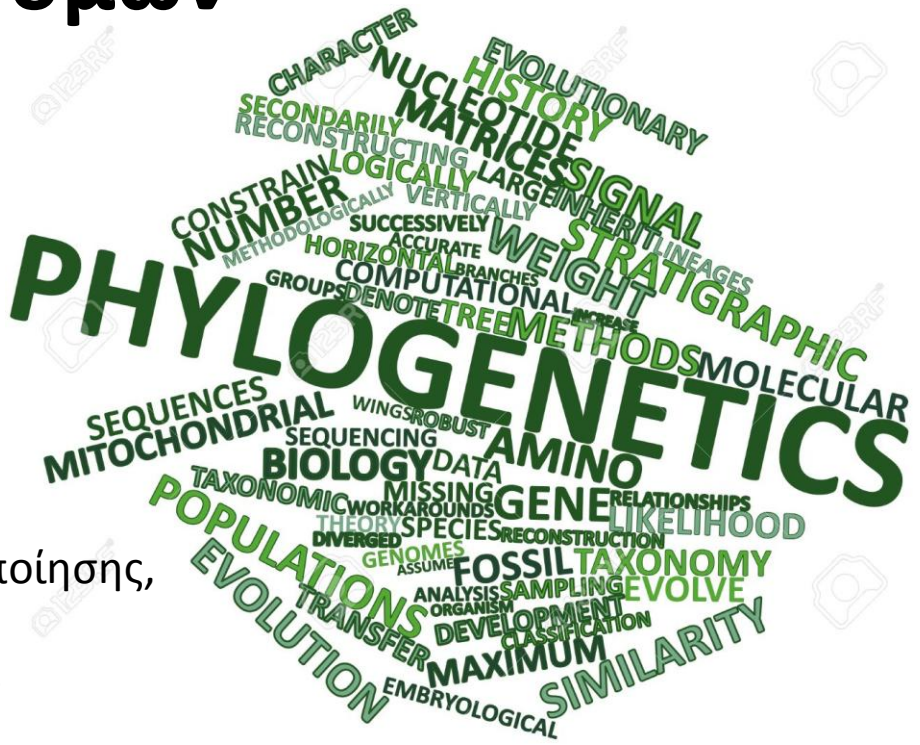
Πρόγραμμα Μεταπτυχιακών Σπουδών «Βιοϊατρικές Μέθοδοι και Τεχνολογία στη Διάγνωση» Τμήμα Ιατρικών Εργαστηρίων, Τ.Ε.Ι. ΑΘΗΝΑΣ

Μοριακός χαρακτηρισμός και φυλογενετική ανάλυση βακτηριακών πληθυσμών



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Εργαστήριο Δημόσιας Υγείας, ΚΕ.ΕΛ.Π.ΝΟ.

Βακτηριακός Πληθυσμός

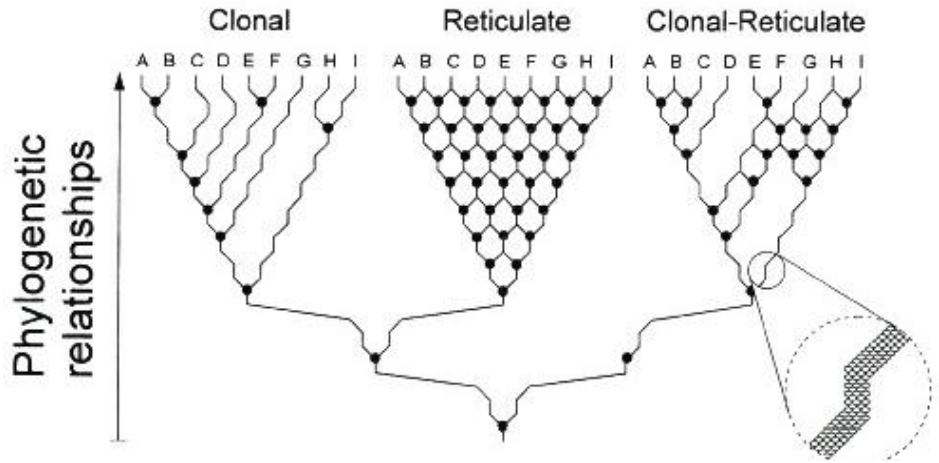
Ένας βακτηριακός πληθυσμός χαρακτηρίζεται από την φαινοτυπική και την γενετική του ποικιλομορφία

κλωνικός

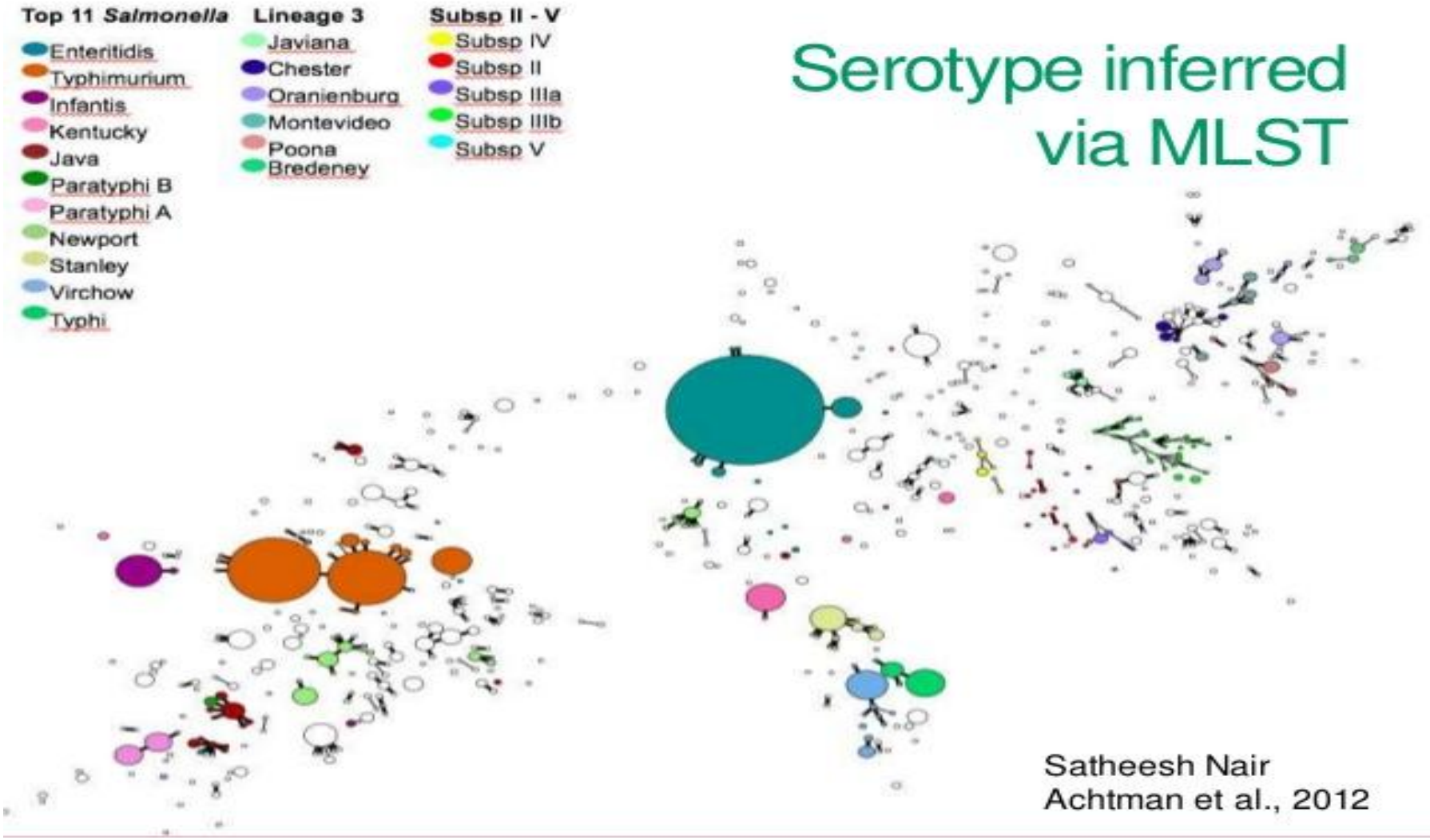
Χαρακτηρίζεται από χαμηλή ή και καθόλου γενετική ποικιλομορφία ανάμεσα στα επικρατή στελέχη του πληθυσμού σε οποιαδήποτε χρονική στιγμή π.χ. *Salmonella typhi*

μη κλωνικός

Χαρακτηρίζεται από υψηλή γενετική ποικιλομορφία ανάμεσα στα επικρατή και μη στελέχη του πληθυσμού π.χ. *Pseudomonas aeruginosa*

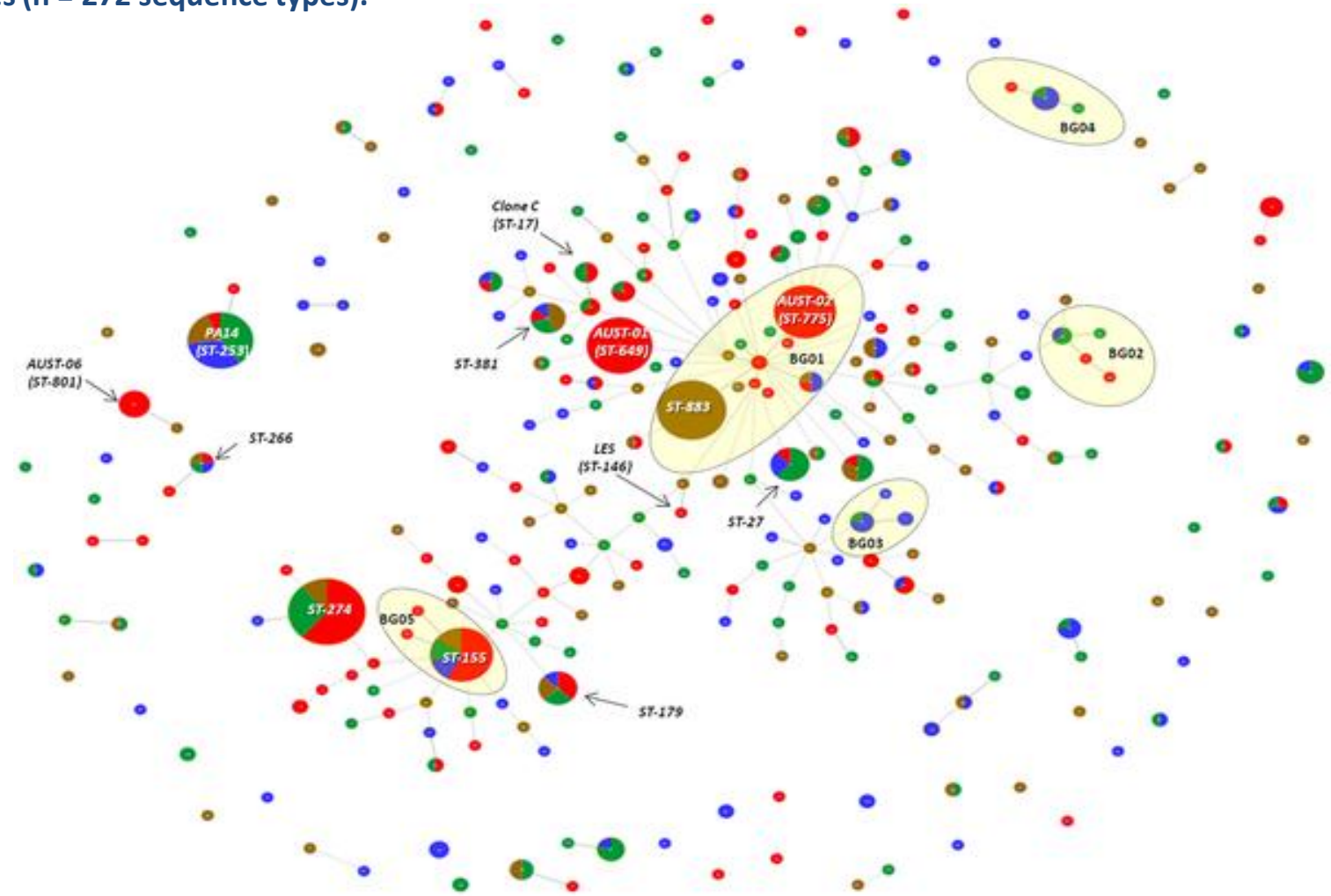


Κλωνικός Βακτηριακός Πληθυσμός

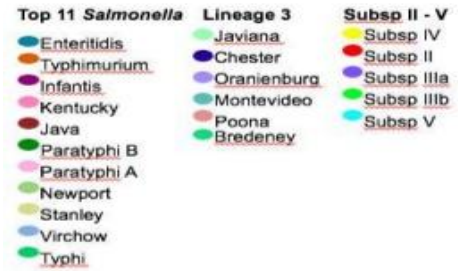


Μη Κλωνικός Βακτηριακός Πληθυσμός

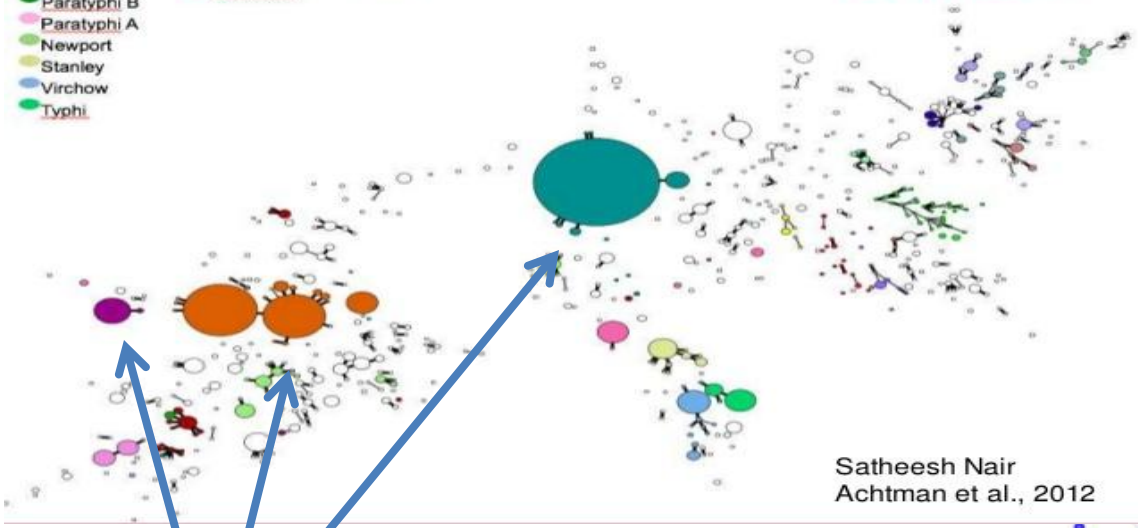
499 typeable Pseudomonas aeruginosa isolates (n = 272 sequence types).



Kidd TJ, Ritchie SR, Ramsay KA, Grimwood K, Bell SC, et al. (2012) Pseudomonas aeruginosa Exhibits Frequent Recombination, but Only a Limited Association between Genotype and Ecological Setting. PLOS ONE 7(9): e44199. doi:10.1371/journal.pone.0044199
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0044199



Serotype inferred via MLST

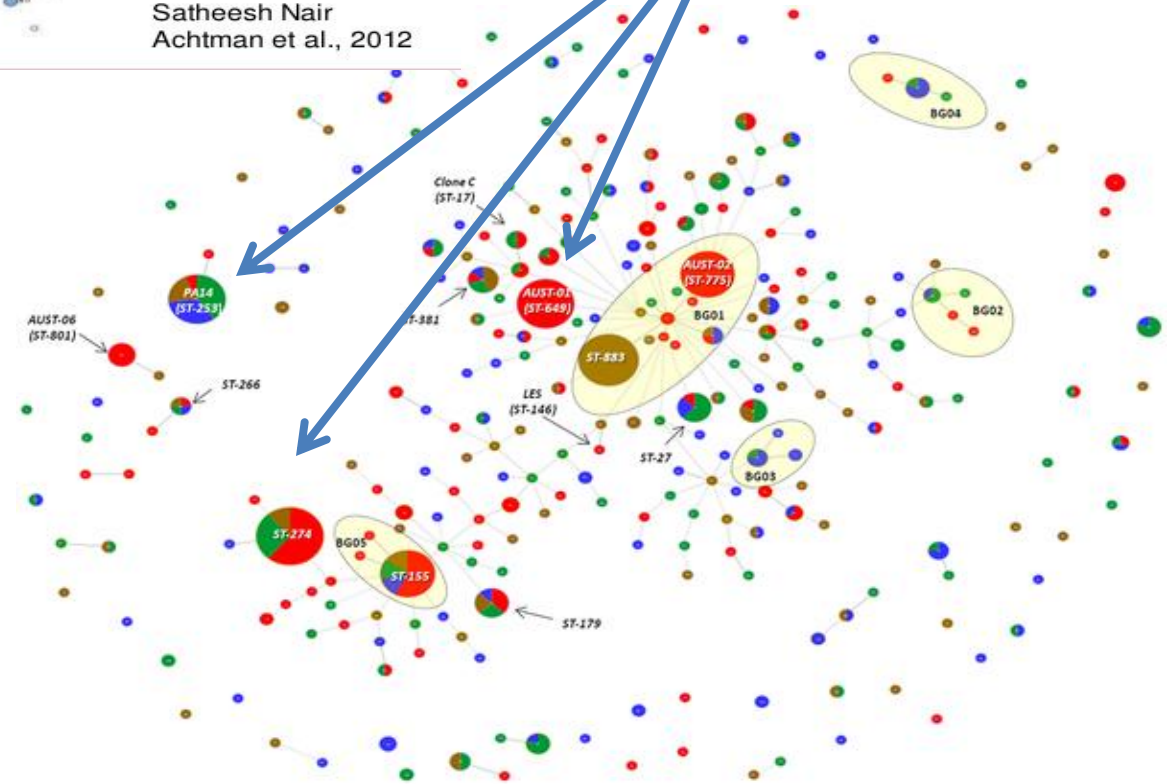


Satheesh Nair
Achtman et al., 2012

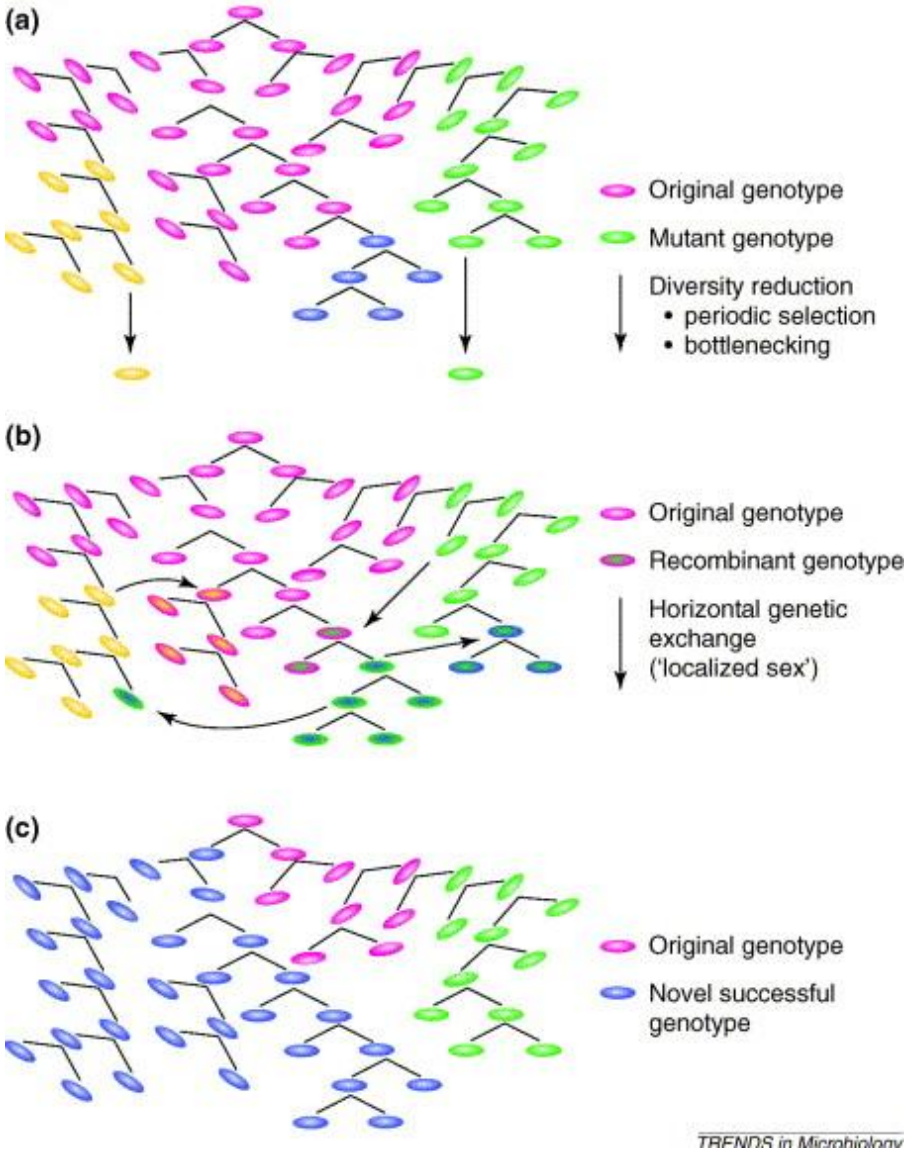
272 sequence types

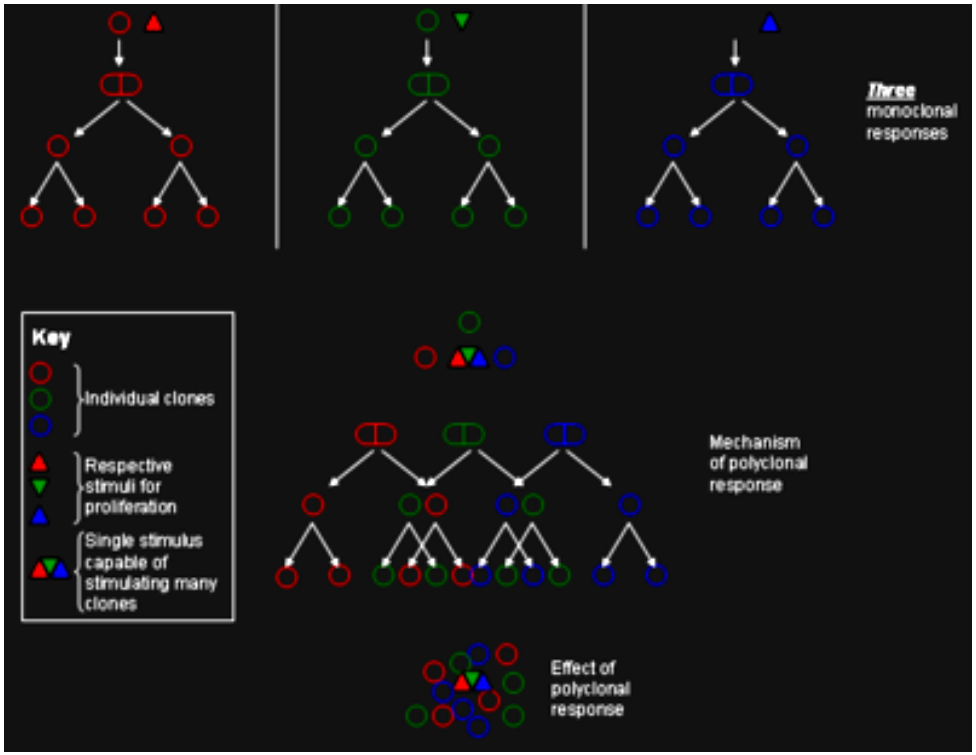
high genetic diversity

3 major (monoclonal) ST's



Κλωνικός → Μη κλωνικός





Τυποποίηση

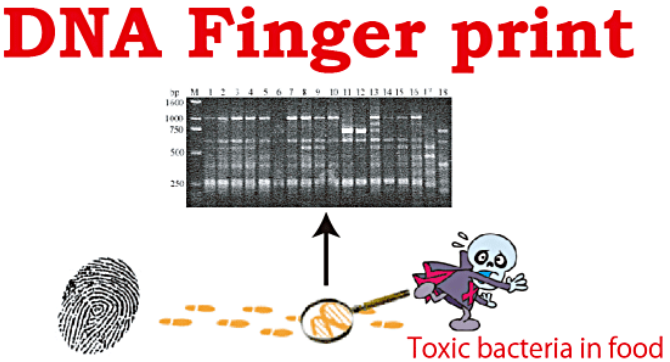
ορίζεται η διαδικασία κατά την οποία πραγματοποιείται ανάλυση φαινοτυπικών ή/και γονοτυπικών χαρακτηριστικών των στελεχών του είδους που μελετάται.

