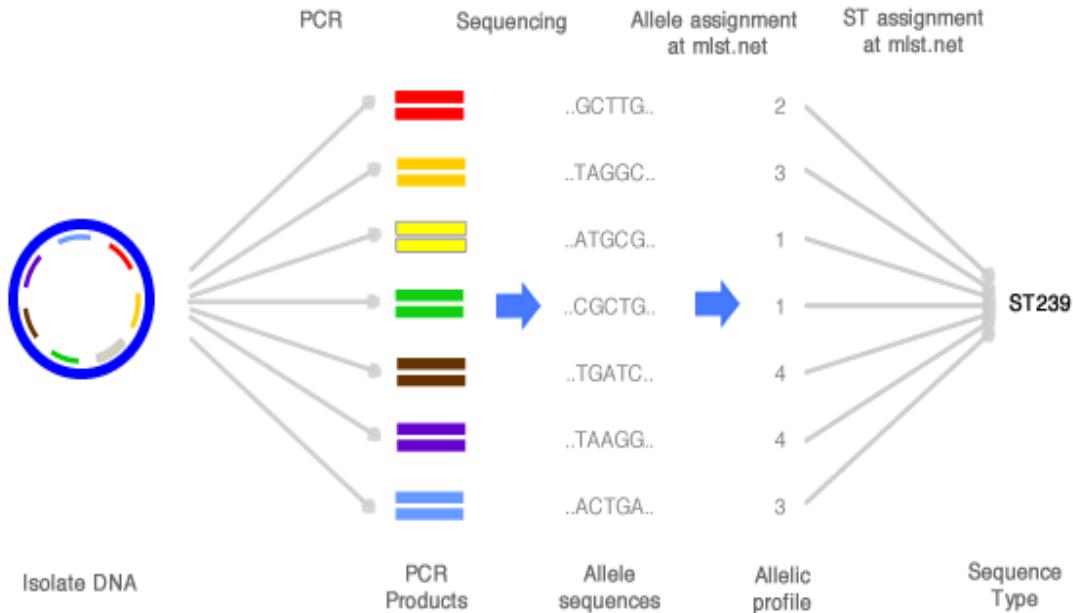
NGS Application for Ab investigation

TABLE 4 Numbers of discrepancies between whole-genome sequencing and PFGE for paired-strain comparisons

Organism	No. of strains							
	Indistinguishable		Closely related		Possibly related		Different	
	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS
VRE	55	9	0	81	0	8	0	18
MRSA	5	15	0	23	0	58	0	35
<i>Acinetobacter baumannii</i>	4	2	12	32	4	23	0	28
All organisms	64	26	12	136	4	89	0	81

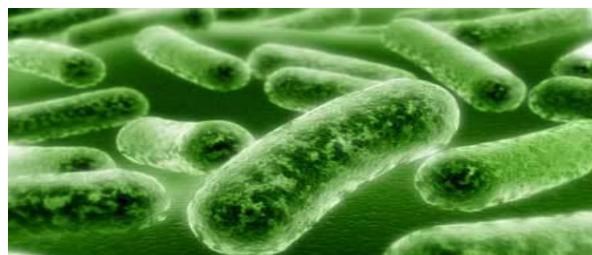
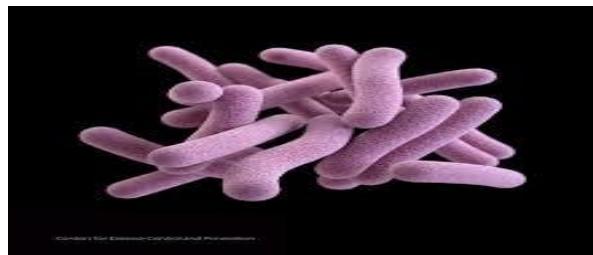
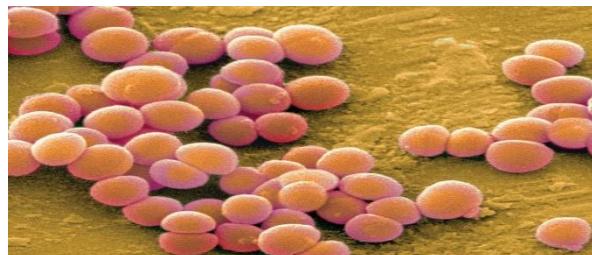
Poor correlation between PFGE and
WGS clonal analysis