Φαινοτυπικές Μέθοδοι

- Μικροβιακή Ευαισθησία
- Αντιγονική τυποποίηση

Μοριακές Μέθοδοι

- Pulsed-field gel electrophoresis
- Multi Locus Sequence Typing
- Double Locus Sequence Typing



Φυλογενετική ανάλυση

ορίζεται η διαδικασία κατά την οποία πραγματοποιείται η μελέτη της εξέλιξης γενετικά κοντινών βακτηριακών στελεχών ενός είδους

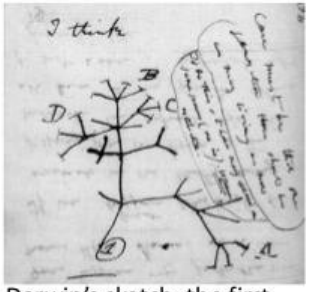
Αναλύονται οι γενετικές αλλαγές που συμβαίνουν κατά τη διάρκεια της εξελικτικής πορείας ενός πληθυσμού

Κατανοούνται οι σχέσεις μεταξύ της αρχέγονης αλληλουχίας με αυτές των απογόνων της

Γίνεται μία εκτίμηση της χρονικής στιγμής/διάρκειας κατά την οποία πραγματοποιήθηκε η γενετική αλλαγή σε ένα σύνολο στελεχών που μοιράζονται τον ίδιο πρόγονο

The goal of phylogenetics is to infer evolutionary relationships between species.

This includes both information about order of branching, .e.g., *did humans and chimpanzees share a common ancestor more recently than humans, chimps and gorillas?* And information about timing of events, e.g., *how long ago did humans and chimps share an ancestor?*



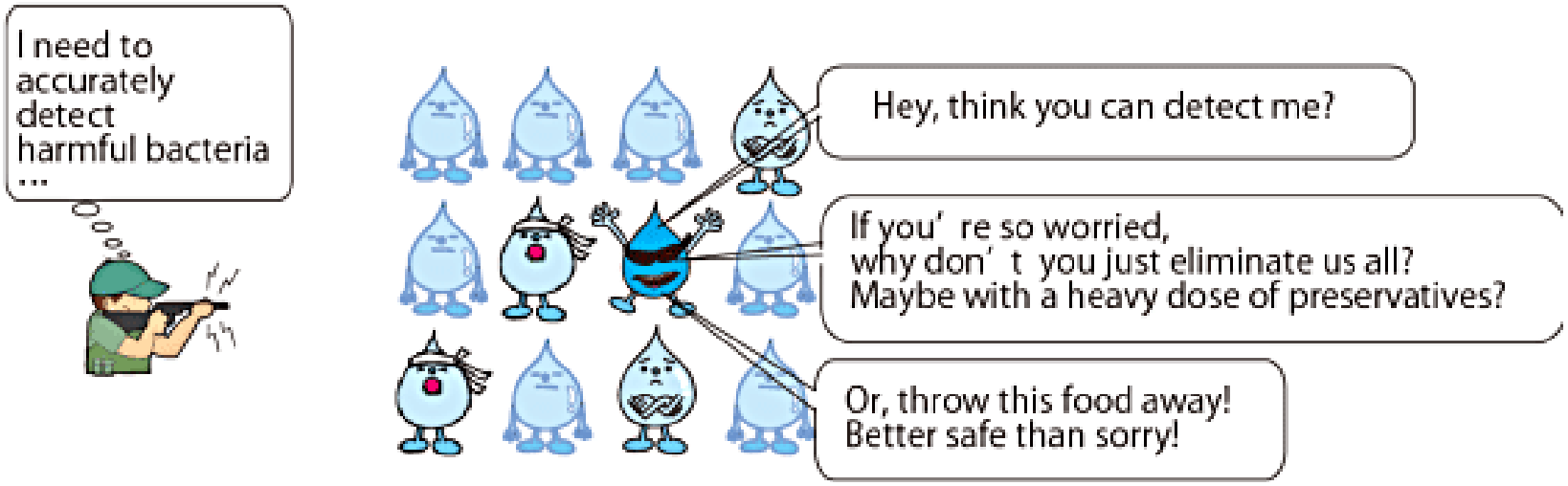
Darwin's sketch: the first phylogenetic tree?

ΚΛΙΝΙΚΑ ΒΑΚΤΗΡΙΑΚΑ ΣΤΕΛΕΧΗ

•Αναγνωρίζεται ο επιδημικός κλώνος
•Πλήρης επιδημιολογική μελέτη/επιτήρηση
•Αντιμετωπίζεται/προλαμβάνεται μια επιδημία εν εξελίξει

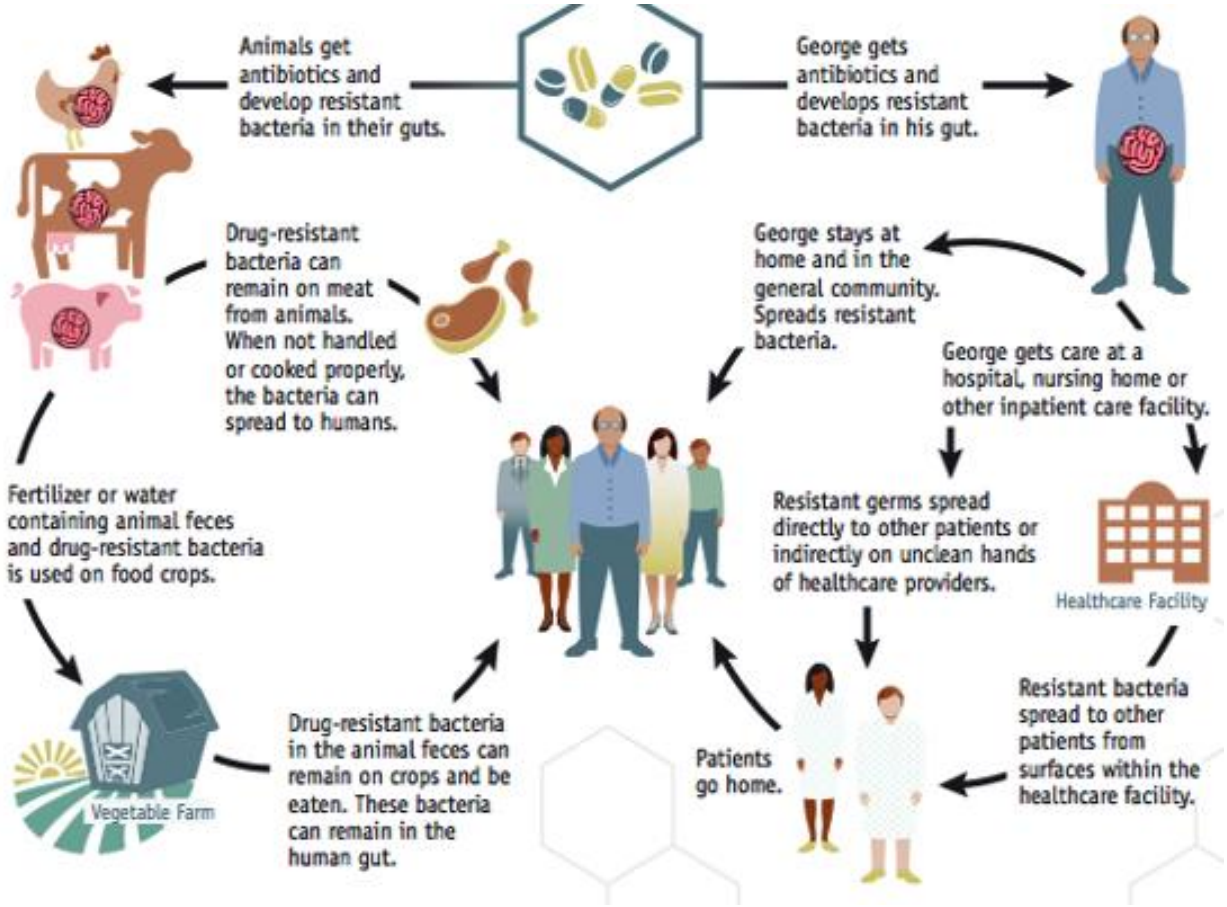
ΠΕΡΙΒΑΛΛΟΝΤΙΚΑ ΒΑΚΤΗΡΙΑΚΑ ΣΤΕΛΕΧΗ

•…..αλλά και η πηγή προέλευσης
•…….και περιβαλλοντική….
•…τροφιμογενής, υδατογενής….

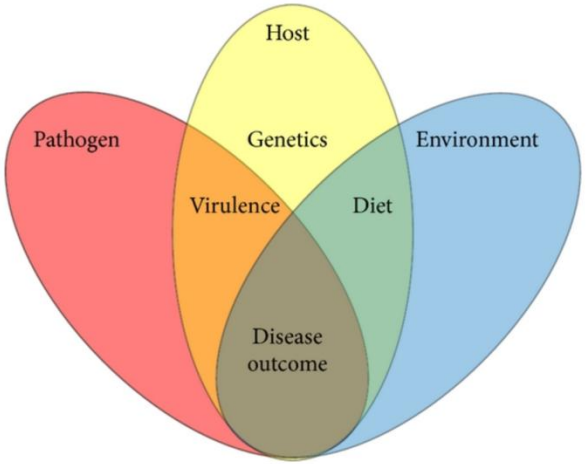


ΚΛΙΝΙΚΑ ΒΑΚΤΗΡΙΑΚΑ ΣΤΕΛΕΧΗ

ΠΕΡΙΒΑΛΛΟΝΤΙΚΑ ΒΑΚΤΗΡΙΑΚΑ ΣΤΕΛΕΧΗ



ONE HEALTH!!!



Outbreaks
clinical + environmental

Molecular Epidemiology of Chronic *Pseudomonas aeruginosa* Airway Infections in Cystic Fibrosis

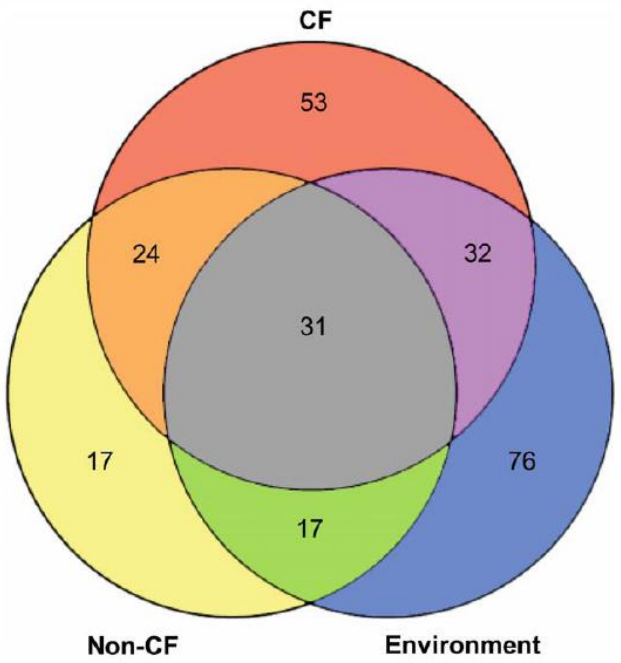
Nina Cramer[1], Lutz Wiehlmann[1], Oana Ciofu[2], Stephanie Tamm[1], Niels Høiby[2], Burkhard Tümmler[1,3]*

Abstract

Background/Methods: The molecular epidemiology of the chronic airway infections with *Pseudomonas aeruginosa* in individuals with cystic fibrosis (CF) was investigated by cross-sectional analysis of bacterial isolates from 51 CF centers and by longitudinal analysis of serial isolates which had been collected at the CF centers Hanover and Copenhagen since the onset of airway colonization over 30 years.

Results: Genotyping revealed that the *P. aeruginosa* population in CF is dominated by a few ubiquitous clones. The five most common clones retrieved from the CF host also belonged to the twenty most frequent clones in the environment and in other human disease habitats. Turnover of clones in CF airways was rare. At the Hanover clinic more than half of the patient cohort was still harbouring the initially acquired clone after twenty years of airway colonization. At the Copenhagen clinic, however, two rare clones replaced the initially acquired individual clones in all but one patient.

Conclusion: The divergent epidemiology at the two sites is explained by their differential management of hygiene and antipseudomonal chemotherapy. Hygienic measures to prohibit patient-to-patient transmission and the modalities of antipseudomonal chemotherapy modify the epidemiology of the chronic *P. aeruginosa* infections in CF.



Outbreaks
clinical + environmental

Eur J Clin Microbiol Infect Dis. 2016 May;35(5):741-6. doi: 10.1007/s10096-016-2591-2. Epub 2016 Feb 10.

Comparative study of all Salmonella enterica serovar Enteritidis strains isolated from food and food animals in Greece from 2008 to 2010 with clinical isolates.

Papadopoulos T[1], Petridou E[2], Zdragas A[3], Mandilara G[4], Nair S[5], Peters T[5], Chattaway M[6], de Pinna E[5], Passiotou M[7], Vatopoulos A[4].

Author information

Abstract

The aim of the present work was to study the epidemiology of Salmonella enterica serovar Enteritidis (S. Enteritidis) in Greece, comparing all the food and food animal isolates during a 3-year period with clinical isolates. Submission of the generated data to the PulseNet Europe database was carried out in order to study the population structure of this particular serovar and indicate possible connections with European strains. One hundred and sixty-eight (168) S. Enteritidis strains of human, animal, and food origin, isolated during the period 2008-2010 in Greece, were studied. Strains were characterized by phenotypic (antibiotic resistance) and molecular [pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)] methods. PFGE revealed 39 XbaI, 48 BlnI, and 80 XbaI-BlnI distinct pulsotypes, suggesting several clones circulating through the food chain and multiple sources of transmission. Submission to the PulseNet Europe database indicated that PFGE profile SENTXB.0001, the most common PFGE profile in Europe, was also predominant in Greece (33.3 %). MLST showed that all the strains studied shared the same sequence type (ST11), representing the most common ST in Europe. High rates of resistance to nalidixic acid were observed among human and poultry isolates (~25 %), indicating the potential fluoroquinolone treatment failure. Our data suggest that strains originating from multiple reservoirs circulated in Greece through the food chain during the study period. Predominant profiles in Greece were common to PulseNet Europe profiles, indicating similarities between the S. Enteritidis populations in Greece and Europe.

PMID: 26864044 DOI: 10.1007/s10096-016-2591-2

[PubMed - in process]

ΜΟΡΙΑΚΕΣ ΤΕΧΝΙΚΕΣ

- Οι σημαντικότερες μοριακές τεχνικές που έχουν χρησιμοποιηθεί στην τυποποίηση βακτηρίων και σε διαγνωστικές μελέτες:
- ➢Τεχνικές PCR και οι εφαρμογές της π.χ. ERIC-pcr, RAPD-pcr, AFLP, Real Time pcr

MLST
Multi Locus Sequence Typing

- **➢Ηλεκτροφόρηση μεταβλητού πεδίου (Pulsed Field Gel**
- **Electrophoresis)**
- **➢Τυποποίηση μέσω αλληλούχισης (Sequence Based Typing π.χ.**
- **MLST, DLST)**

PulseNet™ International
The International Molecular Subtyping Network for Foodborne Disease Surveillance
USA, Canada, Europe, Asia Pacific, Latin America, Middle East
CDC

ΜΟΡΙΑΚΕΣ ΤΕΧΝΙΚΕΣ

Pcr vs sequencing

Ειδικοί/Τυχαίοι εκκινητές

- Μικρά τμήματα του γενετικού υλικού

- Δεν ανιχνεύεται επαρκώς η συγγένεια δυο ή κ περισσοτέρων στελεχών

- Ελλιπής επιδημιολογική διερεύνηση

Πλήρης πρόσβαση στο γενετικό υλικό

- PubMed, MLST (**http://www.mlst.net/misc/new_schemes.asp**)

- Αλλαγές στα γονίδια (insertion, deletions, duplications κ.α.)

- Επαρκής επιδημιολογική προσέγγιση

Pulsed Field Gel Electrophoresis (PFGE)

- **Χρησιμοποιείται για την τυποποίηση στελεχών του ιδίου είδους**
- ✓ Δημιουργία παλσοτύπων (Pulsotypes)
- ✓ διευκολύνει τη διαδικασία προσδιορισμού «ομοιότητας /συγγένειας» μεταξύ καλλιεργημάτων που προέκυψαν:
  - από τη διερεύνηση μιας επιδημίας
  - έλεγχος πορείας μετάδοσης ενός βακτηρίου

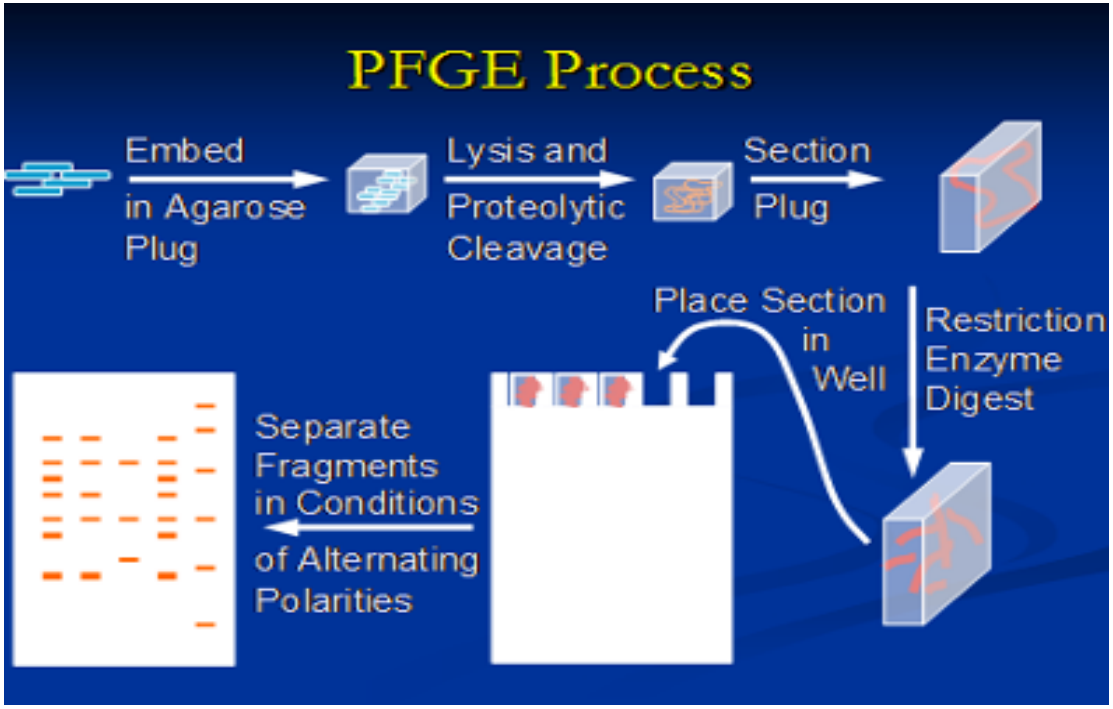
} **επιβεβαιώνει ή απορρίπτει την υπόθεση ότι τα καλλιεργήματα προήλθαν από κοινή εστία**

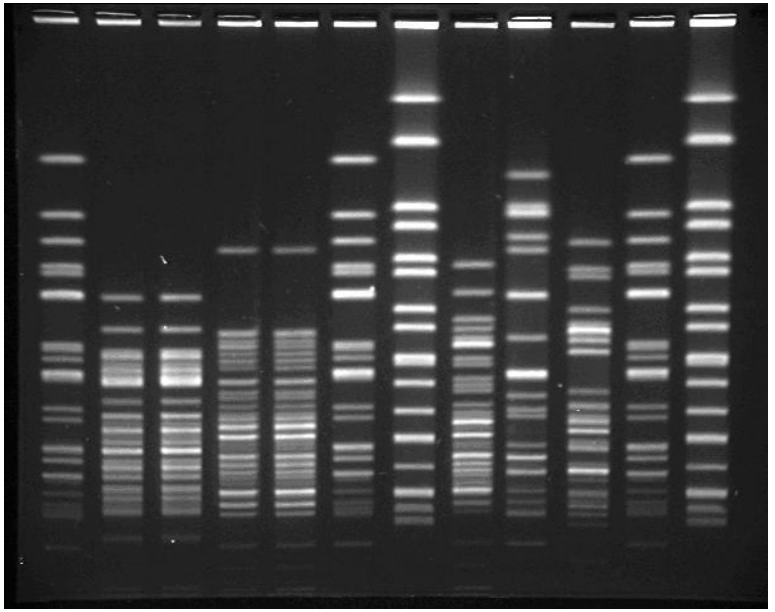
- Πληροφορίες για τρόπο μετάδοσης βακτηρίου
- Εύρεση reservoir (δεξαμενής) ή εστία μόλυνσης
- Χαρακτηρίζεται από υψηλή τυποποιητική ικανότητα και διακριτική ικανότητα
- Αποτελεί την **‘gold standard method’**

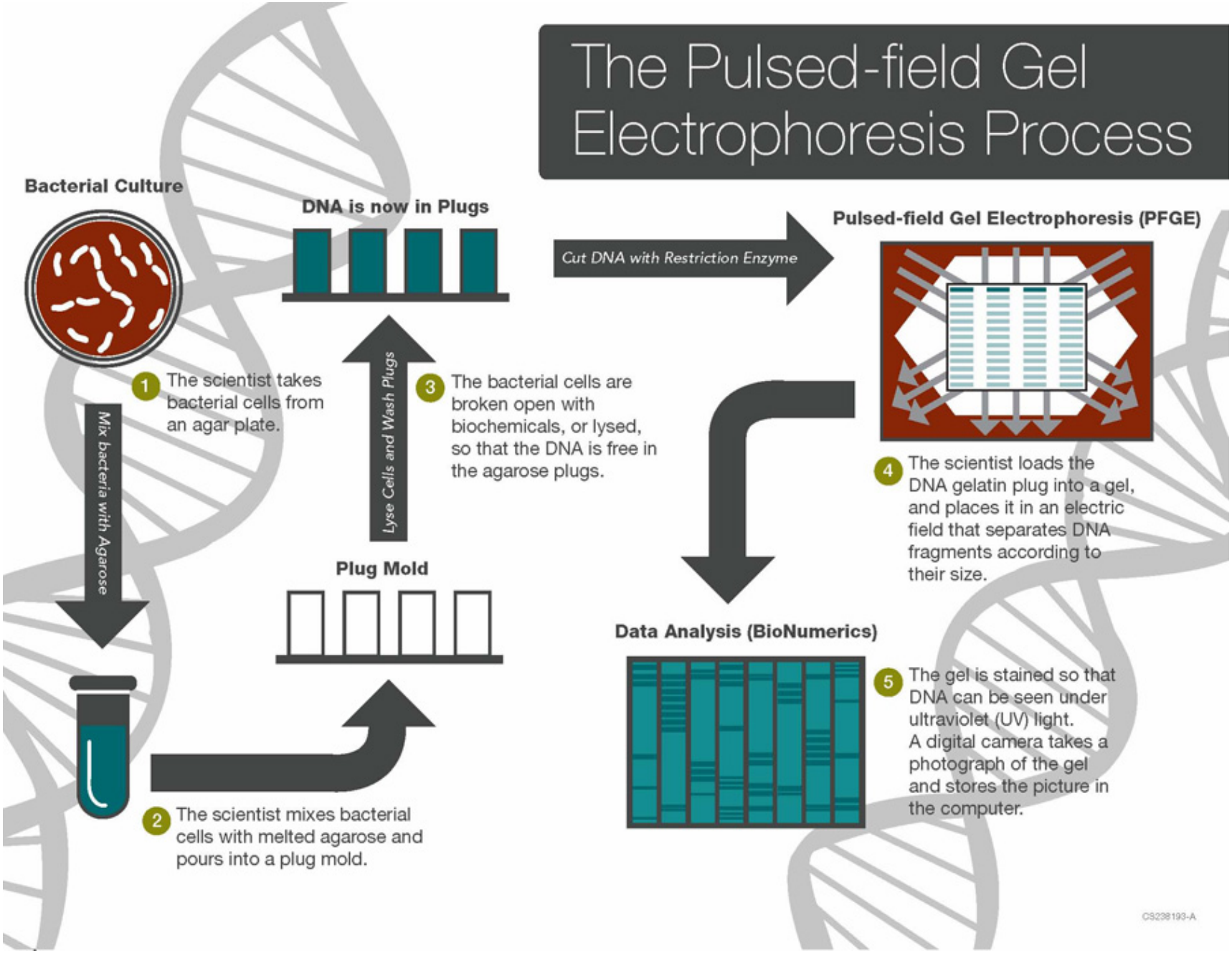
Pulsed Field Gel Electrophoresis (PFGE)

❖ **Αρχή μεθόδου**

- Διαχωρίζονται μεγάλα τμήματα χρωμοσομικού DNA εφαρμόζωντας ηλεκτρικό πεδίο, το οποίο περιοδικά αλλάζει κατεύθυνση μέσα στο gel (παλλόμενο πεδίο)



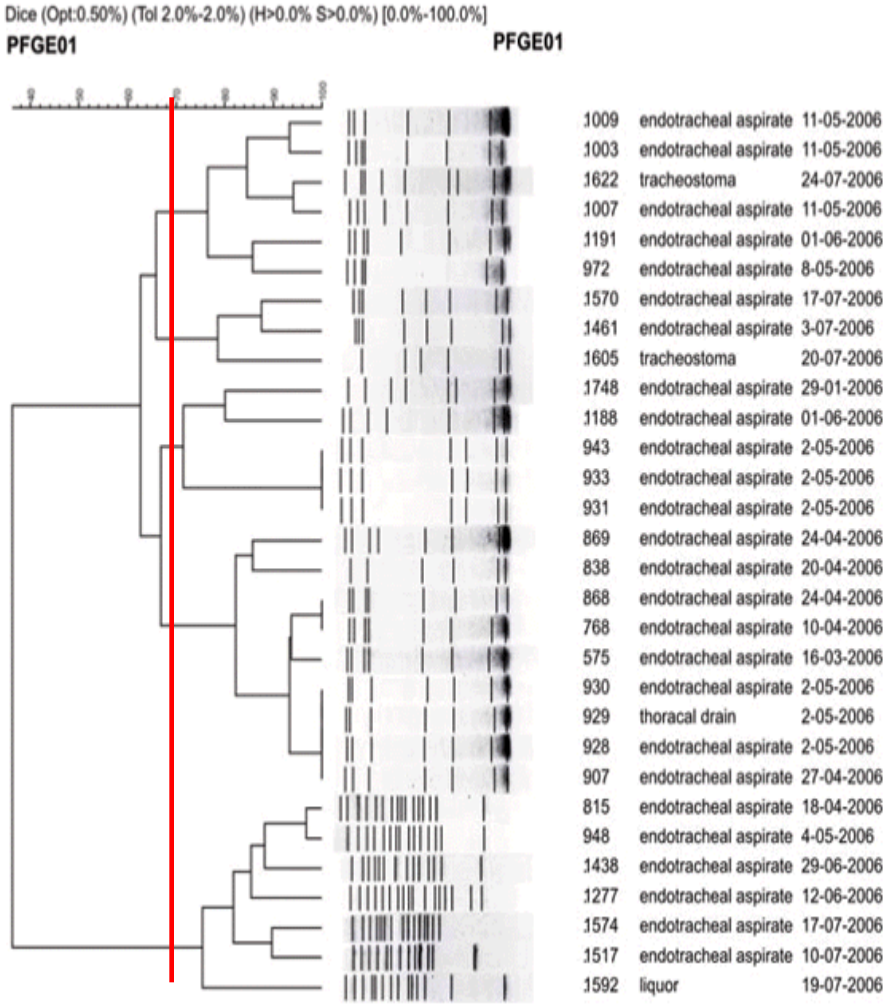




http://www.cdc.gov/pulsenet/pathogens/protocolimages.html#pfge

Pulsed Field Gel Electrophoresis (PFGE)

Figure 2. Dendrogram depicting 30 representative isolates of *Acinetobacter baumannii* species obtained from CICU.



- **Gel Compar 2**
- **Δενδρόγραμμα**

Tenover's κριτήρια (διάκριση σε κλώνους/υποτύπους) (Tenover et al., 1995)

❖**CDC/PulseNet** (http://www.cdc.gov/pulsenet/)

PulseNet™ International
The International Molecular Subtyping Network for Foodborne Disease Surveillance
USA, Canada, Europe, Asia Pacific, Latin America, Middle East
CDC

Pulsed Field Gel Electrophoresis (PFGE)

Advantages of PFGE

- High concordance with epidemiological relatedness
- Can be applied as a universal generic subtyping method for many different bacteria with only the choice of the restriction enzyme and electrophoresis conditions optimized for each species
- Stable and reproducible DNA restriction patterns
- More discriminating than methods such as ribotyping or multi-locus sequence typing for many bacteria

1. **Cholera epidemic** associated with consumption of unsafe drinking water and street-vended water--Eastern Freetown, Sierra Leone, 2012, Nguyen VD et al, **2014**
2. Epidemiological characteristics and **molecular typing of *Salmonella enterica* serovar Typhi** during a waterborne outbreak in Eastern Anatolia, Bayram Y et al, 2011
3. **A waterborne *Campylobacter jejuni*** outbreak on a Greek island, Karagiannis I et al, 2010
4. Isolation of ***Pseudomonas aeruginosa*** **from open ocean** and comparison with freshwater, clinical, and animal isolates, Khan NH et al, 2008

frontiers in Microbiology

ORIGINAL RESEARCH
published: 21 October 2016
doi: 10.3389/fmicb.2016.01664

Molecular Characterization of *Salmonella* Serovars Anatum and Ealing Associated with Two Historical Outbreaks, Linked to Contaminated Powdered Infant Formula

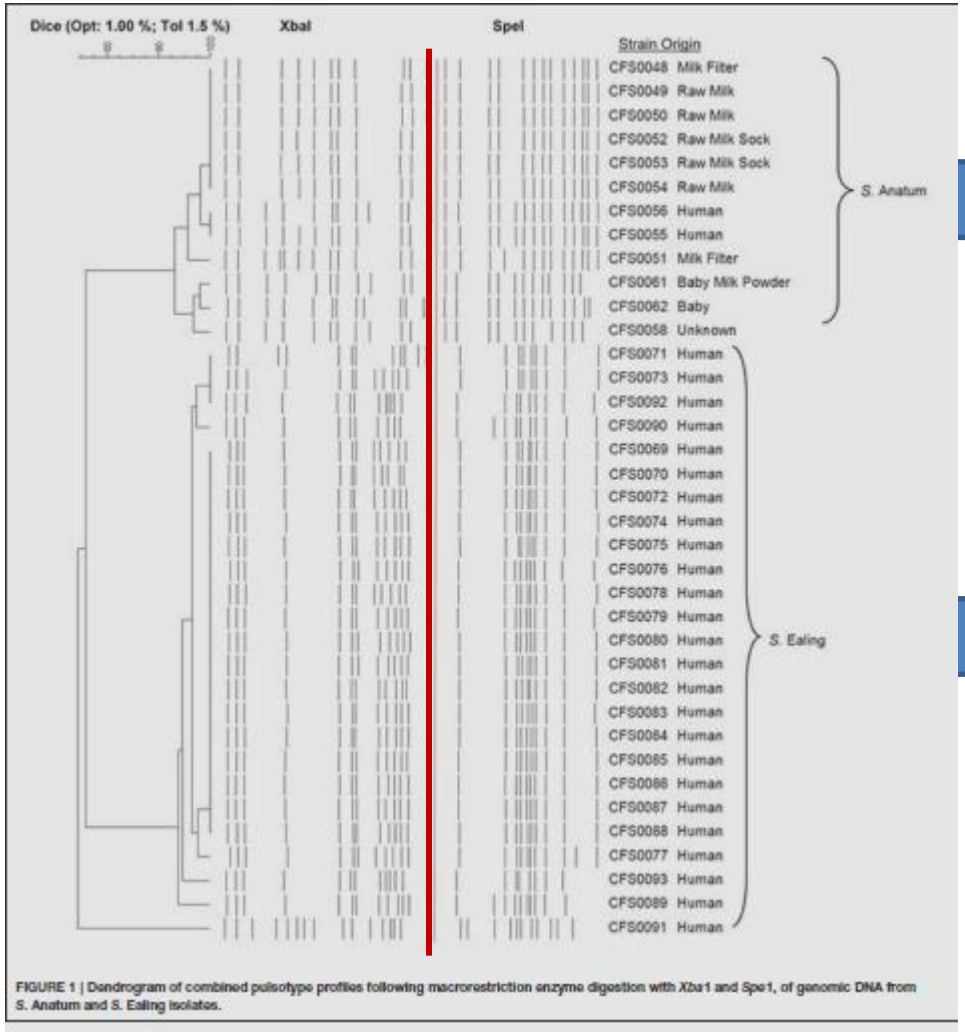


FIGURE 1 | Dendrogram of combined pulsotype profiles following macrorestriction enzyme digestion with *Xba*1 and *Spe*1, of genomic DNA from S. Anatum and S. Ealing isolates.

Human strains, milk, baby powder: **genetically related!!!**

Human strains **genetically distant from the epidemic clone!!**

Pulsed Field Gel Electrophoresis (PFGE)

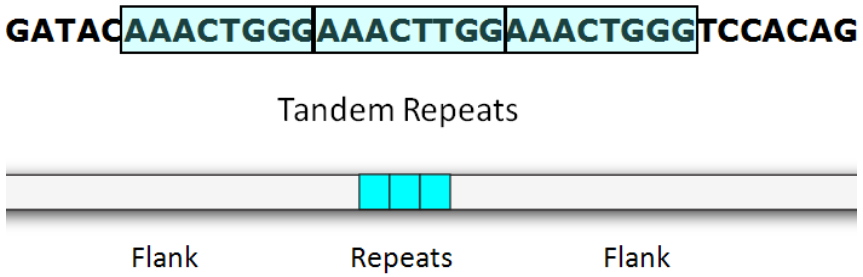
Limitations of PFGE

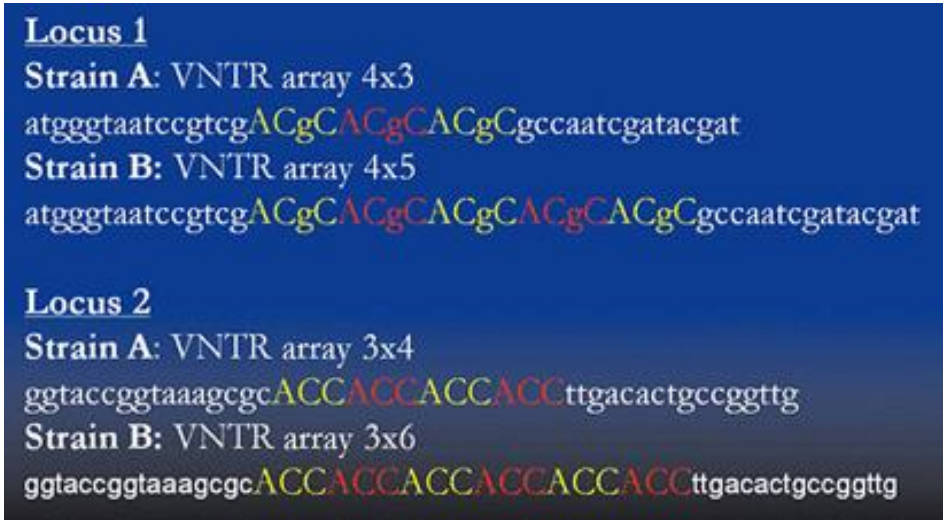
- Time consuming (7 days)
- **Does not discriminate between ALL unrelated isolates**
- DNA restriction patterns can vary slightly between technicians
- Cannot optimize separation in every part of the gel at the same time
- Bands of same size may not come from the same part of the chromosome
- Change in one restriction site can result in more than one band change
- **"Relatedness" should be used as a guide, not as a true phylogenetic measure**
- **Some strains cannot be typed by PFGE**
- **Clonal bacterial populations do not discriminate by PFGE such as *Salmonella typhi***

Multiple-Locus Variable number tandem repeat (MLVA)

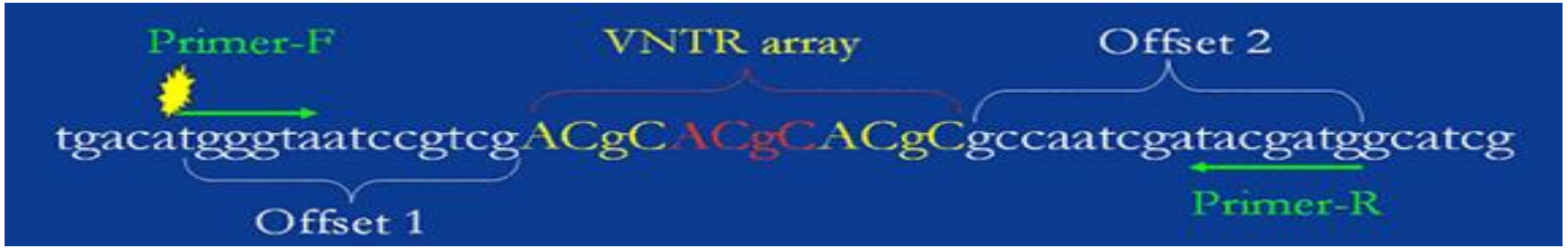
- Είναι μια μέθοδος που εκμεταλλεύεται τον πολυμορφισμό και τις διαδοχικά επαναλαμβανόμενες αλληλουχίες του DNA σε έναν συγκεκριμένο μ.ο.

- "**VNTR" σημαίνει "Variable Number of Tandem Repeats" = ποικίλος** αριθμός επαναλαμβανόμενων αλληλουχιών
- μια σειρά από καλά επιλεγμένους και χαρακτηρίσμένους (από άποψη ρυθμού μετάλλαξης και της ποικιλομορφίας) γονιδιακούς τόπους πολλαπλασιάζεται με PCR.
- Το καθορισμένο μέγεθος (μήκος της αλληλουχίας) του κάθε γονιδιακού τόπου χρησιμοποιείται για να υπολογιστεί ο αριθμός των επαναλαμβανόμενων μονάδων σε κάθε γενετικό τόπο
- Ο αριθμός αυτός αποτελεί το MLVA προφίλ του κάθε στελέχους, το οποίο μπορεί να συγκριθεί εύκολα με βάσεις δεδομένων αναφοράς.





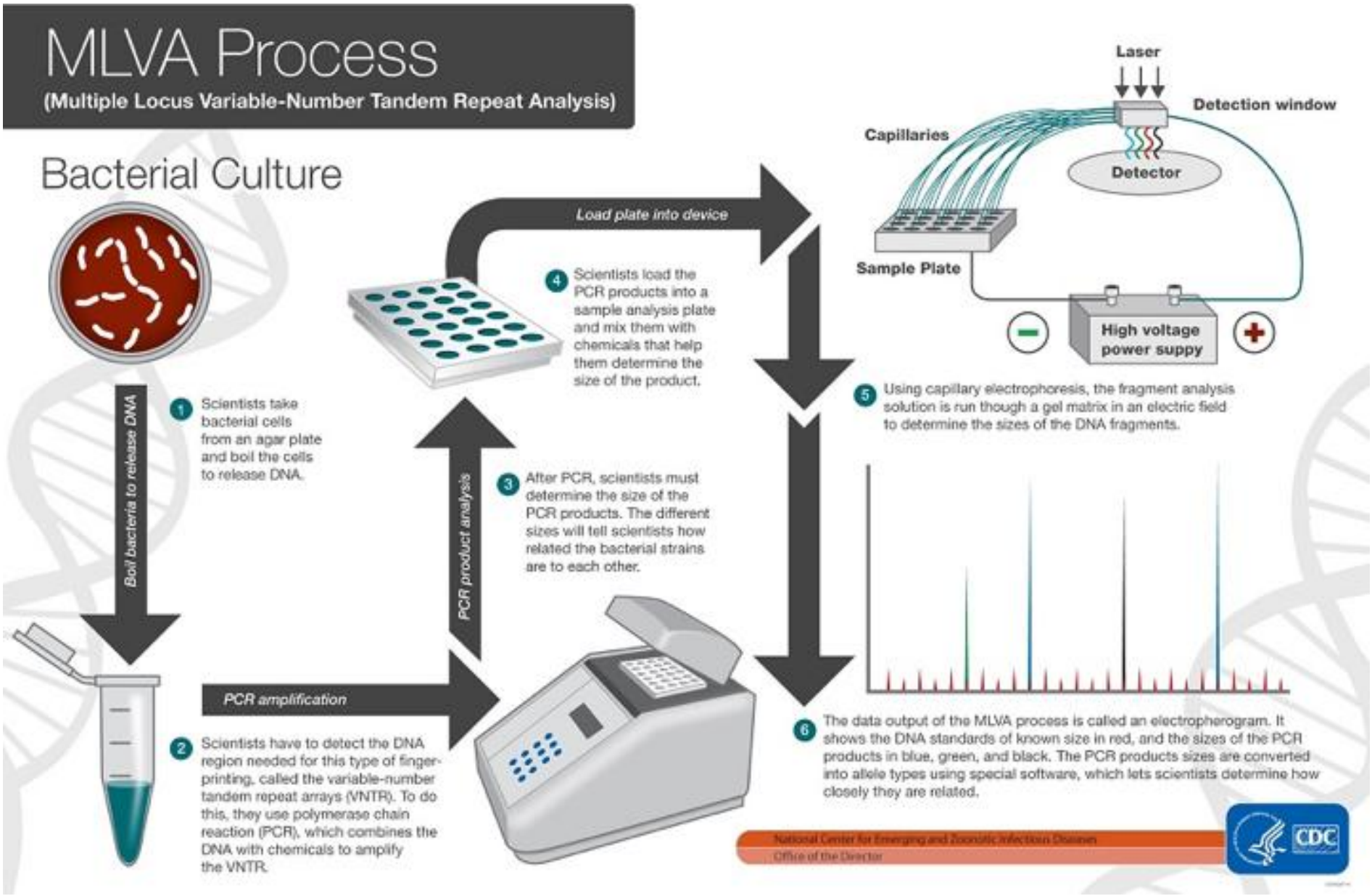
Δύο 'τόποι' από διαφορετικά στελέχη του ίδιου είδους. Οι επαναλαμβανόμενες αλληλουχίες είναι οι χρωματιστές.