NGS Application in Public Health Micro



A gene-by-gene approach is portable, scalable and standardised

Current cgMLST Schemes



Development of cgMLST scheme for Lp

TABLE 1

Finished genomes^a and assembled raw reads^b used for *Legionella pneumophila* core genome definition (n=17)

Strain	SBT BAPS cluster	SBT ST (by Sanger sequencing)	Mean coverage	NCBI/EBI accession number
Philadelphia ^c	13	36	N/A	NC_002942.5
Lens	2	15 ^d	N/A	NC_006369.1
Lorraine	3	47	N/A	NC_018139.1
Paris	6	1 ^d	N/A	NC_006368.1
Alcoy	11	57 ^d	N/A	NC_014125.1
Corby	11	51	N/A	NC_009494.2
H093620212	1	46 ^d	350.32	ERR315646
H090500162	4	61 ^d	447.33	ERR315652
RR08000760	5	37 ^d	359.47	ERR315654
H093380153	7	179	38.27	ERR315657
H100260089	8	44	486.84	ERR315660
Lansing-3	9	336	354.57	ERR315662
H063280001	10	23	387.44	ERR315663
H070840415	12	59 ^d	463.52	ERR315666
H044500045	13	28 ^d	520.93	ERR315669
H074360710	14	68 ^d	418.93	ERR315671
H091960009	15	707 ^e	391.10	ERR315672

BAPS: Bayesian analysis of population structure; EBI: European Bioinformatics Institute; N/A: not applicable; NCBI: National Center for Biotechnology Information; SBT: sequence-based typing; ST: sequence type.

^a Finished genomes were from the NCBI database.

^b Assembled raw reads were from EBI.

^c Reference genome.

^d Extraction of *mompS* allele from genomic data not possible due to multi-copy occurrence.

^e Whole genome sequencing analysis corrected erroneous allelic profile of ST707 compared with original publication [24].

TABLE 2

Finished genomes^a and assembled raw reads^b used for *Legionella pneumophila* core genome validation (n=21)

Strain	SBT BAPS cluster	SBT ST (by Sanger sequencing)	Mean coverage	% MLST ^c good targets	NCBI/EBI accession number
Thunder Bay	13	187 ^e	N/A	99.34	NC_021350
HL06041035	7	734 ^c	N/A	98.29	NC_018140
ATCC43290	13	187	N/A	99.67	NC_016811
LPE509	Not known	New ST (3,10,1,1,-1,-9,1) ^d	N/A	99.67	NC_020521
H053260229	1	74	72.66	97.76	ERR315647
H043940028	2	84	379.34	98.42	ERR315648
LP617	3	47	83.46	98.82	ERR164430
Ho64180002	3	62	73.28	96.98	ERR315651
H065000139	3	54	283.37	97.57	ERR315650
H063920004	3	47	271.57	98.82	ERR315649
H071260094 ^f	5	87	485.82	98.29	ERR315653
LP423	6	1	46.26	98.75	ERR164431
EUL00013	6	5	364.53	98.75	ERR315655
H074360702	6	152 ^c	343.23	98.62	ERR315656
RR08000517	7	337 ^c	339.81	97.24	ERR315658
LC6674	9	154 ^c	356.65	96.32	ERR315661
LC6451	10	78	74.39	97.63	ERR315664
H091960011	11	454 ^c	433.78	98.62	ERR315665
H075160080	12	188	388.03	99.01	ERR315667
H034680035	13	37	84.00	97.96	ERR315668
RR08000134	14	34	435.23	99.80	ERR315670

BAPS: Bayesian analysis of population structure; EBI: European Bioinformatics Institute; N/A: not applicable; NCBI: National Center for Biotechnology Information; SBT: sequence-based typing; ST: sequence type.