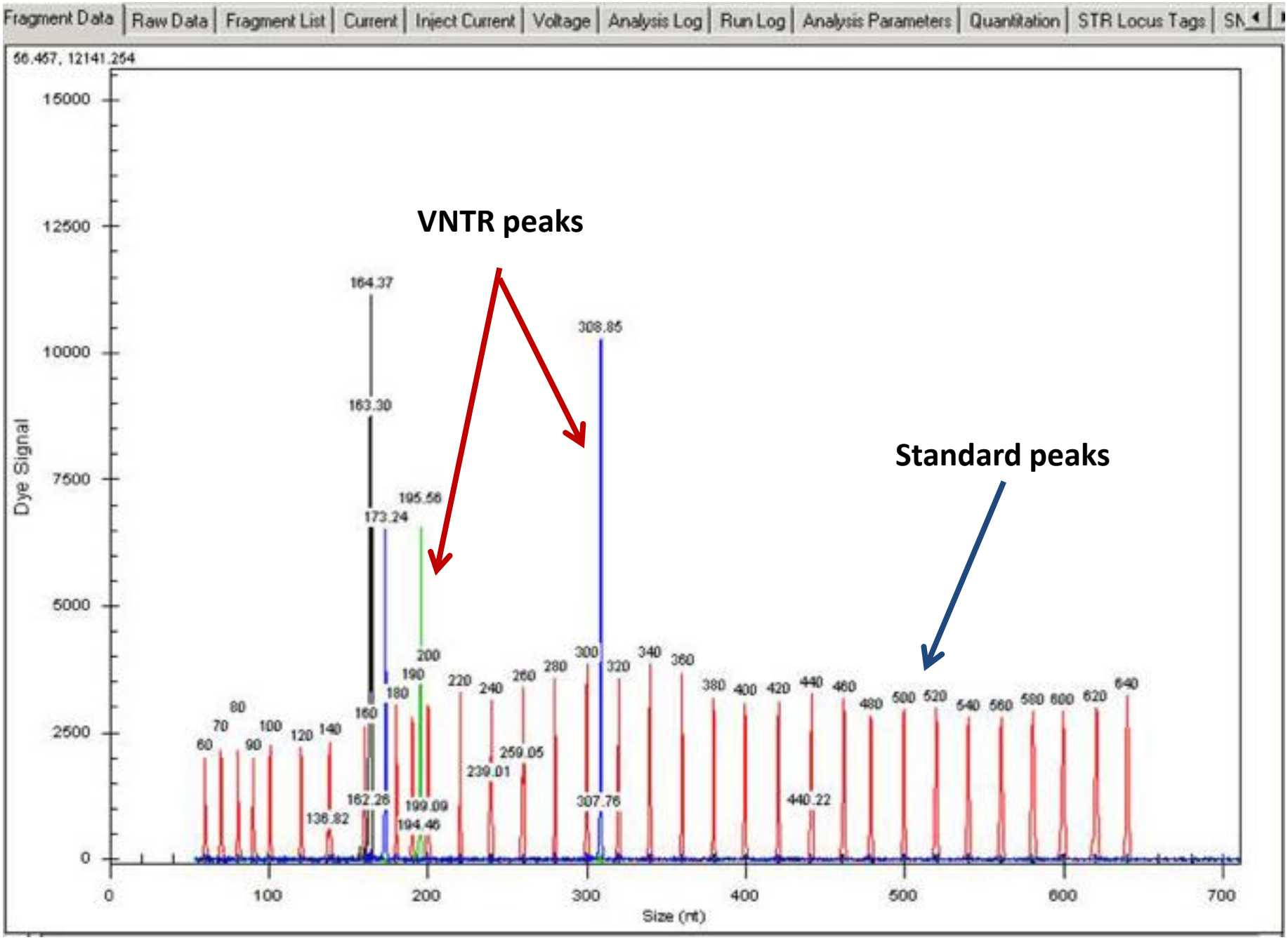
Οι Forward primers είναι σημασμένοι μέ κάποια φθορίζουσα ουσία έτσι ώστε το PCR product να μπορεί να ανιχνευθεί χρησιμοποιώντας μια ειδική ηλεκτροφόρηση, high resolution capillary electrophoresis (automatic sequencer).

MLVA Process

(Multiple Locus Variable-Number Tandem Repeat Analysis)



http://www.cdc.gov/pulsenet/pathogens/protocolimages.html#mlva


Fragment Data
Raw Data
Fragment List
Current
Inject Current
Voltage
Analysis Log
Run Log
Analysis Parameters
Quantitation
STR Locus Tags
56.467, 12141.254
VNTR peaks
Standard peaks
Dye Signal
Size (nt)
164.37
163.30
308.85
195.56
173.24
200
190
180
160
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
80
70
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90
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120
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259.05
239.01
162.26
199.09
194.46
136.82
307.76
440.22
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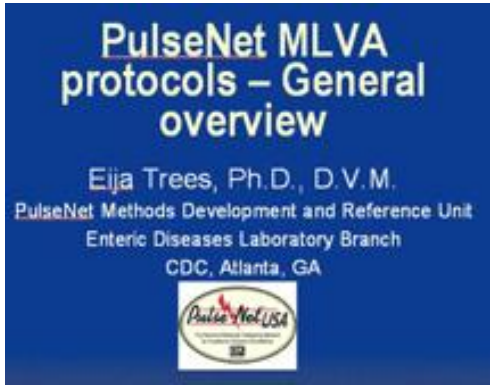
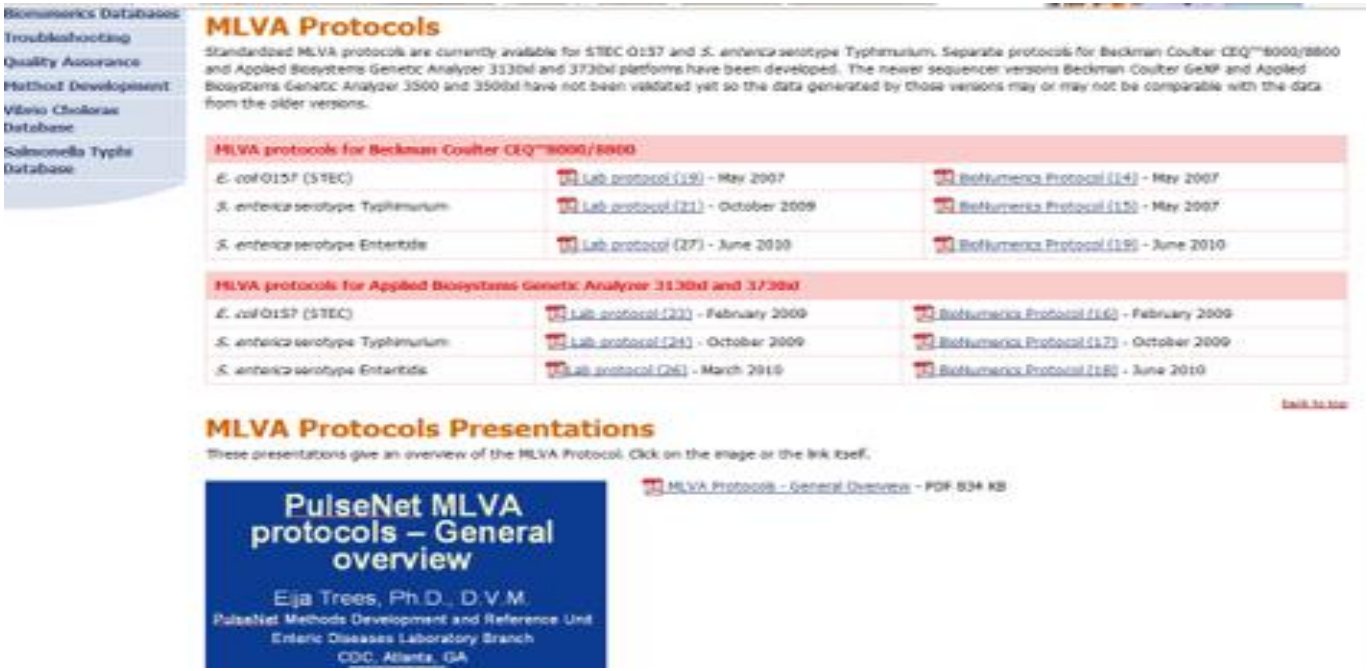
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
600
700

Multiple-Locus Variable number tandem repeat (MLVA)



ΠΛΑΤΦΟΡΜΑ ΤΟΥ ECDC (European Center for Disease and Control)

Πρωτόκολλα τυποποίησης



- Bordetella pertussis
- Haemophilus influenzae
- Neisseria meningitidis
- Staphylococcus aureus
  - Background
  - Protocols and Tables
  - Single profile query
  - Multiple profile query
- Streptococcus pneumoniae

Staphylococcus aureus

Staphylococcus aureus is an important bacterial pathogen that is associated with serious community-acquired and nosocomial diseases. Although *S. aureus* can cause a variety of serious clinical syndromes such as bacteremia, pneumonia and endocarditis, it seems to be omnipresent and is often carried without any clinical symptoms. Carriers may spread the pathogen infecting individuals who may develop disease. The introduction of methicillin has led to the rapid emergence of methicillin-resistant *S. aureus* (MRSA) and is posing a major clinical problem within hospitals worldwide

Multiple-Locus Variable number of tandem repeat Analysis (MLVA) of *Staphylococcus aureus*

In order to understand the population biology of *S. aureus* and to study the impact of measures to control MRSA infections unambiguous characterization of *S. aureus* isolates is required. Many typing techniques have been employed for the analysis of *S. aureus* of which multi-locus sequence typing (MLST) is considered to be the current gold standard for molecular typing of *S. aureus*. However, MLST is expensive, labor intensive and therefore not the method of choice for high throughput typing for most laboratories. Spa-sequence typing is a portable technique and is relatively easy to perform. A disadvantage of this technique is that it is based on a single locus in the genome only and clustering and tree-building of *S. aureus* isolates based on spa-data is complex. For these reasons we developed a typing technique based on the composition of genomic loci containing tandem repeats. Several MLVA schemes for *S. aureus* have been designed and used to type this pathogen. However, these MLVA schemes rely on analysis in agarose gels making them less accurate and often not portable. The MLVA used here is based on accurate band sizing using

Statistics
12/12/2016

Total - 5425 profiles

Total - 62 complexes

Contact:
Leo Schouls

Multiple-Locus Variable number tandem repeat (MLVA)

Advantages of MLVA

•MLVA may be able to differentiate suspected, fast-evolving bacterial strains from an outbreak even though those strains might look the same using other methods of DNA fingerprinting, such as PFGE
•PulseNet suggests MLVA as a complementary technique to PFGE, allowing microbiologists to see more detailed differences between bacteria that have similar PFGE patterns.

Limitations of MLVA

•Requires a trained and skilled technician
•Is not a practical routine subtyping method because a specific protocol must be used for each pathogen
•A few standardized protocols are available, but only the most common pathogens are subtyped by this method

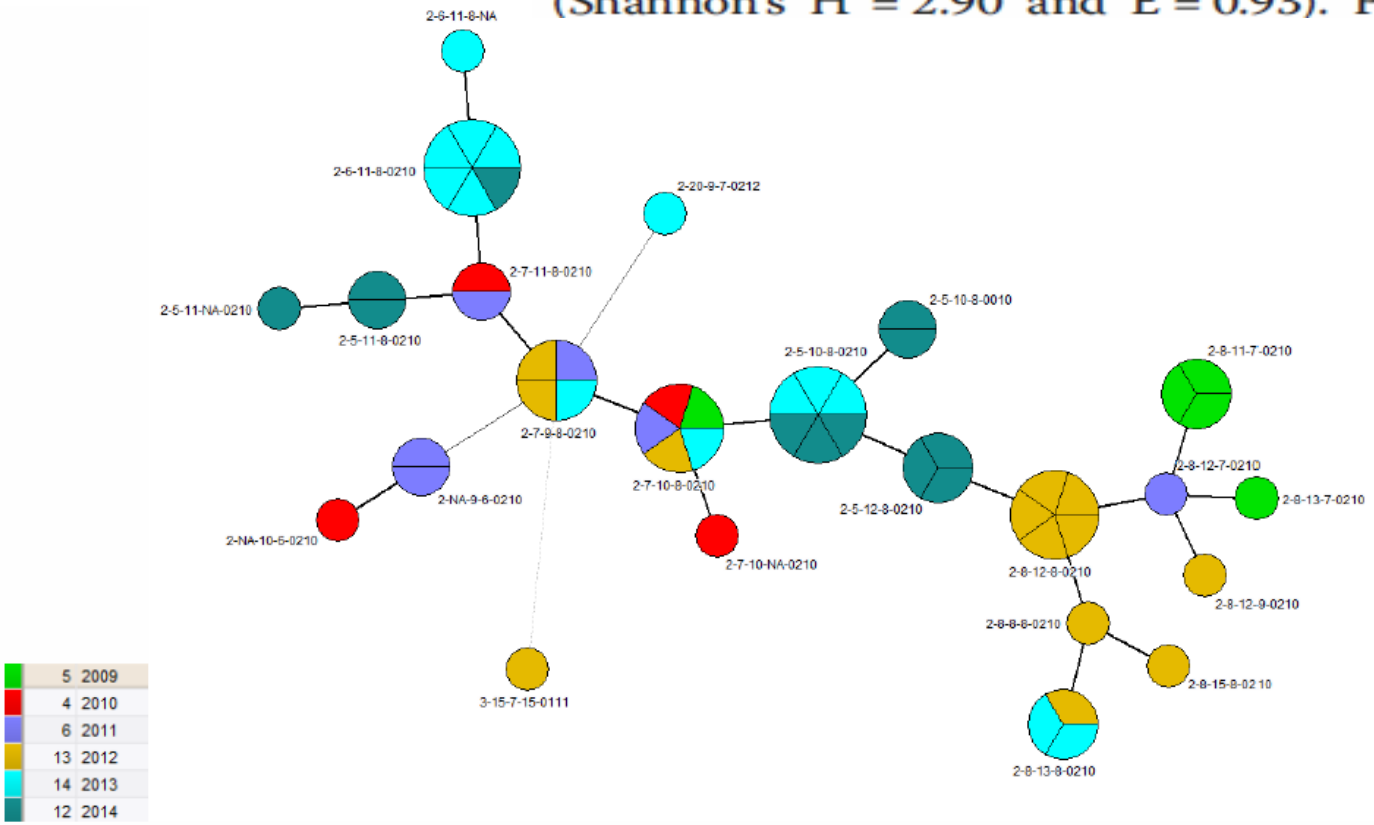
Falay et al. BMC Infectious Diseases (2016) 16:271
DOI 10.1186/s12879-016-1604-1

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Microbiological, clinical and molecular findings of non-typhoidal *Salmonella* bloodstream infections associated with malaria, Oriental Province, Democratic Republic of the Congo

MLVA analysis of the *Salmonella* Typhimurium isolates revealed heterogeneity with 23 different profiles across 54 isolates collected between 2009 and 2014 (Simpson's D = 0.95) (Additional file 1: Figure S1). The *Salmonella* Typhimurium isolates also showed an even distribution (Shannon's H' = 2.90 and E = 0.93). Forty-four of the 54



Lienemann *et al. BMC Microbiology* (2015) 15:131
DOI 10.1186/s12866-015-0467-8

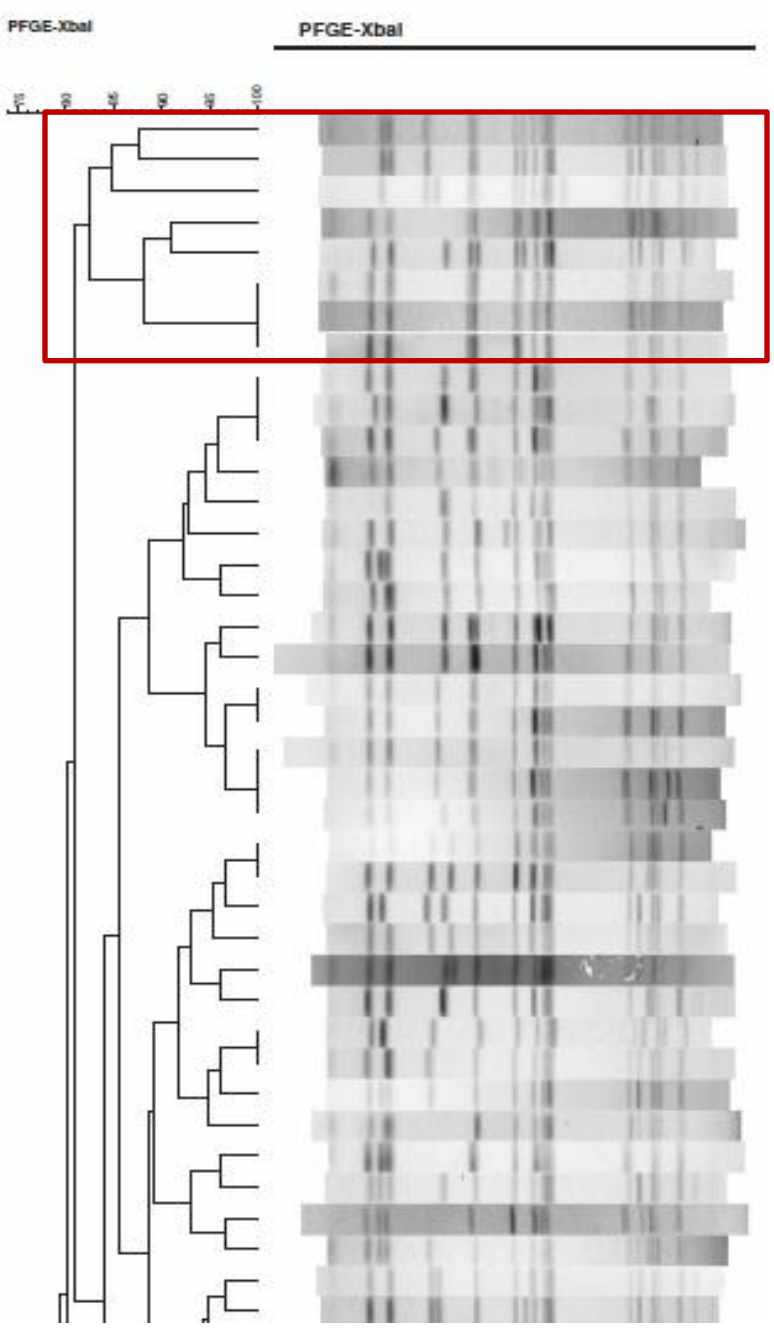
BMC Microbiology

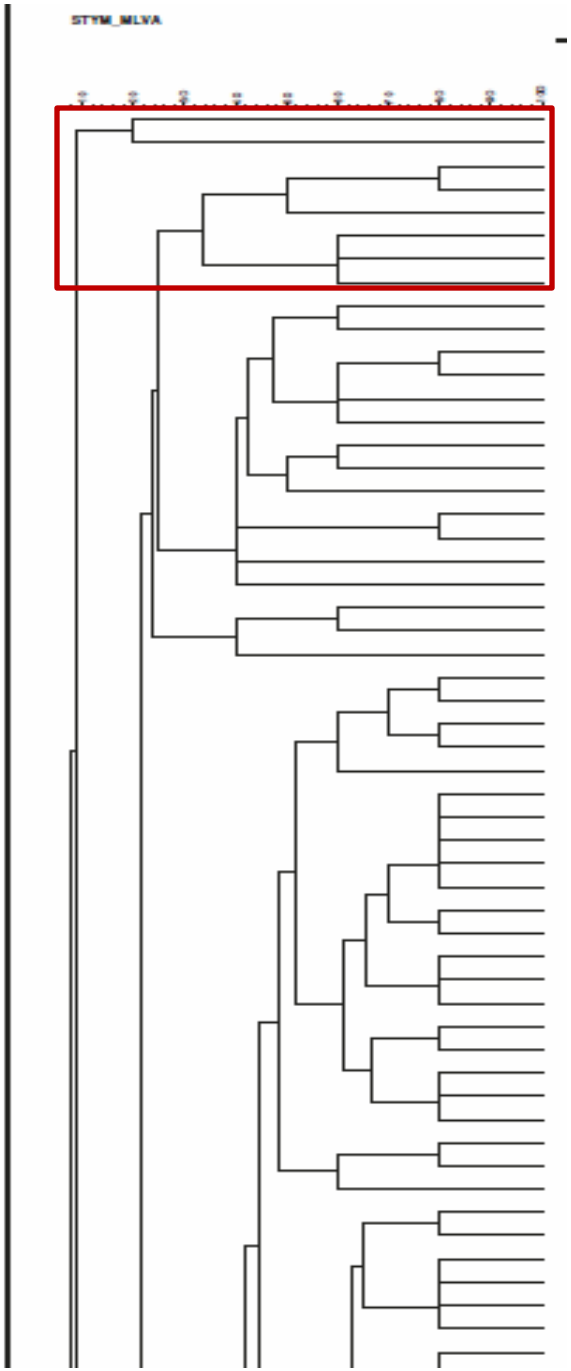
RESEARCH ARTICLE Open Access

Characterization of *Salmonella* Typhimurium isolates from domestically acquired infections in Finland by phage typing, antimicrobial susceptibility testing, PFGE and MLVA

Table 3 Discriminatory power of three typing methods for 375 sporadic *S.* Typhimurium isolates

Method	No. of profiles	Simpson's DI (95 % CI)
PT	31	0.749 (0.703-0.794)
XbaI-PFGE	83	0.829 (0.792-0.865)
5-loci MLVA	111	0.867 (0.835-0.898)