^a Finished genomes were from the NCBI database.

^b Assembled raw reads were from EBI.

^c Extraction of *mompS* allele from genomic data not possible due to multi-copy occurrence.

^d Ordered in accordance with SBT scheme [21]: *flaA*, *pilE*, *asd*, *mlp*, *mompS*, *proA*, *neuA*.

^e Wrongly stated as LC6677 in Underwood et al. [24].

Development of cgMLST scheme for Lp

TABLE 3

Whole genome sequencing data of *Legionella pneumophila* strains included in the study^a

Strain	Source	Epidemiological context	SBT ST (by Sanger sequencing)	Mean coverage	Contig count	MLST-good targets %	ENA accession number
Lp-001	Clinical	Unrelated case; ST4o 'outgroup' strain	40	131.51	43	99.54	ERR593560
Lp-012	Clinical	Unrelated case	23 ^b	48.09	69	98.75	ERR593561
Lp-032	Environmental	Routine inspection; ST1 'outgroup' strain	1	43.61	70	98.29	ERR593562
Lp-56207	Clinical	Case 1; epidemiologically linked to strain Lp-2002694p8	1	93.20	66	98.55	ERR594281
Lp-2002694p7	Environmental	Case 1; concurrent isolate from humidifier; unrelated 'innocent bystander'	40	50.53	39	99.74	ERR593569
Lp-2002694p8	Environmental	Case 1; Isolate from humidifier; last stage in transmission chain	1	74.11	57	98.62	ERR593570
Lp-119	Environmental	Case 2; Isolate from humidifier; last stage in transmission chain	1	77.40	367	98.29	ERR632205, ERR632206
Lp-120	Environmental	Case 2; Isolate from domestic water filtering device; middle stage in transmission chain	1	49.73	92	98.49	ERR593565
Lp-121	Environmental	Case 2; Isolate from domestic water; initial stage in transmission chain	1	34.62	89	98.49	ERR593566
Lp-122	Environmental	Case 2; Isolate from domestic water filtering device's filter; middle stage in transmission chain	1	109.62	409	98.22	ERR593567, ERR593568
Lp-282-1	Environmental	Case 3; Isolate from domestic water; middle stage in transmission chain	1	68.83	113	98.75	ERR593571
Lp-283	Environmental	Case 3; Isolate from domestic water; initial stage in transmission chain	1	42.20	77	98.22	ERR593572
Lp-284	Environmental	Case 3; Isolate from domestic water filtering device's filter; middle stage in transmission chain	1	52.66	233	97.57	ERR593573
Lp-285	Environmental	Case 3; Isolate from domestic water filtering device's filter; middle stage in transmission chain	1	55.89	284	98.49	ERR593574
Lp-286-1	Environmental	Case 3; Isolate from humidifier; last stage in transmission chain	1	122.55	87	98.55	ERR593575

ENA: European Nucleotide Archive; MLST: multilocus sequence typing; SBT: sequence-based typing; ST: sequence type.

^a ENA study number PRJEB7140.

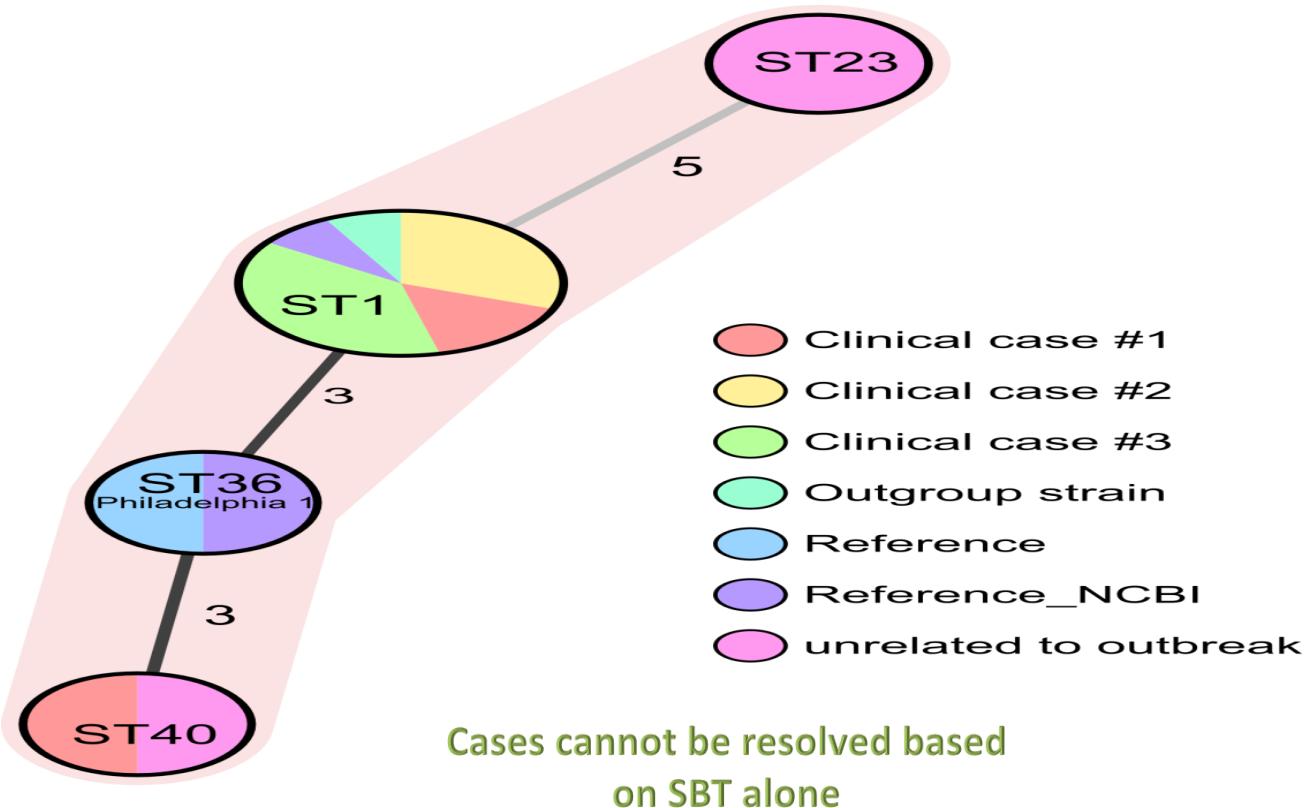
^b Extraction of *mompS* allele from whole genome sequence data not possible due to multi-copy occurrence.