PFGE-XbaI
PFGE-XbaI


STYM_MLVA

frontiers in Microbiology

ORIGINAL RESEARCH
published: 11 September 2015
doi: 10.3389/fmicb.2015.00882

Staphylococcus aureus strains associated with food poisoning outbreaks in France: comparison of different molecular typing methods, including MLVA

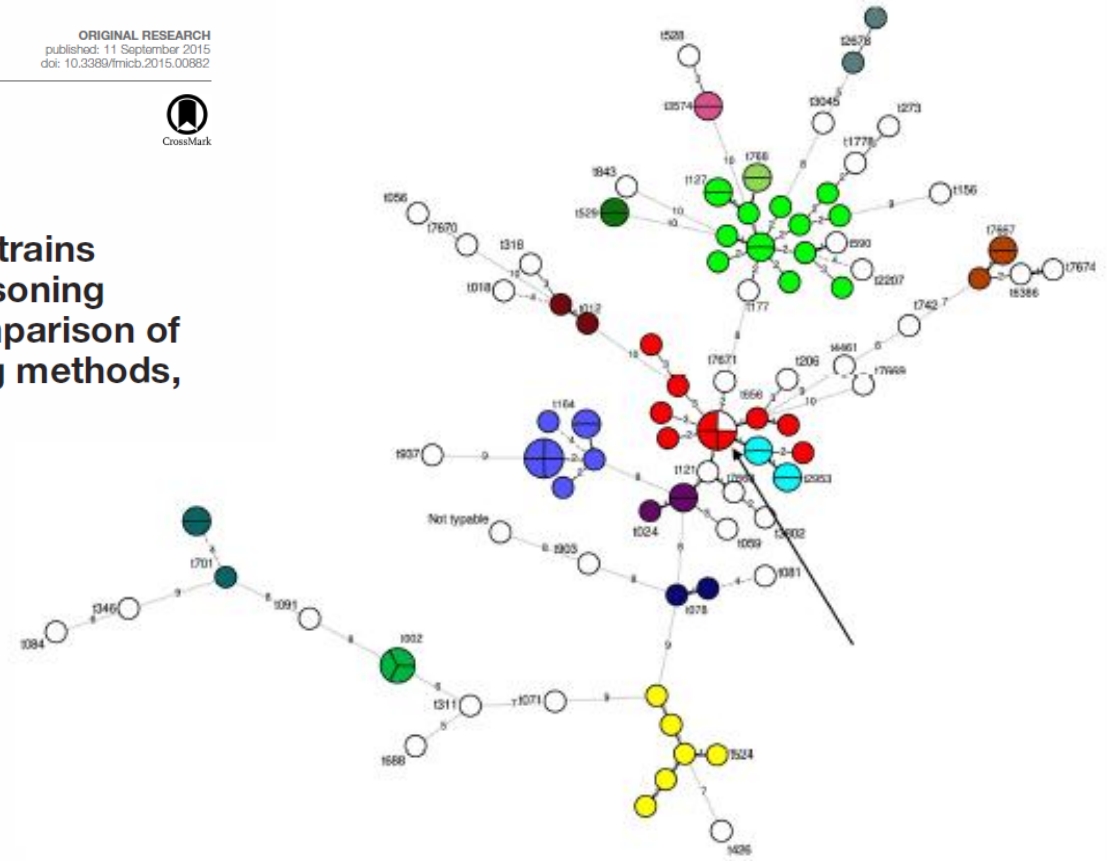


TABLE 4 | Multiple-locus variable-number tandem-repeat analysis, PFGE and spa-typing results from a panel of 146 not epidemiologically related strains.

	Number of analyzed strains	Number of successfully typed strains	ID and CI 95%	Total number of distinct types	Total number of unique types
MLVA	146	146	0.997 (0.995–0.999)	125	109
PFGE	146	146	0.995 (0.992–0.998)	118	100
spa-typing	146	142	0.970 (0.959–0.982)	71	50

Multiple-Locus Variable number tandem repeat (MLVA)

❖ **ΠΛΕΟΝΕΚΤΗΜΑΤΑ:**

✓ Είναι PCR-based μέθοδος, κάτι που την καθιστά απλή, γρήγορη και φτηνή

✓ Υψηλή διακρτική ικανότητα για τυποποίηση μ.ο.

ΜΕΙΟΝΕΚΤΗΜΑΤΑ:

✓ Οι επαναλαμβανόμενες περιοχές γενετικού υλικού υπόκεινται συχνά σε μετταλάξεις, οπότε και δεν μπορούν στη συνέχεια να ανιχνευθούν από τους εκκινητές

Multiple-Locus Variable number tandem repeat (MLVA)

•Wang H. et al, Genotyping of ***Salmonella Typhi*** *using 8-loci multi locus VNTR* analysis, *Gut Pathog, 2016, doi: 10.1186/s13099-016-0094-4*

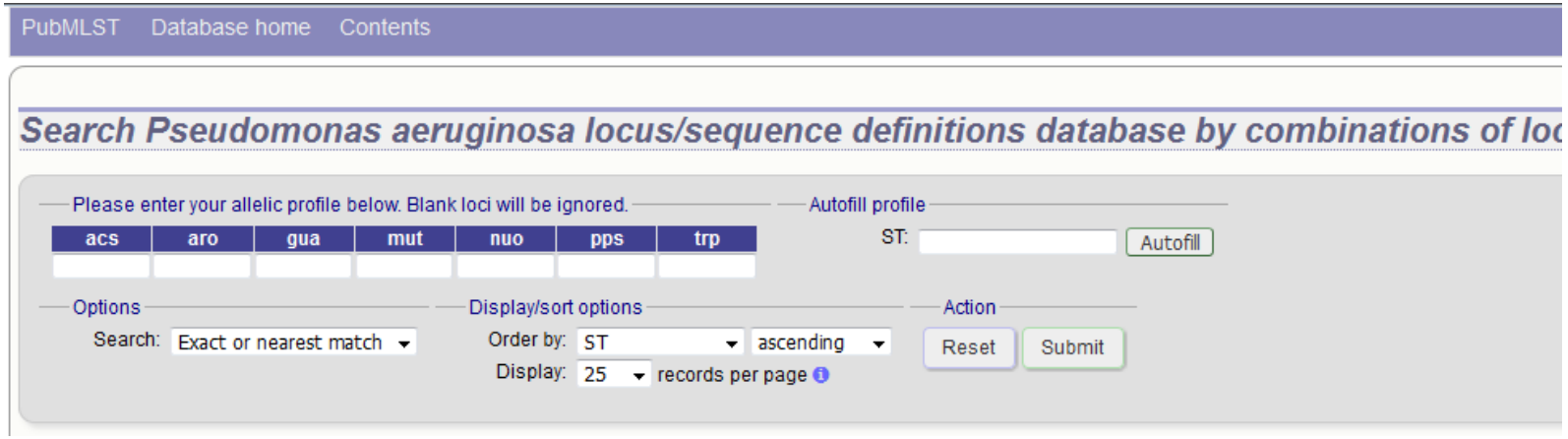
•Johansson E. et al, Genotyping of ***Pseudomonas aeruginosa*** *reveals high diversity,* stability over time and good outcome of eradication, *J Cyst Fibros, 2015, doi:* 10.1016/j.jcf.2014.09.016.

•Rodríguez-Martínez S. et al, Spatial distribution of ***Legionella pneumophila*** *MLVA genotypes* in a drinking water system, *Water Res, 2015, doi: 10.1016/j.watres.* 2015.03.010

•Krüger A. et al, Genetic characterization of Shiga toxin-producing ***Escherichia coli O26:H11*** *strains isolated from animal, food, and clinical samples, Front Cell Infect Microbiol, 2015, doi: 10.3389/fcimb.2015.00074.*

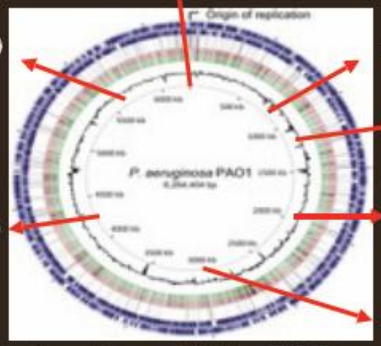
Multi Locus Sequence Typing (MLST)

- Τυποποίηση μέσω αλληλούχισης
- Αναγνωρίζει άμεσα τις διαφορές σε αλληλουχίες DNA ‘συντηρημένων γονιδίων’ (**housekeeping genes**) που εντοπίζονται σε στελέχη του ιδίου είδους
- Έτσι χαρακτηρίζει τα στελέχη απο το μοναδικό τους ‘γονιδιακό προφίλ’ (allelic profile) (http://pubmlst.org/paeruginosa)
- περιλαμβάνει 1707(1686) αλληλουχίες με 2342 (2265) διαφορετικά προφίλ (STs)
- Υψηλή τυποποιητική ικανότητα και αναπαραγωγιμότητα, αλλά σχετικά χαμηλή διακριτική ικανότητα
- **Δύκολη στην εφαρμογή της με υψηλό κόστος και απαιτεί πολύ χρόνο!!**

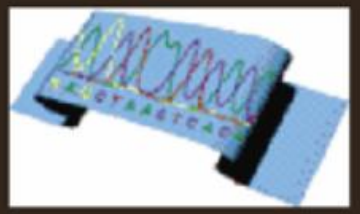


Multi Locus Sequence Typing (MLST)

Multi Locus Sequencing Typing of *Pseudomonas aeruginosa*



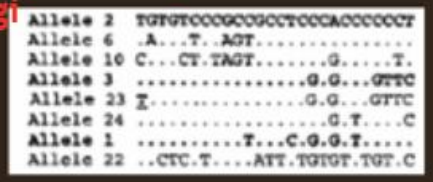
Select and amplify by PCR seven essential or housekeeping genes from genomic DNA



Sequencing

Sequence internal fragments 800-1000 bp of the genes in numerous isolates

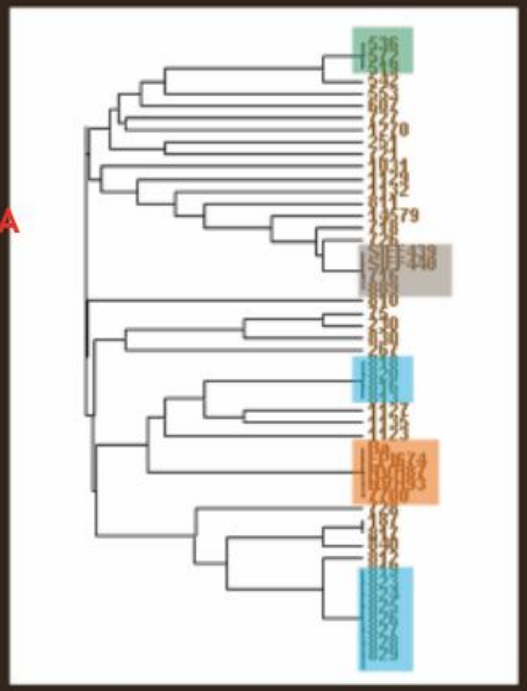
http://blast.ncbi.nlm.nih.gov/Blast.cgi



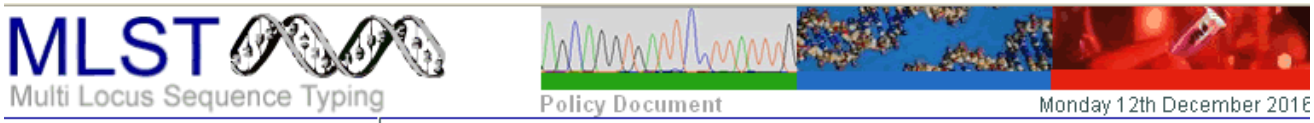
Compare sequences and assign allele numbers and sequence types (STs)

START PROGRAM/UPGMA (http://www.mlst.net)

Conduct a phylogenetic analysis based on differences between allelic profiles or sequences to infer genetic relatedness between isolates



Multi Locus Sequence Typing (MLST)



DATA ANALYSIS

DATABASES

SUBMISSIONS

NEWS

LINKS

NEW MLST SCHEMES IN DEVELOPMENT

Site requirements

Welcome to the Multi Locus Sequence Typing home page

MLST is a nucleotide sequence based approach for the unambiguous characterisation of isolates of bacteria and other organisms via the internet.

The aim of MLST is to provide a portable, accurate, and highly discriminating typing system that can be used for most bacteria and some other organisms. It is envisaged that this approach will be particularly helpful for the typing of bacterial pathogens.

To achieve this aim we have taken the proven concepts of multilocus enzyme electrophoresis (MLEE) and have adapted them so that alleles at each locus are defined directly, by nucleotide sequencing, rather than indirectly from the electrophoretic moblity of their gene products.

MLST was developed in the laboratories of Martin Maiden, Dominique Caugant, Ian Feavers, Mark Achtman and Brian Spratt.

This site is hosted at Imperial College with funding from the Wellcome Trust.
The location of the subsites for the individual species are shown on their respective front pages.

For general information please Click here or to register feedback or interest Click here

News

C. trachomatis MLST scheme now available
Click here to visit the site

Leptospira spp MLST scheme launched
Click here to visit the site

Borrelia burgdorferi MLST scheme launched
Click here to visit the site

MLST-maps : a facility for mapping the global distribution of Sequence Types. The MLST databases have been made available to view using either Google Maps or Google Earth. Click here to visit the site

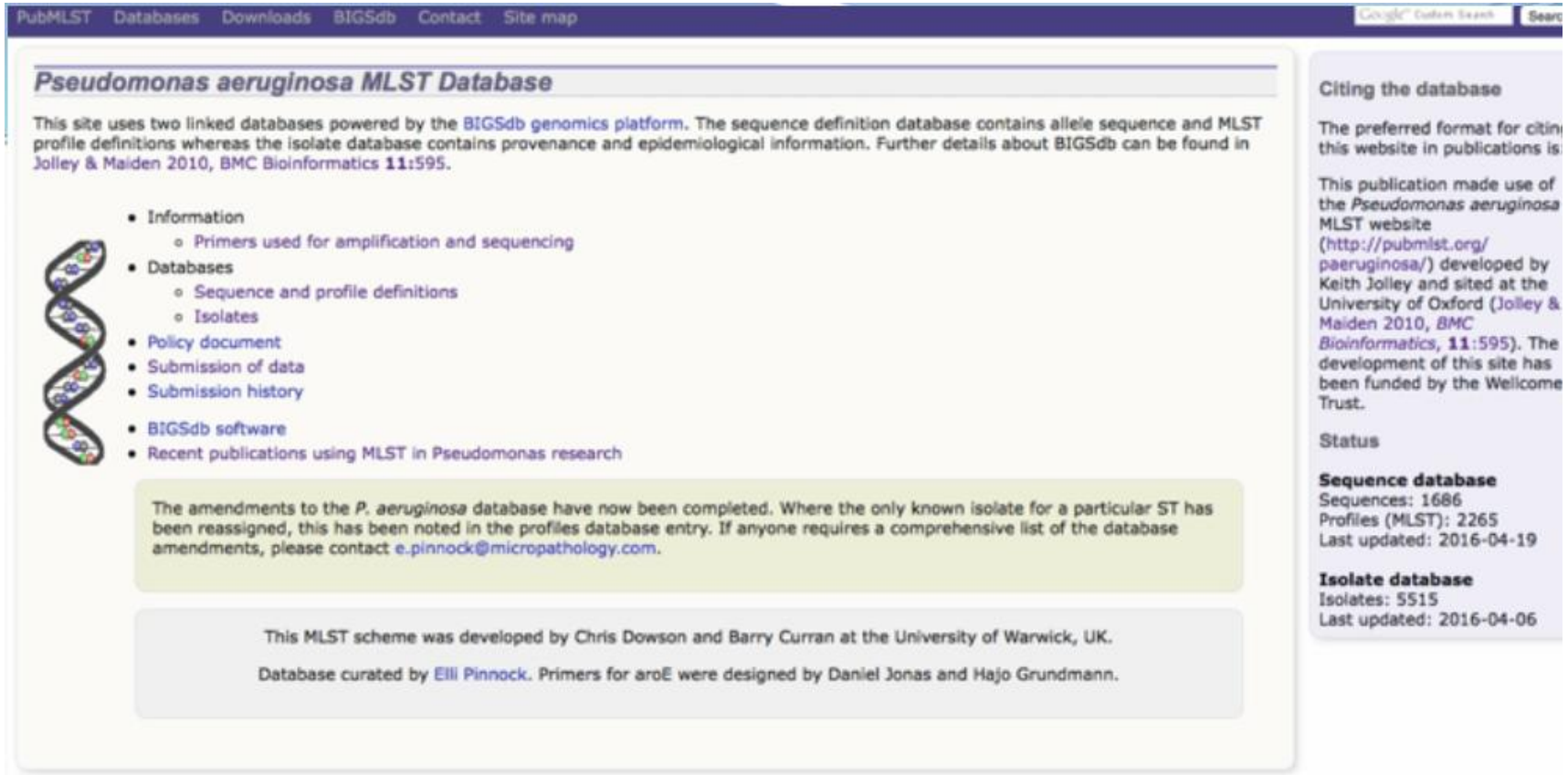
For comments, queries, bugs or suggestions please contact David Aanensen

DATA ANALYSIS

DATABASES

- *B.burgdorferi*
- *B.cereus*
- *B.henselae*
- *B.pseudomallei*
- *C.albicans*
- *C.glabrata*
- *C.trachomatis*
- *C.krusei*
- *C.tropicalis*
- *C.jejuni*
- *C.neoformans var grubii*
- *E.coli*
- *E.faecalis*
- *E.faecium*
- *H.influenzae*
- *H.pylori*
- *Leptospira spp.*
- *M.catarrhalis*
- *N.meningitidis*
- *P.acnes*
- *S.agalactiae*
- *S.aureus*
- *S.dysgalactiae*
- *S.enterica*
- *S.epidermidis*
- *S.pneumoniae*
- *S.pyogenes*
- *S.suis*
- *V.vulnificus*

Multi Locus Sequence Typing (MLST)



- Curran B. et al, Development of a multi locus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*, *J Clin Microbiol*, 2004, 42(12):5644-9
- Rosa van Mansfeld et al, *Pseudomonas aeruginosa* Genotype Prevalence in Dutch Cystic Fibrosis Patients and Age Dependency of Colonization by Various P. aeruginosa Sequence Types, *J Clin Microbial*, doi:10.1128/JCM.01462-09

Multi Locus Sequence Typing (MLST)

THE UNIVERSITY OF WARWICK

MLST Databases at UoW

Warwick Medical School

For citation please refer to: Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., Karch, H., Reeves, P. R., Maiden, M. C., Ochman, H., and Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol.Microbiol.* **60(5)**, 1136-1151.

Update information: ST complexes have been updated again on 17.05.2007. There are currently 600 STs and 54 ST complexes. The criteria have also been changed and are now groups of at least 3 STs sharing 6 alleles in pair-wise comparisons. The assignments of STs to some of the previous ST complexes have changed as a result, although we have tried to maintain consistency.

ST complexes have been updated again on 24.08.2005. Multiple new ST Complexes have been assigned and multiple STs have been assigned to known complexes. Due to the increased number of strains assigned to the ST29 Complex, it has become unclear whether these bacteria are closely related or only linked by one intermediate recombinant. Therefore, this has now been split into the ST23 and ST29 Complexes.

ST complexes have been updated on 23.11.2004. This includes the merging of ST21, 29 and 90 Complexes into ST29 Complex and ST3 and 17 Complexes into ST20 Complex. Multiple new ST complexes have been assigned. A number of STs have been merged with other STs due to curation of the database.