Development of cgMLST scheme for Lp

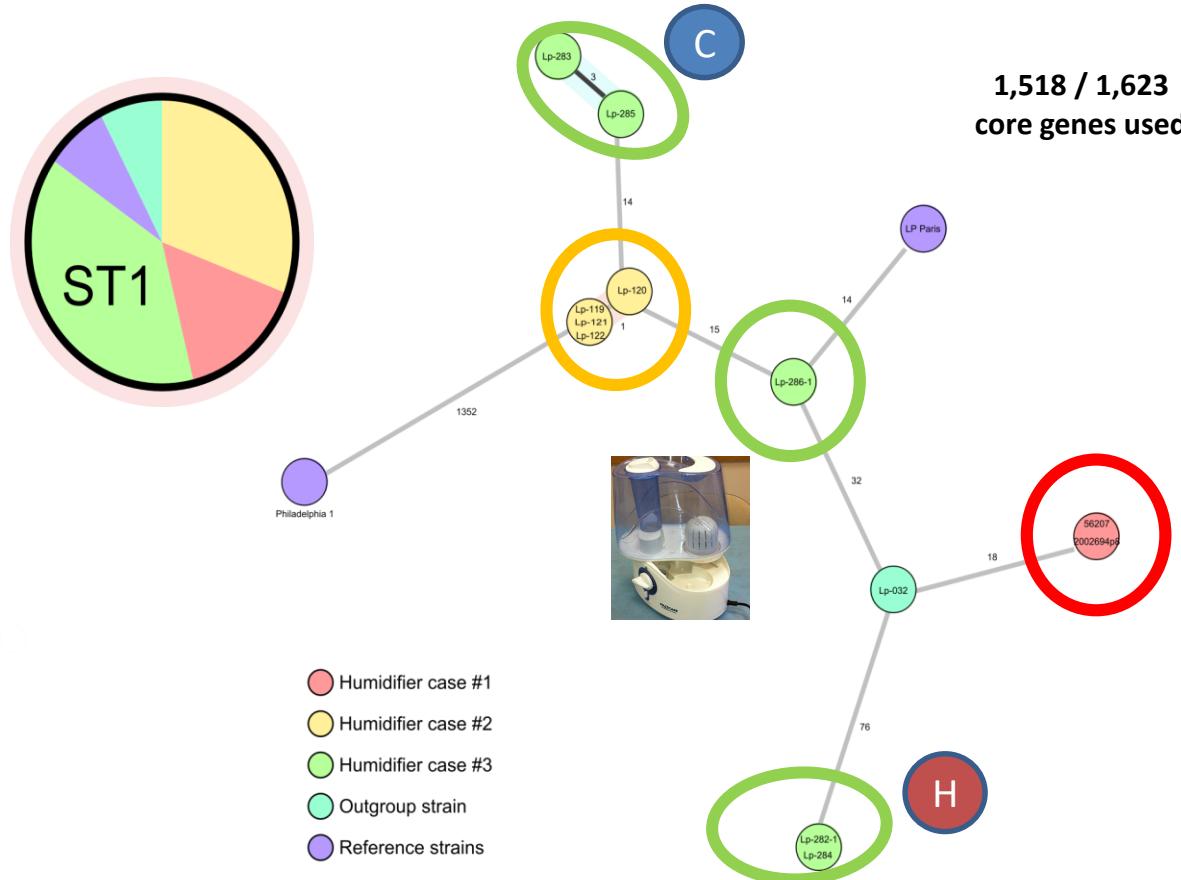


Novel chain of transmission

Development of cgMLST scheme for Lp



Development of cgMLST scheme for Lp



cgMLST changes our understanding of epi

H

282-1 (ST1)



C

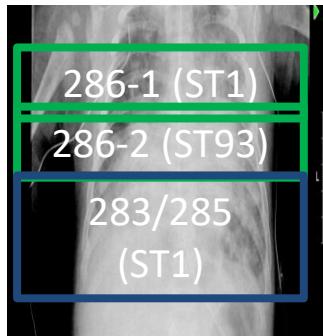
283/285
(ST1)

284 (ST1)

N

283/285
(ST1)

Triple Lp (including double ST1)
infection stemming from cold
water system feeding the filtrator
and aerosolised via humidifier



286-1 (ST1)

286-2 (ST93)