Protocols used for MLST of *Escherichia coli*

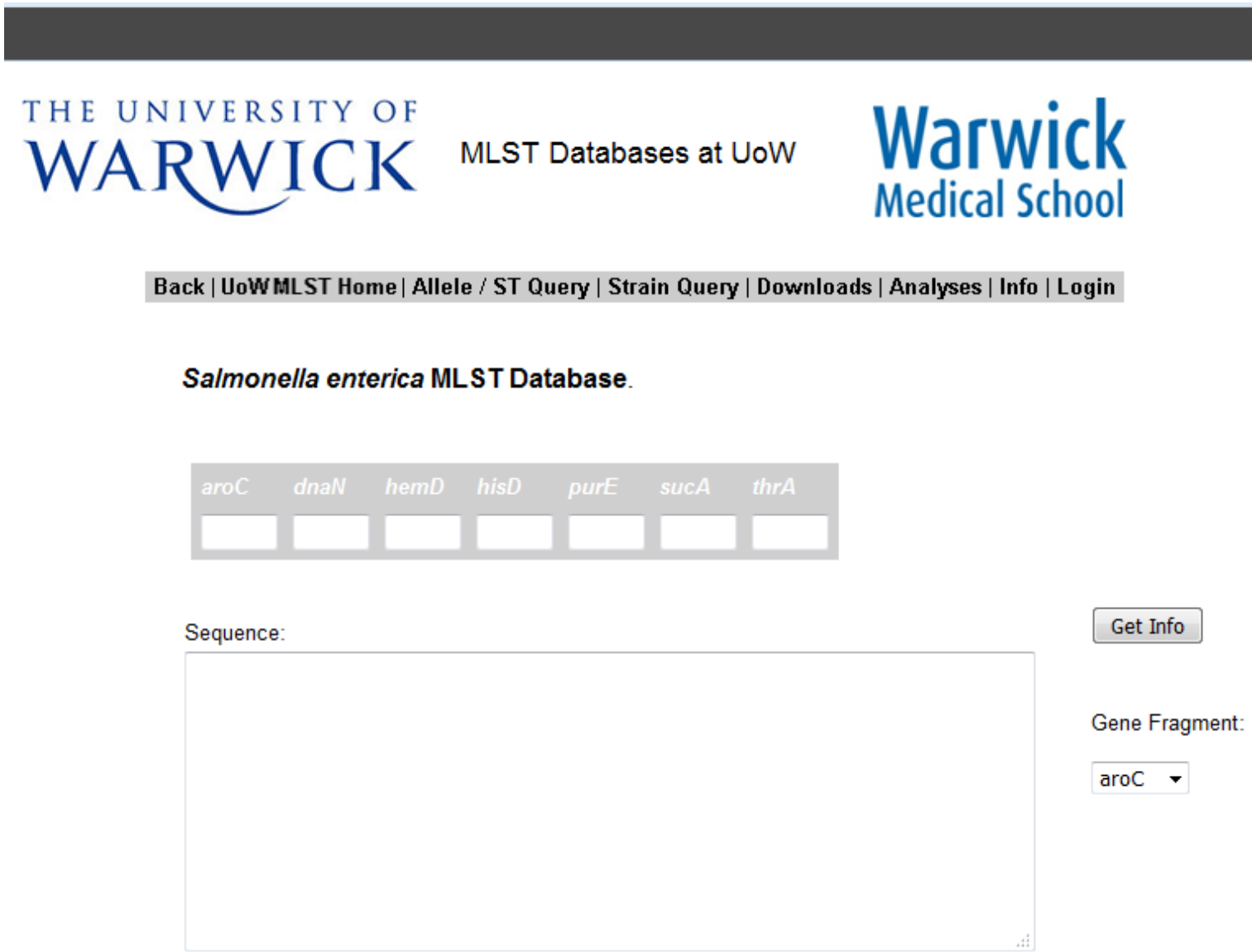
Genes

The E. coli MLST scheme uses internal fragments of the following seven house-keeping genes:

adk (adenylate kinase)
fumC (fumarate hydratase)
gyrB (DNA gyrase)
icd (isocitrate/isopropylmalate dehydrogenase)
mdh (malate dehydrogenase)
purA (adenylosuccinate dehydrogenase)
recA (ATP/GTP binding motif)

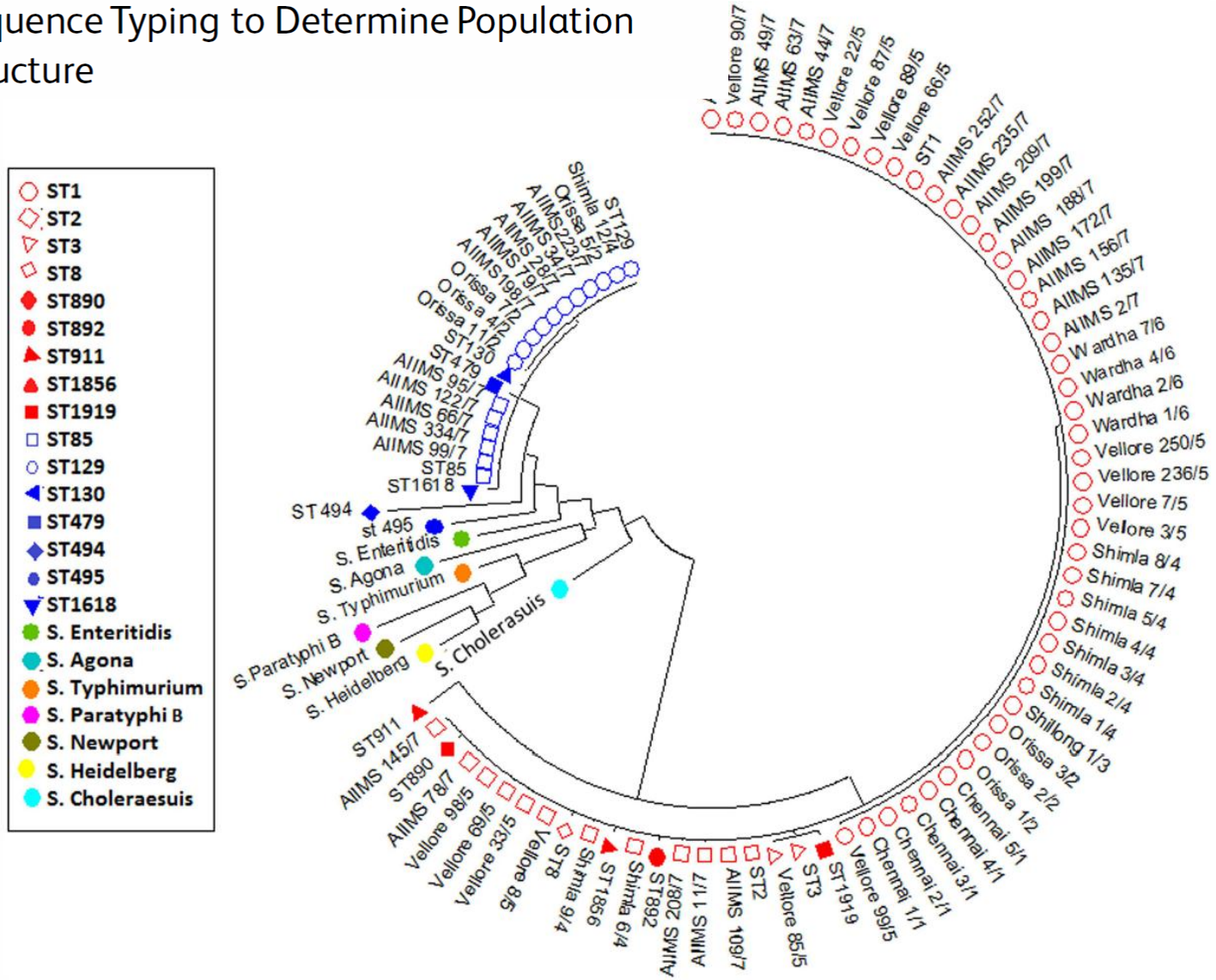
PCR Amplification

Multi Locus Sequence Typing (MLST)



RESEARCH ARTICLE

Typhoidal Salmonellae: Use of Multi-Locus Sequence Typing to Determine Population Structure

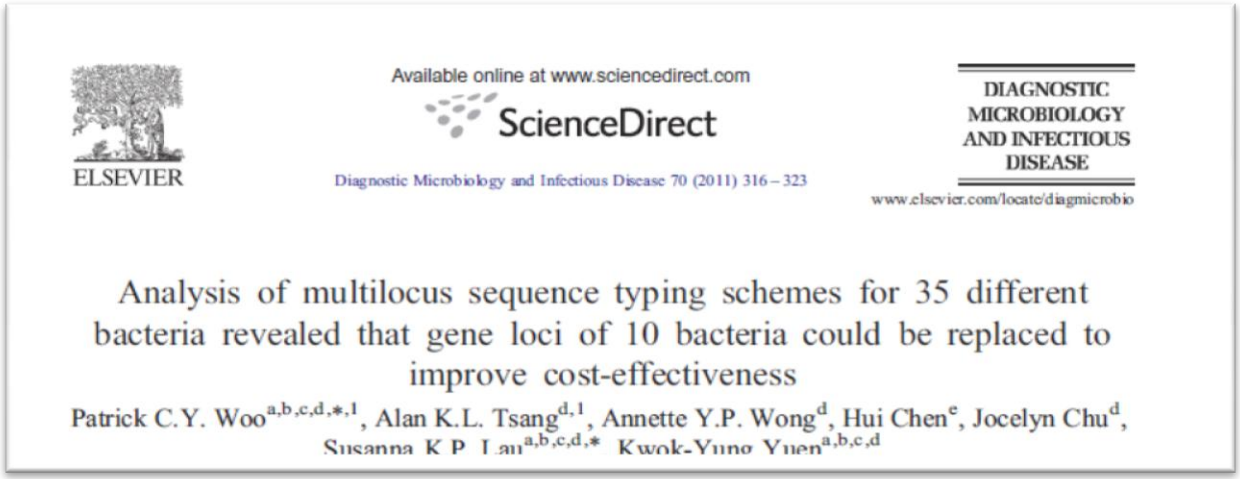


Multi Locus Sequence Typing (MLST)

MLST
Multi Locus Sequence Typing

•Νέους STs
•Αλλαγές στο αρχικό πρωτόκολλο → αύξηση χρόνου και κόστους
•Το πρόβλημα ενισχύθηκε καθώς η μέθοδος βρήκε εφαρμογή και σε *P. aeruginosa* στελέχη προερχόμενα από περιβαλλοντικά ενδιαιτήματα όπως υδάτινα οικοσυστήματα, χώμα, φυτά κ.λ.π.

•η διακριτική ικανότητα της μεθόδου δεν εξαρτάται από τον αριθμό των γονιδίων που χρησιμοποιούνται
•ανάλυση λιγότερων γονιδίων
•μείωση κόστους και χρόνου
•αύξηση αριθμού των υπό μελέτη στελεχών



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ScienceDirect
Diagnostic Microbiology and Infectious Disease 70 (2011) 316 – 323

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Analysis of multilocus sequence typing schemes for 35 different bacteria revealed that gene loci of 10 bacteria could be replaced to improve cost-effectiveness

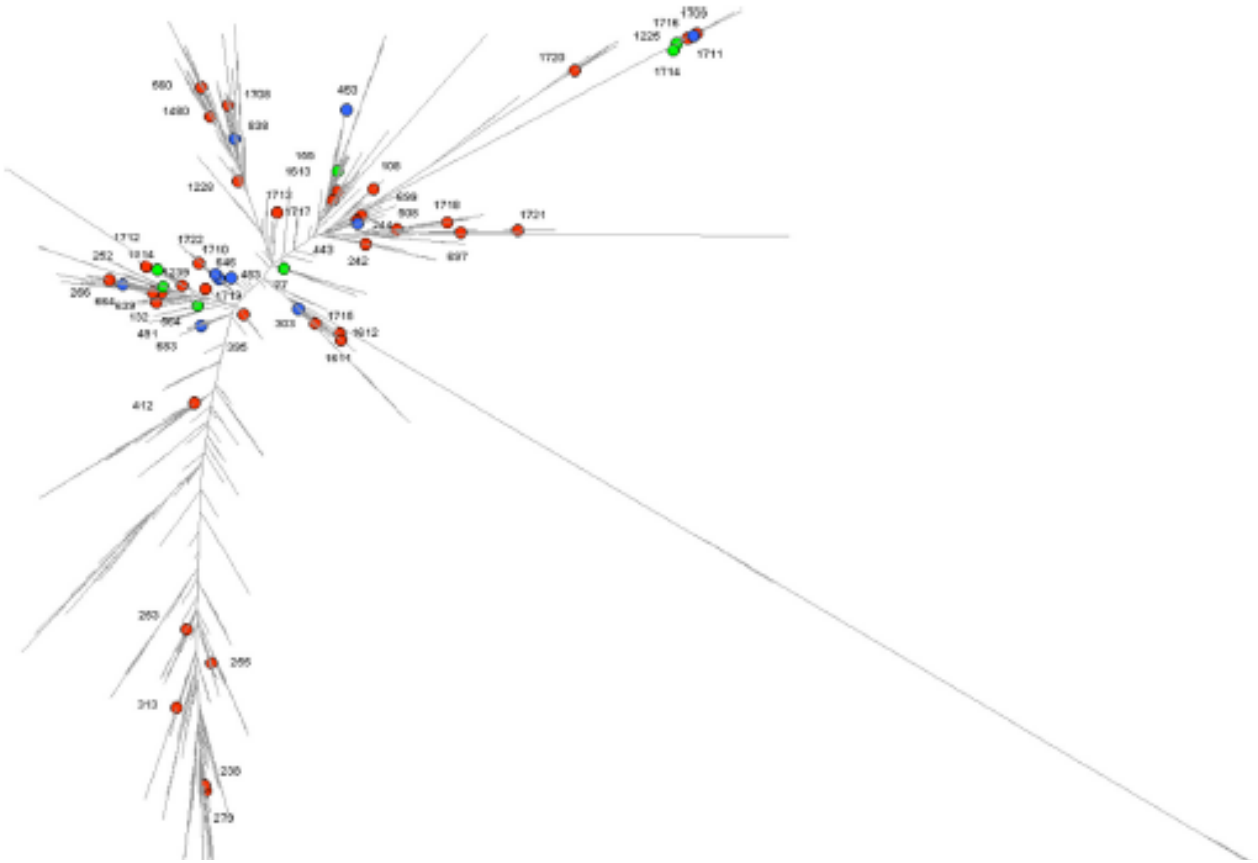
Patrick C.Y. Woo[a,b,c,d,*,1], Alan K.L. Tsang[d,1], Annette Y.P. Wong[d], Hui Chen[e], Jocelyn Chu[d], Susanna K.P. Lau[a,b,c,d,*], Kwok-Yung Yuen[a,b,c,d]

Haenni et al. BMC Veterinary Research (2015) 11:9
DOI 10.1186/s12917-015-0324-x

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Population structure and antimicrobial susceptibility of *Pseudomonas aeruginosa* from animal infections in France



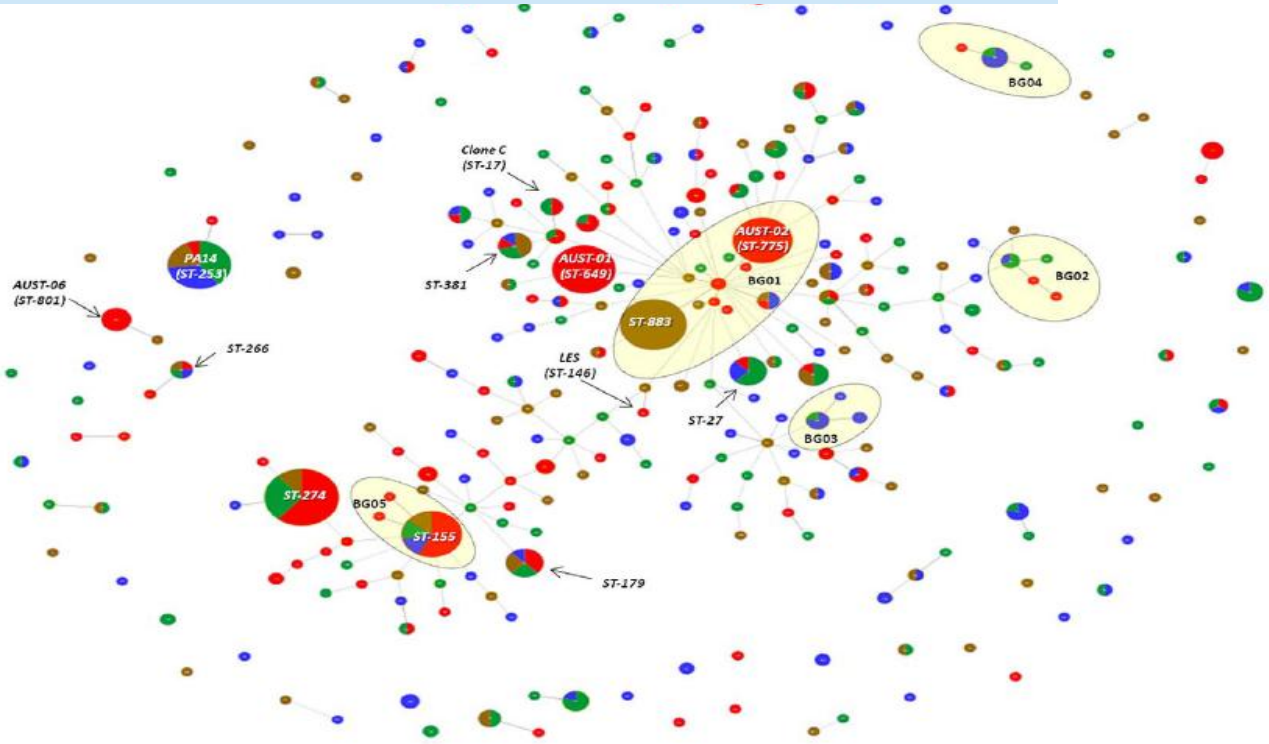
Results: Thirty-four already registered and 19 new MLST profiles were identified. Interestingly, a few clones were more prevalent, and clones associated to human outbreaks were also detected. Multidrug resistance phenotypes were overall rare.

OPEN ACCESS Freely available online

PLOS | ONE

Pseudomonas aeruginosa Exhibits Frequent Recombination, but Only a Limited Association between Genotype and Ecological Setting

geographical region. Overall, MLST identified 274 different sequence types, of which 53 were shared between one or more ecological settings. Our analysis revealed a limited association between genotype and environment and evidence of frequent recombination. We also found that genetic diversity of *P. aeruginosa* in Queensland, Australia was indistinguishable from that of the global *P. aeruginosa* population. Several CF strains were encountered frequently in multiple ecological settings; however, the most frequently encountered CF strains were confined to CF patients. Overall, our data confirm a non-clonal epidemic structure and indicate that most CF strains are a random sample of the broader *P. aeruginosa* population. The increased abundance of some CF strains in different geographical regions is a likely product of chance colonisation events followed by adaptation to the CF lung and horizontal transmission among patients.



Multi Locus Sequence Typing (MLST)

•Molecular Typing of CTX-M-producing *Escherichia coli Isolated from* Environmental Water, Swine Feces, Healthy Human Specimens and Human Patients, Hu Y.Y. et al, 2013

•High Prevalence of ESBL-Producing *Klebsiella pneumoniae* Causing Community-Onset Infections in China, Zhang et al, 2016

•Clonal Dissemination of *Pseudomonas aeruginosa* Sequence Type 235 Isolates Carrying blaIMP-6 and Emergence of blaGES-24 and blaIMP-10 on Novel Genomic Islands PAGI-15 and -16 in South Korea, Hong et al, 2016

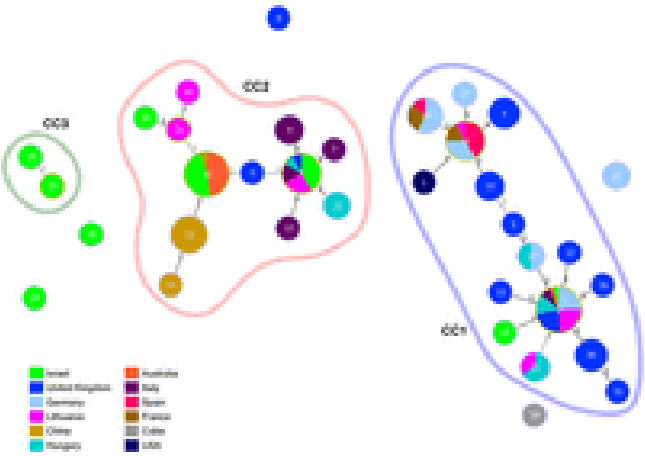
•Phenotyping and genetic characterization of *Salmonella enterica* isolates from Turkey revealing arise of different features specific to geography, , Acar et al, 2017

Double Locus Sequence Typing (DLST)

ΣΥΝΔΥΑΣΜΟΣ MLVA+MLST

Τυποποίηση μέσω αλληλούχισης

- Αναγνωρίζει άμεσα τις διαφορές σε αλληλουχίες DNA επαναλαμβανόμενων γενετικών τόπων (VNTR) που εντοπίζονται σε στελέχη του ιδίου είδους
- Χρησιμοποιούνται εκκινητές που 'πιάνουν' μεγάλα τμήματα του DNA (>1000bp)
- Χαρακτηρίζει τα στελέχη απο το μοναδικό τους 'γονιδιακό προφίλ' (allelic profile)
- Υψηλή τυποποιητική ικανότητα και διακριτική ικανότητα, αλλά χαμηλή αναπαραγωγιμότητα
- Γρήγορη, εύκολη στην εφαρμογή της με **ΧΑΜΗΛΟ κόστος**



Double Locus Sequence Typing (DLST)

❖ Διαδικασία:

✓ PCR (2 γονίδια-VNTR με ειδικούς εκκινητές)

✓ Αλληλούχιση των προϊόντων της PCR

✓ Upload τις αλληλουχίες στις βάσεις δεδομένων

✓ Έτσι προκύπτουν οι DLST- τύποι για το κάθε στέλεχος

✓ Στελέχη που έχουν το ίδιο γονιδιακό προφίλ ανήκουν στον ίδιο DLST - τύπο.

Οι DLST data bases περιλαμβάνουν την αλληλουχία αναφοράς και αλληλουχίες από πολλά διαφορετικά στελέχη του ίδιου μ.ο.

- *Οι βάσεις δεδομένων είναι ελεύθερα προσβάσιμες στο διαδίκτυο:*

✓ *http://www.dlst.org*

CHUV Centre hospitalier universitaire vaudois

UNIL | Université de Lausanne

Double locus sequence typing

A sequence based typing scheme for epidemiological investigation of bacterial pathogens

Home

Staphylococcus aureus

Pseudomonas aeruginosa

Contact

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BioNumerics Server

Welcome to the Double Locus Sequence Typing (DLST) webpage!

DLST is a fast and simple sequence based method to type bacteria for epidemiological investigation. This method is based on the sequencing of c.a. 500bp of two highly variable loci.

DLST has been shown to offer a high resolution and it allows studying both local and international datasets .

For each locus, an arbitrary number is assigned to each allele and the combination of both alleles constitutes the DLST type.

The purpose of this DLST website is to synchronize the DLST alleles from various laboratories and geographical regions.

For comments, queries, or suggestions please contact the curator.

Double Locus Sequence Typing (DLST)

Eur J Clin Microbiol Infect Dis (2014) 33:927–932
DOI 10.1007/s10096-013-2028-0

ARTICLE

Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method

P. Basset · D. S. Blanc

Diagnostic Microbiology and Infectious Disease 82 (2015) 274–277

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Comparison of double-locus sequence typing (DLST) and multilocus sequence typing (MLST) for the investigation of *Pseudomonas aeruginosa* populations☆

Pascal Cholley [a,1], Milos Stojanov [b,1], Didier Hocquet [a], Michelle Thouverez [a], Xavier Bertrand [a], Dominique S. Blanc [b,*]

[a] Infection Control Department, UMR Chrono-environnement (CNRS), University Hospital of Besançon, Besançon, France
[b] Service of Hospital Preventive Medicine, Lausanne University Hospital, Lausanne, Switzerland

Double Locus Sequence Typing (DLST)

ms172, ms217* και *oprD

Eur J Clin Microbiol Infect Dis. 2014 Jun;33(6):927-32. doi: 10.1007/s10096-013-2028-0. Epub 2013 Dec 11.

Fast and simple epidemiological typing of Pseudomonas aeruginosa using the double-locus sequence typing (DLST) method.

Basset P[1], Blanc DS.