***Acinetobacter baumannii* cgMLST**

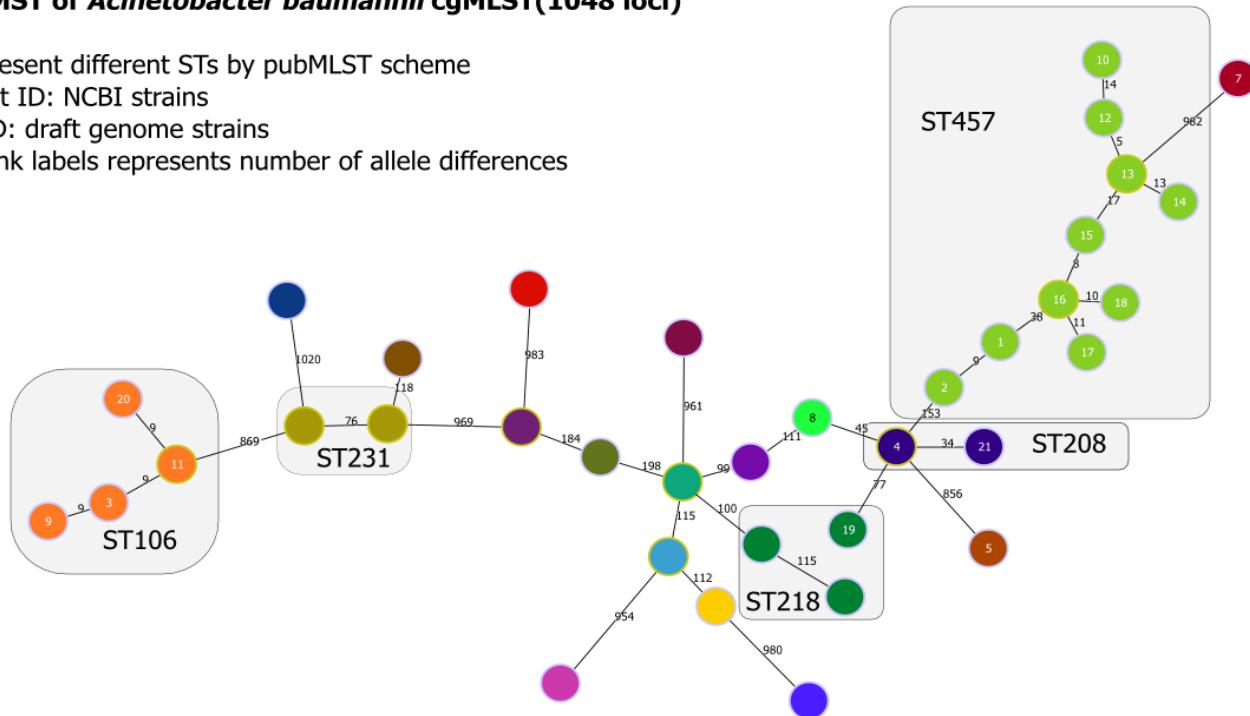
goeBURST MST of *Acinetobacter baumannii* cgMLST(1048 loci)