⊕ **Author information**

Abstract

Although the molecular typing of Pseudomonas aeruginosa is important to understand the local epidemiology of this opportunistic pathogen, it remains challenging. Our aim was to develop a simple typing method based on the sequencing of two highly variable loci. Single-strand sequencing of three highly variable loci (ms172, ms217, and oprD) was performed on a collection of 282 isolates recovered between 1994 and 2007 (from patients and the environment). As expected, the resolution of each locus alone [number of types (NT) = 35-64; index of discrimination (ID) = 0.816-0.964] was lower than the combination of two loci (NT = 78-97; ID = 0.966-0.971). As each pairwise combination of loci gave similar results, we selected the most robust combination with ms172 [reverse; R] and ms217 [R] to constitute the double-locus sequence typing (DLST) scheme for P. aeruginosa. This combination gave: (i) a complete genotype for 276/282 isolates (typability of 98%), (ii) 86 different types, and (iii) an ID of 0.968. Analysis of multiple isolates from the same patients or taps showed that DLST genotypes are generally stable over a period of several months. The high typability, discriminatory power, and ease of use of the proposed DLST scheme makes it a method of choice for local epidemiological analyses of P. aeruginosa. Moreover, the possibility to give unambiguous definition of types allowed to develop an Internet database (http://www.dlst.org) accessible by all.

Double Locus Sequence Typing (DLST)

UNIL | Université de Lausanne

Home

Staphylococcus aureus

Pseudomonas aeruginosa

Contact

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BioNumerics Server

This site was implemented by Applied Maths NV.

Last updated on 2014-10-22

Pseudomonas aeruginosa

The DLST scheme is based on the analysis of a 400 bp fragment of the ms172 locus and a 350 bp fragment of the ms217 locus.

Trace files | FASTA file | Plain text sequences

Paste sequences into the text boxes below.

ms172 sequence: CTTGCACCCGAACCGATGTTGGCGCCGC TCTGGCTCTGCACTGCGGAGATACCGTTG GCGC

ms217 sequence: CGCCAGGCGCATCTCGAACTCCGCCGGA TCGTGGATCAGCACGTCCTGTCCGGCGA ACG

Submit Clear

Protocol for DLST of *P. aeruginosa*

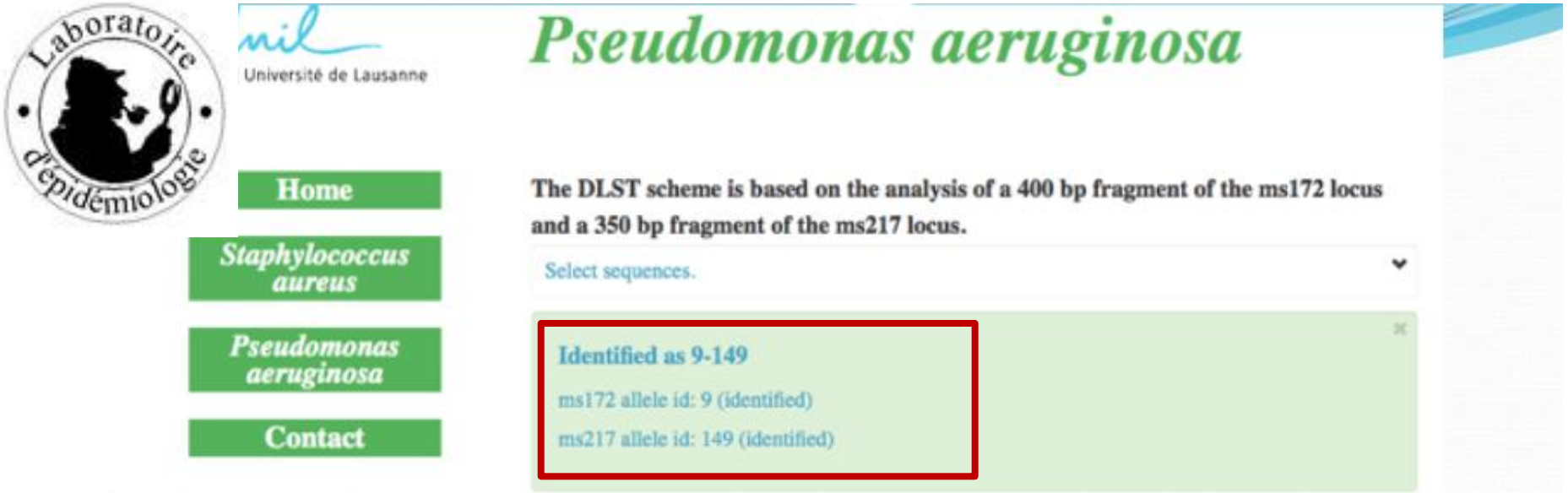
References for DLST of *P. aeruginosa*

Allele definitions of ms172

Allele definitions of ms217

[http://www.dlst.org/Paeruginosa/]

Double Locus Sequence Typing (DLST)

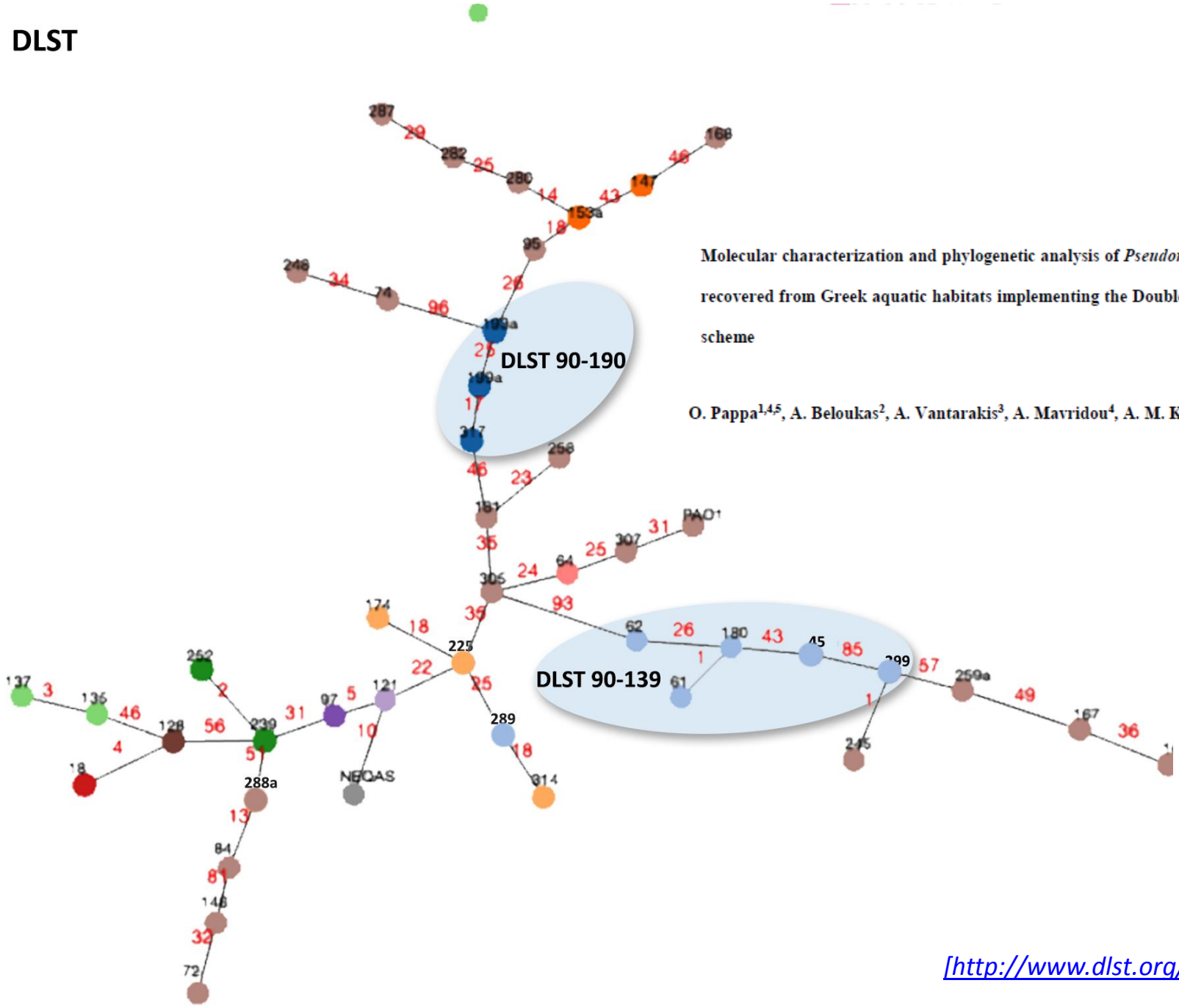


ms172: 115 alleles
ms217:190 alleles

- Basset P, Blanc DS. Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method. Eur J Clin Microbiol Infect Dis. 2014 Dec 11
- Pascal Cholley et al, Comparison of double-locus sequence typing (DLST) and multi locus sequence typing (MLST) for the investigation of *Pseudomonas aeruginosa* populations, Diagn Microbiol Infect Dis 82 (2015) 274-277

[http://www.dlst.org/Paeruginosa/]

DLST



Molecular characterization and phylogenetic analysis of *Pseudomonas aeruginosa* isolates recovered from Greek aquatic habitats implementing the Double Locus Sequence Typing scheme

O. Pappa[1,4,5], A. Beloukas[2], A. Vantarakis[3], A. Mavridou[4], A. M. Kefala[5], A. Galanis[5*]

[http://www.dlst.org/Paeruginosa/]

Double Locus Sequence Typing (DLST)

Eur J Clin Microbiol Infect Dis. 2016 Feb;35(2):175-81. doi: 10.1007/s10096-015-2525-4. Epub 2015 Nov 18.

Development and evaluation of double locus sequence typing for molecular epidemiological investigations of Clostridium difficile.

Stojanov M[1,2], Magalhaes B[3], Terletsky V[3], Basset P[3], Prod'hom G[4], Greub G[4], Senn L[3], Blanc DS[3,4].

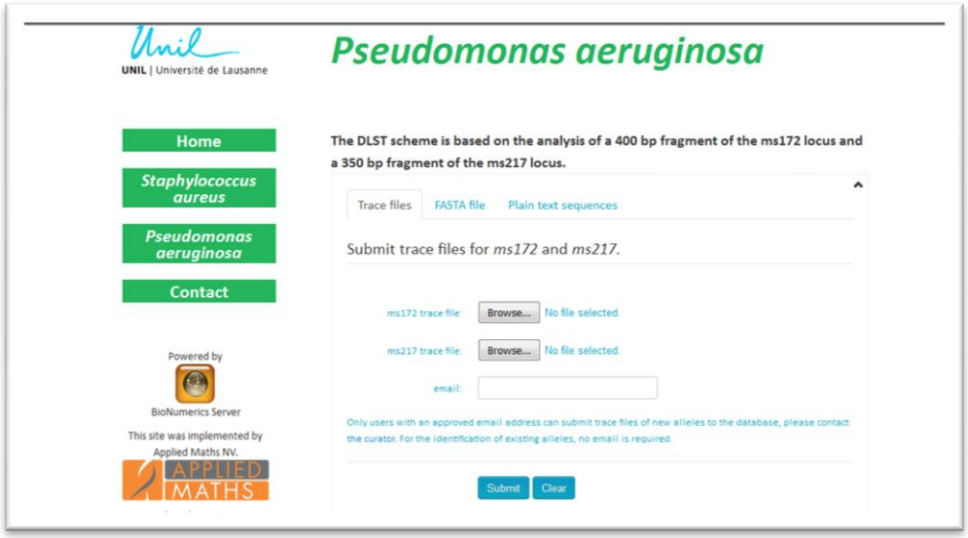
⊕ Author information

Abstract

Despite the development of novel typing methods based on whole genome sequencing, most laboratories still rely on classical molecular methods for outbreak investigation or surveillance. Reference methods for Clostridium difficile include ribotyping and pulsed-field gel electrophoresis, which are band-comparing methods often difficult to establish and which require reference strain collections. Here, we present the double locus sequence typing (DLST) scheme as a tool to analyse C. difficile isolates. Using a collection of clinical C. difficile isolates recovered during a 1-year period, we evaluated the performance of DLST and compared the results to multilocus sequence typing (MLST), a sequence-based method that has been used to study the structure of bacterial populations and highlight major clones. DLST had a higher discriminatory power compared to MLST (Simpson's index of diversity of 0.979 versus 0.965) and successfully identified all isolates of the study (100 % typeability). Previous studies showed that the discriminatory power of ribotyping was comparable to that of MLST; thus, DLST might be more discriminatory than ribotyping. DLST is easy to establish and provides several advantages, including absence of DNA extraction [polymerase chain reaction (PCR) is performed on colonies], no specific instrumentation, low cost and unambiguous definition of types. Moreover, the implementation of a DLST typing scheme on an Internet database, such as that previously done for Staphylococcus aureus and Pseudomonas aeruginosa (http://www.dlst.org), will allow users to easily obtain the DLST type by submitting directly sequencing files and will avoid problems associated with multiple databases.

DLST vs MLST

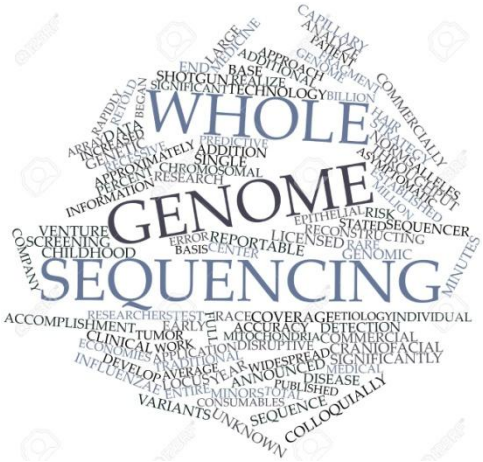
1. **χρησιμοποιούνται δυο γονίδια αντί για 7 (μείωση χρόνου και κόστους)**
2. **απαιτείται αλληλούχιση μόνο στη μία κατεύθυνση του γονιδιώματος (μονό διάβασμα, Reverse/μείωση χρόνου και κόστους)**
3. **η βιβλιογραφία έχει δείξει οτι η διακριτική ικανότητα και η αναπαραγωγιμότητα της μεθόδου είναι ίδια με αυτή της MLST** ***[****Cholley P. et al, 2015****]***
4. **η χρήση γενετικών τόπων όχι τόσο συντηρημένων λειτουργιών ίσως αντικατοπτρίζει καλύτερα και πιο αξιόπιστα τη γενετική ποικιλομορφία του βακτηριακού πληθυσμού**



Whole Genome Sequencing (WGS)

❖Είναι μια εργαστηριακή διαδικασία που καθορίζει την πλήρη αλληλουχία του γονιδιώματος ενός οργανισμού. Πραγματοποιείται πλήρης αλληλούχιση του χρωμοσωμικού DNA ενός οργανισμού, καθώς και του DNA που περιέχεται στα μιτοχόνδρια

❖Χρησιμοποιείται επίσης για την ανίχνευση σημειακών μεταλλαγών σε ένα γονιδίωμα (SNPs) βελτιώνοντας έτσι τη πληροφορία (μέγεθος + ποιότητα) που λαμβάνεται σε εξελικτικές μελέτες ενώ παράλληλα θέτει τις βάσεις για την πρόβλεψη της ευαισθησίας μιας νόσου και την ανταπόκριση στα διαθέσιμα φάρμακα.



❖Βρίσκει εφαρμογή τόσο στην έρευνα όσο και στη κλινική πράξη

Fig. 8.13, Shotgun sequencing a genome

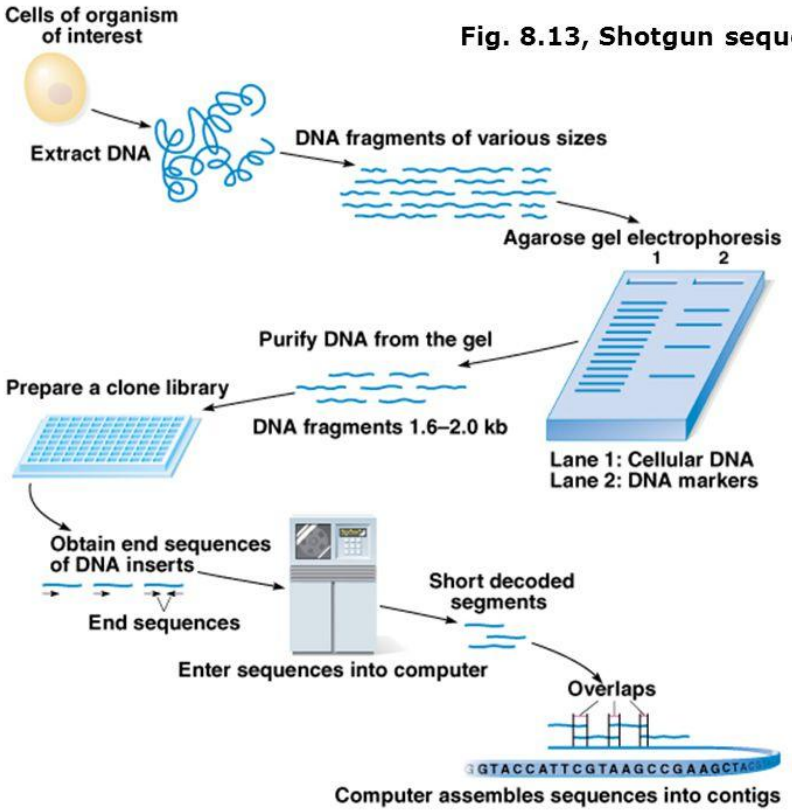
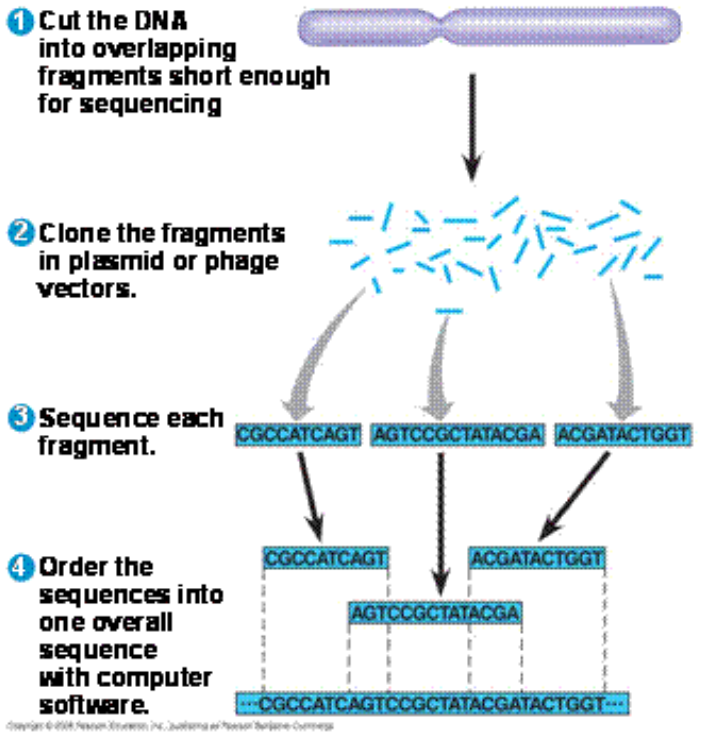


Fig. 21-3-3



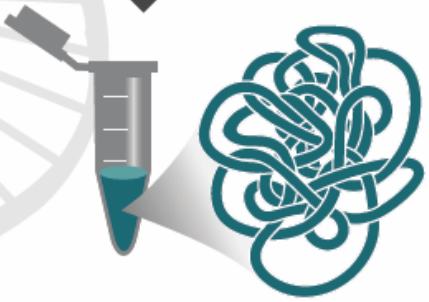
The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

Bacterial Culture

1. DNA Extraction

1. Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.



2. DNA Shearing

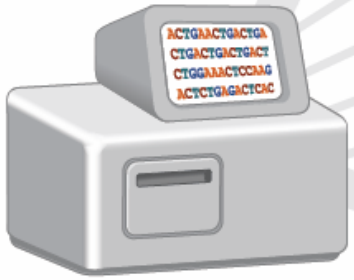
2. DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.

3. DNA Library Preparation

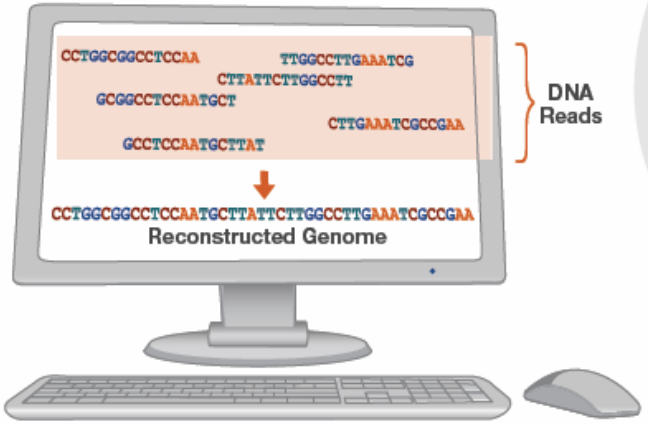
3. Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."