Colours represent different STs by pubMLST scheme

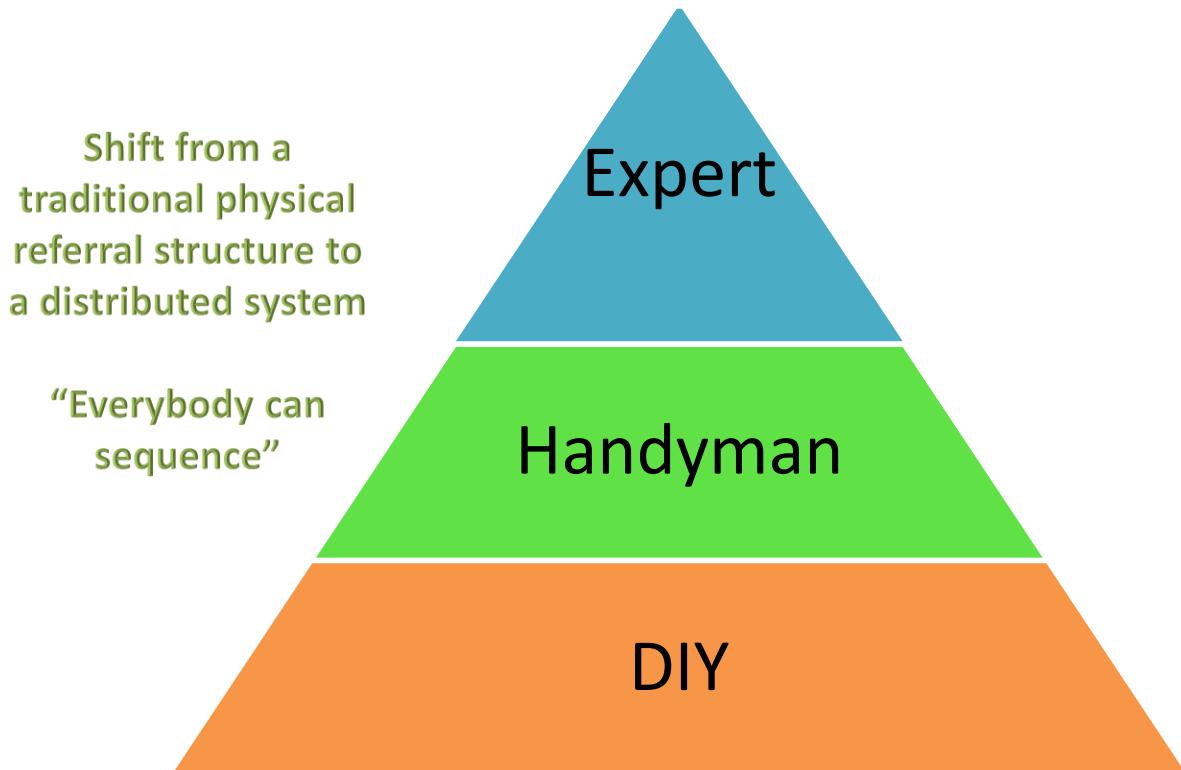
Nodes without ID: NCBI strains

Nodes with ID: draft genome strains

Numbers in link labels represents number of allele differences



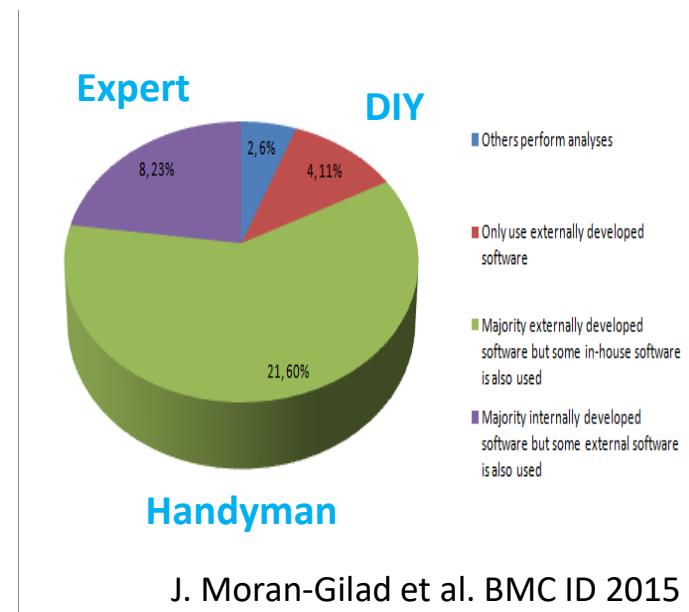
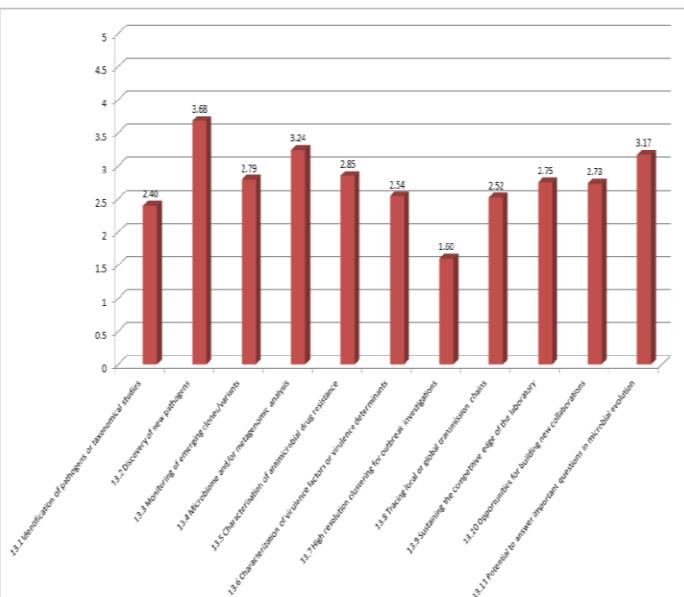
Diagnostic hierarchy in the era of NGS is driven by bioinformatics C&C



Proficiency testing for bacterial whole genome sequencing: an end-user survey of current capabilities, requirements and priorities



Jacob Moran-Gilad^{1,2}, Vitali Sintchenko^{3,4}, Susanne Karlsmose Pedersen⁵, William J Wolfgang⁶, James Pettengill⁷, Errol Strain⁷, Rene S Hendriksen^{5*} and on behalf of the Global Microbial Identifier initiative's Working Group 4 (GMI-WG4)



Current Public Health Challenges for Choosing the Right (Bioinformatics) Tools

- Adoption of **cgMLST** schemes as a **standard tool** and making them **publicly available** and widely implemented
- Continuously **optimise** cgMLST schemes (**refined** core genome, backward **compatibility**, setting thresholds for **interpretability**)
- **Build capacity** for *add-on* SNP-based analyses for enhanced resolution (micro-evolution, tree directionality)
- Ensure **quality, validation** and **harmonisation** of WGS typing (create nomenclature, proficiency testing, reference materials)
- **IT Infrastructure**, data **integration**, genome repositories
- **Human resource** - Cloning of bioinformaticians?

The ESGLI NGS WG

Remit for the ESGLI NGS WG

1. Identify and prioritise relevant public health and clinical applications of Whole Genome Sequencing (WGS) in the field of legionellosis (e.g. outbreak investigation, national and international surveillance etc.).
2. Decide which species other than *L. pneumophila* (if any) should be covered?
3. Review and compare existing genomic approaches for phylogenetic analysis, characterisation and typing of Legionella (e.g. core genome MLST, whole genome MLST, SNP mapping, other techniques or modifications of those approaches).
4. Suggest ways to create and sustain an agreed global nomenclature for Legionella strain typing and continuously validate, update and refine a WGS-based typing scheme.
5. Evaluate and harness genomic approaches to introduce fit-for-purpose, robust, reproducible and practicable analytical tools that would meet public health, clinical and medicolegal requirements.
6. Discuss the architecture, use, management and curation of a shared public WGS database and its associated metadata.
7. Create a shared Legionella genome repository accessible to WG members for scheme development purposes.
8. Explore modalities for quality control and quality assurance of legionella sequence data determination, deposition and sharing / comparison.
9. Discuss possible efforts for proficiency testing / EQA to scheme users.
10. Ensure reverse compatibility with current SBT scheme is maintained.
11. Recommend bioinformatics tools and interfaces in support of the above tasks and objectives.
12. Develop joint research initiatives

Group members:

- Kathy Bernard, Winnipeg, Canada
Alex Ensminger, Toronto, Canada
Norman Fry, London, UK
Sophie Jarraud, Lyon, France
Natalia Kozak-Muiznieks, Atlanta, USA
Christian Lück, Dresden, Germany
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Brian Raphael, Atlanta, USA
Rodney Ratcliff, Adelaide, Australia
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Bioinformatics expert advisors:

- Sophia David, Cambridge, UK
Anthony Underwood, London, UK

Future Challenges for Developing the Right Tools

- **Transferable** pipelines to support routine NGS implementation globally— **diffuse from Experts to DIY's**
- **Metagenomic** FW&E microbiology
- **Metagenomic** clinical sample analysis
- Exploit BIG data, machine learning, cloud computing for **real time global genomic epidemiology**
- Embrace, develop and train '**Public Health Informatics**' as an allied discipline

Capacity-building Workshop: Rapid NGS for Characterization and Typing of Resistant Gram-Negative Bacilli



umcg



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THANK YOU

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