4. DNA Library Sequencing

4. The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."



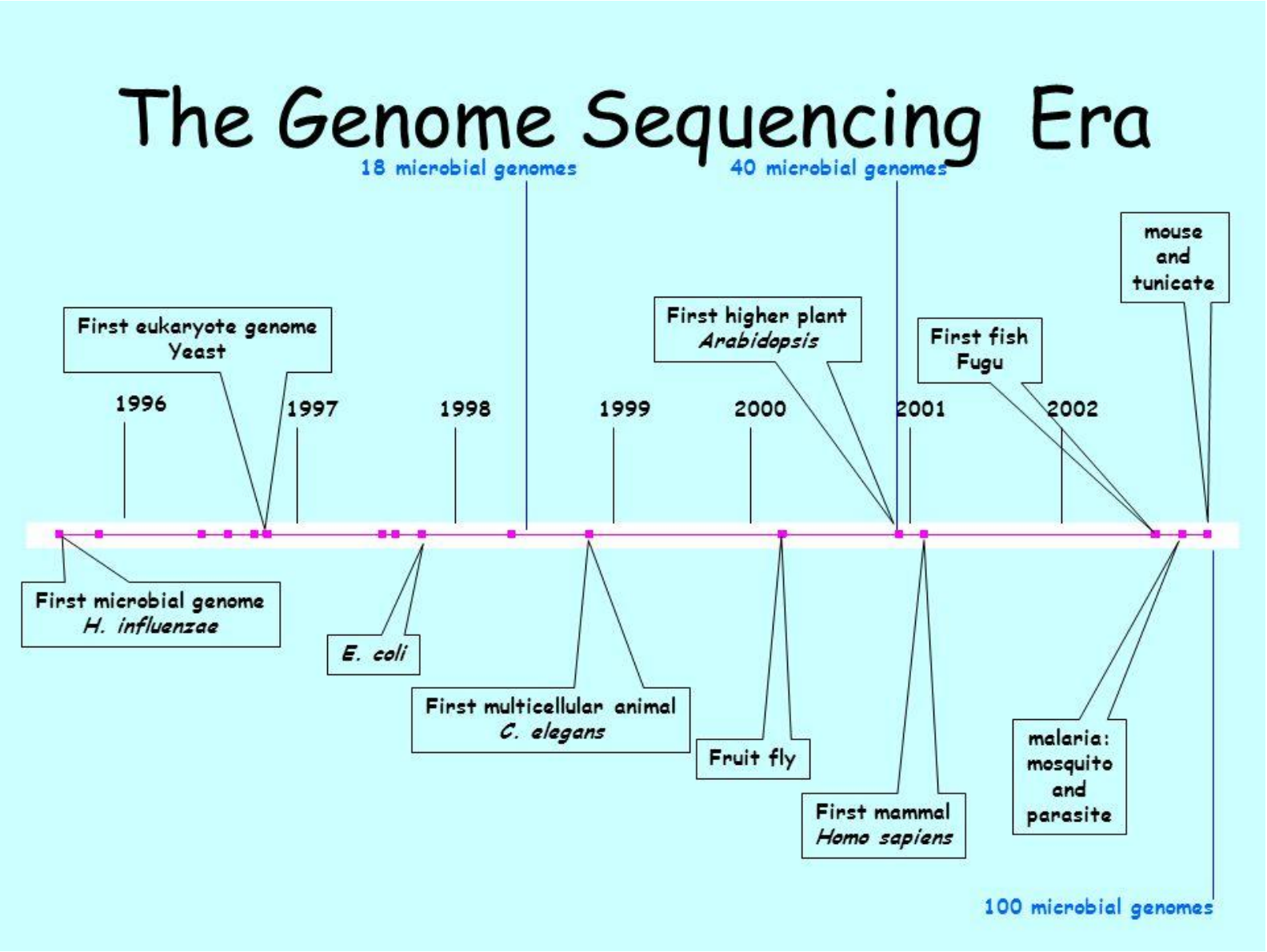
5. DNA Sequence Analysis



5. The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

CS264780-A

Whole Genome Sequencing (WGS)



Whole Genome Sequencing (WGS)

Το πρώτο βακτηριακό γονιδίωμα που αλληλουχίθηκε πλήρως ήταν αυτό του *Haemophilus influenzae*

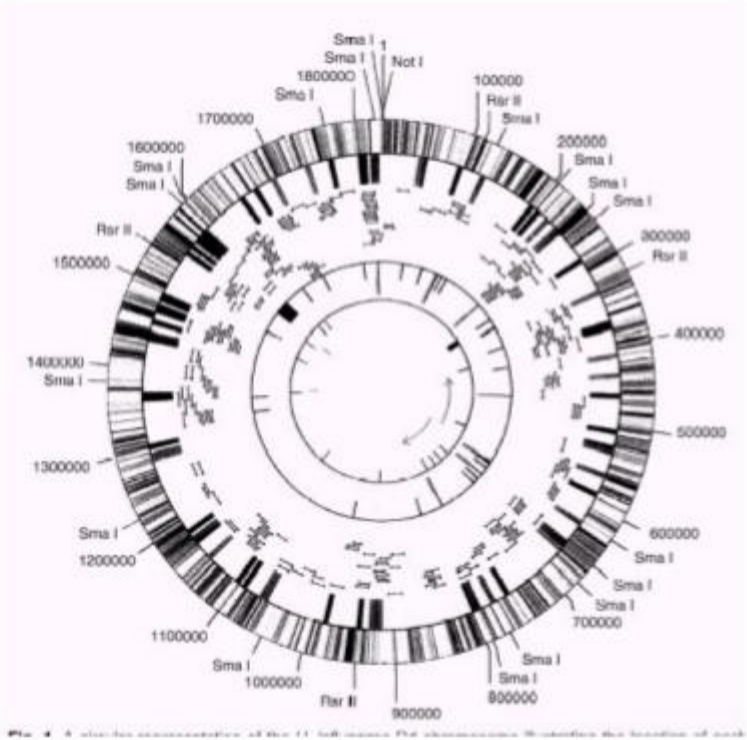
First microbial genome was completely sequenced in 1995 by The Institute for Genomic Research (TIGR)

Genome of *Haemophilus influenzae* Rd

single circular chromosome 1,860,137 bp

Outer circle – coding sequences with database matches

40% of genes at the time had no match in the databases



Fleishmann, R.D. et al. 1995. Science 269:496-512.

Whole Genome Sequencing (WGS)

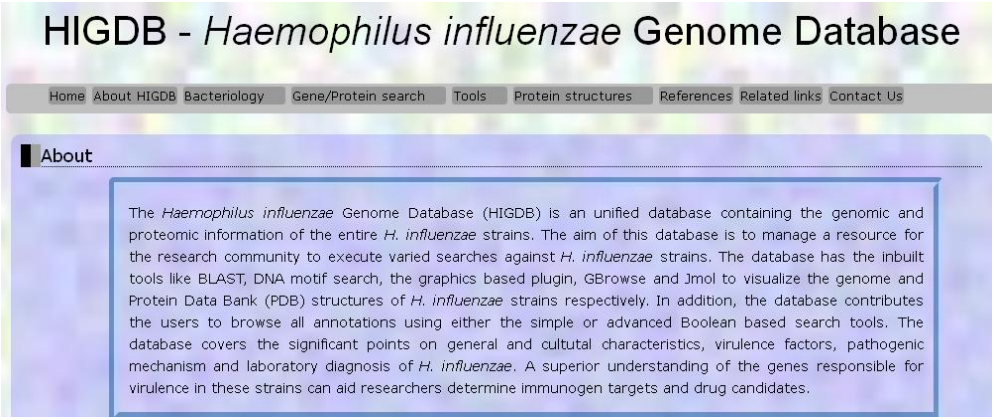
Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11121–11125, October 1996
Microbiology

DNA repeats identify novel virulence genes in *Haemophilus influenzae*

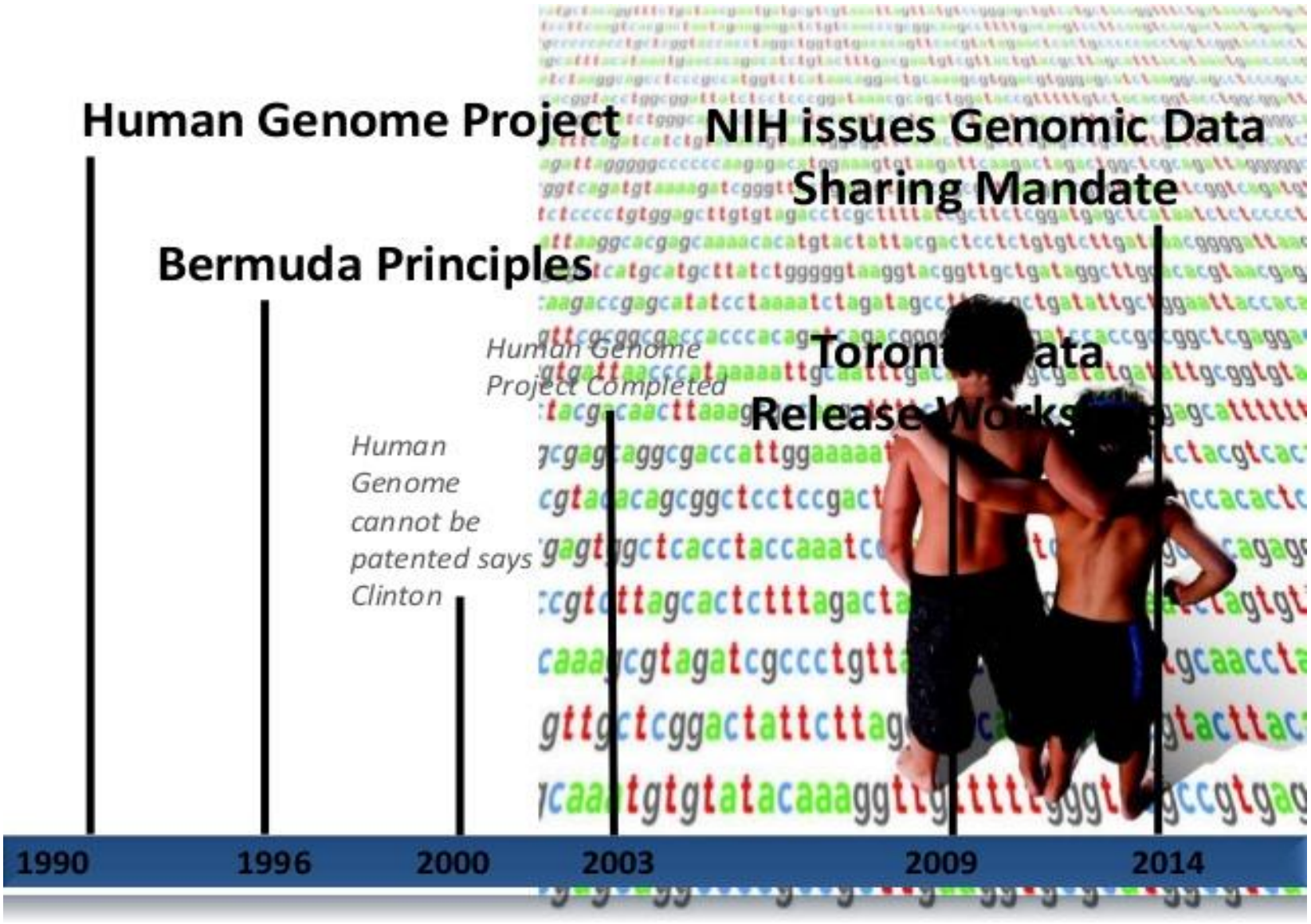
(microbiology/whole genome sequencing/pathogenicity)

DEREK W. HOOD*†, MARY E. DEADMAN*, MICHAEL P. JENNINGS*‡, MARINA BISERCIC*, ROBERT D. FLEISCHMANN§, J. CRAIG VENTER§, AND E. RICHARD MOXON*

Searching for DNA Repeats in the *H. influenzae* Genome Sequence. All possible combinations of mononucleotide, dinucleotide, trinucleotide, and tetranucleotide motifs were searched in early versions of the *H. influenzae* strain Rd genome data base prior to completion of sequencing and annotation. Three or more repeats of tetranucleotide and trinucleotide motifs were searched by using the program BLAST (17), and mononucleotide (homopolymeric) tracts comprising two or more nucleotides of A(T) or C(G) and dinucleotide



Whole Genome Sequencing (WGS)



Whole Genome Sequencing (WGS)

Community agreements to share data

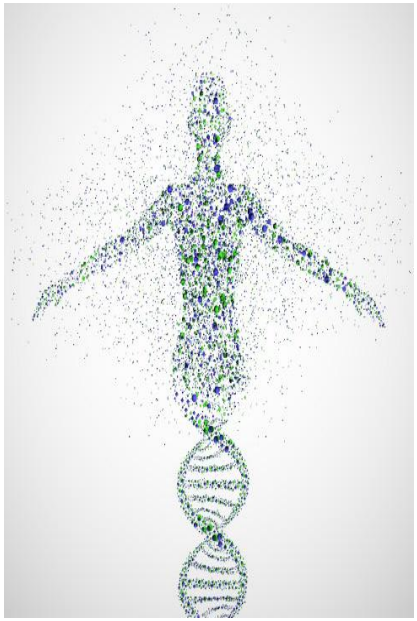
the Bermuda Principles for sharing DNA seq. data

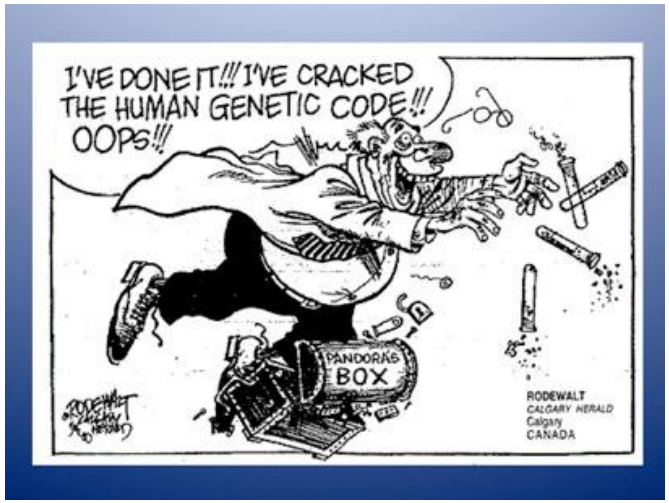
- Automatic release of sequence assemblies larger than 1 kb (preferably within 24 hours).
- Immediate publication of finished annotated sequences.
- Aim to make the entire sequence freely available in the public domain

"Bermuda Principles" drafted for Human Genome Project free data access

At a 1996 summit in Bermuda, leaders of the Human Genome Project agreed that all human genomic sequence information generated by centers funded for large-scale human sequencing should be made freely available and in the public domain within 24 hours after generation. The "Bermuda Principles" were drafted to encourage research and development and to maximize the Human Genome Project's benefits to society – in contrast to the standard practice in scientific research of making experimental data available only after its publication. These principles reshaped the practices of an entire industry and have established rapid prepublication data release as the norm in genomics and other fields.

Whole Genome Sequencing (WGS)





Finishing the euchromatic sequence of the human genome

International Human Genome Sequencing Consortium*

NATURE | VOL 431 | 21 OCTOBER 2004 | www.nature.com/nature

The sequence of the human genome encodes the genetic instructions for human physiology, as well as rich information about human evolution. In 2001, the International Human Genome Sequencing Consortium reported a draft sequence of the euchromatic portion of the human genome. Since then, the international collaboration has worked to convert this draft into a genome sequence with high accuracy and nearly complete coverage. Here, we report the result of this finishing process. The current genome sequence (Build 35) contains 2.85 billion nucleotides interrupted by only 341 gaps. It covers, 99% of the euchromatic genome and is accurate to an error rate of, 1 event per 100,000 bases. Many of the remaining euchromatic gaps are associated with segmental duplications and will require focused work with new methods. The near-complete sequence, the first for a vertebrate, greatly improves the precision of biological analyses of the human genome including studies of gene number, birth and death. Notably, the human genome seems to encode only 20,000-25,000 protein-coding genes. The genome sequence reported here should serve as a firm foundation for biomedical research in the decades ahead.

www.nature.com/ejhg

POLICY

Whole-genome sequencing in health care

Recommendations of the European Society of Human Genetics

Carla G van El[1], Martina C Cornel[1,2,3], Pascal Borry[4], Ros J Hastings[5], Florence Fellmann[6], Shirley V Hodgson[7], Heidi C Howard[8,9], Anne Cambon-Thomsen[8,9], Bartha M Knoppers[10], Hanne Meijers-Heijboer[11], Hans Scheffer[12], Lisbeth Tranebjaerg[13,14,15], Wybo Dondorp[16,17], Guido MWR de Wert[3,16,17]
on behalf of the ESHG Public and Professional Policy Committee

First published in European Journal of Human Genetics (2013) **21**, 580–584; doi:10.1038/ejhg.2013.46

Keywords: recommendations; whole-genome sequencing; policy

.....More services based on these technologies are now becoming available for patients, **raising the issue of how to ensure that these are provided appropriately.** .....The **Public and Professional Policy Committee (PPPC)** and the **Quality Committee of the European Society of Human Genetics (ESHG)** addressed these challenges at a joint workshop in Gothenburg, Sweden, in 2010.3 PPPC also organized workshops in Amsterdam, the Netherlands (January 2011 in collaboration with the EU-funded project TECHGENE, January 2012). **Focusing on the clinical diagnostics setting, this paper is intended to contribute to the discussion and the development of guidelines in this fast-moving field, and provide recommendations for health-care professionals.** The paper and recommendations were posted on the ESHG website from 20 June to 1 August 2012 for comment by the membership. The final version was approved by the ESHG Board in December 2012.

should the raw data obtained through WGS be stored and if so under what conditions?

how to deal with outcomes that may be clinically relevant but will or may only affect the child in its adult life?

how to deal with information that patients or parents have indicated they would not want to receive, but that may still be important for their own health, for that of their children or any close relatives.

Whole Genome Sequencing (WGS)

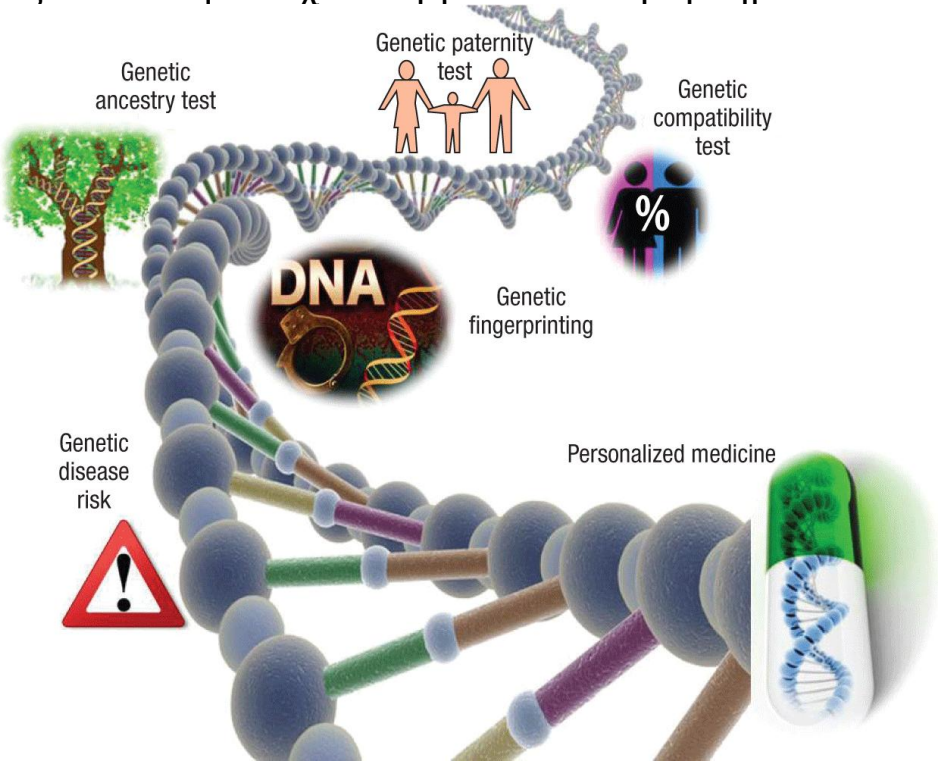
ΠΛΕΟΝΕΚΤΗΜΑΤΑ

• Η μελέτη μιας ασθένειας δεν βασίζεται πια μόνο στην ανίχνευση του μεταλλαγμένου γονιδίου που προκαλεί την ασθένεια, αλλά και σε άλλα γονίδια του ασθενή.

• Η τυποποίηση των καρκινικών κυττάρων επιτρέπει πια στους γιατρούς να επιλέξουν την αποδοτικότερη χημειοθεραπεία και ακόμη και να μειώσουν την έκθεση του ασθενή σε τοξικές θεραπείες

• Γονίδια άγνωστα έως τώρα, πλέον αναγνωρίζονται και ίσως να συμβάλλουν στην εμφάνιση και τη πορεία μιας ασθένειας. Οι παραδοσιακές τεχνικές βασίζονται στην ανίχνευση μόνο του προβληματικού γονιδίου.

•Περιβαλλοντικοί παράγοντες που μπορεί να επηρεάζουν ή ακόμη και να ενισχύουν την εμφάνιση και τη πορεία μιας νόσου είναι δυνατόν πλέον να αναγνωρίζονται και να τροποποιούνται



A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases

While the cost of whole genome sequencing (WGS) is approaching the realm of routine medical tests, it remains too tardy to help guide the management of many acute medical conditions. Rapid WGS is imperative in light of growing evidence of its utility in acute care, such as in diagnosis of genetic diseases in very ill infants, and genotype-guided choice of chemotherapy at cancer relapse. In such situations, delayed, empiric, or phenotype-based clinical decisions may meet with substantial morbidity or mortality.

For 18-h WGS performed in Essex, isolated genomic DNA was prepared using a modification of the standard Illumina TruSeq sample preparation

Participants were principally parent-child trios enrolled in a research biorepository who received WGS in addition to standard diagnostic tests to diagnose monogenic disorders of unknown etiology in affected infants

J Clin Microbiol. 2016 Apr 20. pii: JCM.00349-16. [Epub ahead of print]

Application of WGS data for O-specific antigen analysis and in silico serotyping of Pseudomonas aeruginosa isolates.

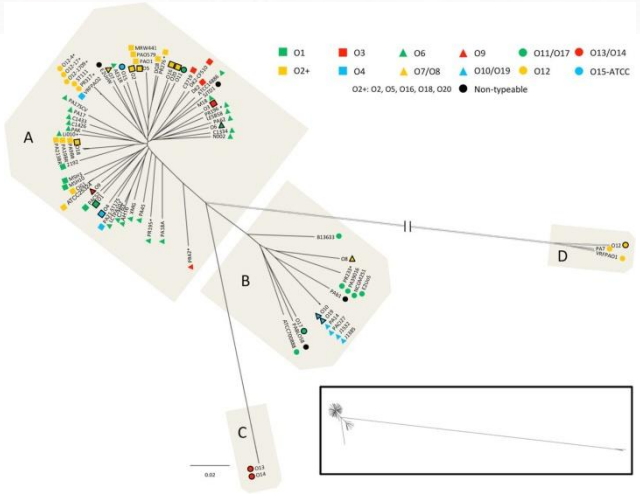
Thrane SW[1], Taylor VL[2], Lund O[3], Lam JS[2], Jelsbak L[4].

⊕ Author information

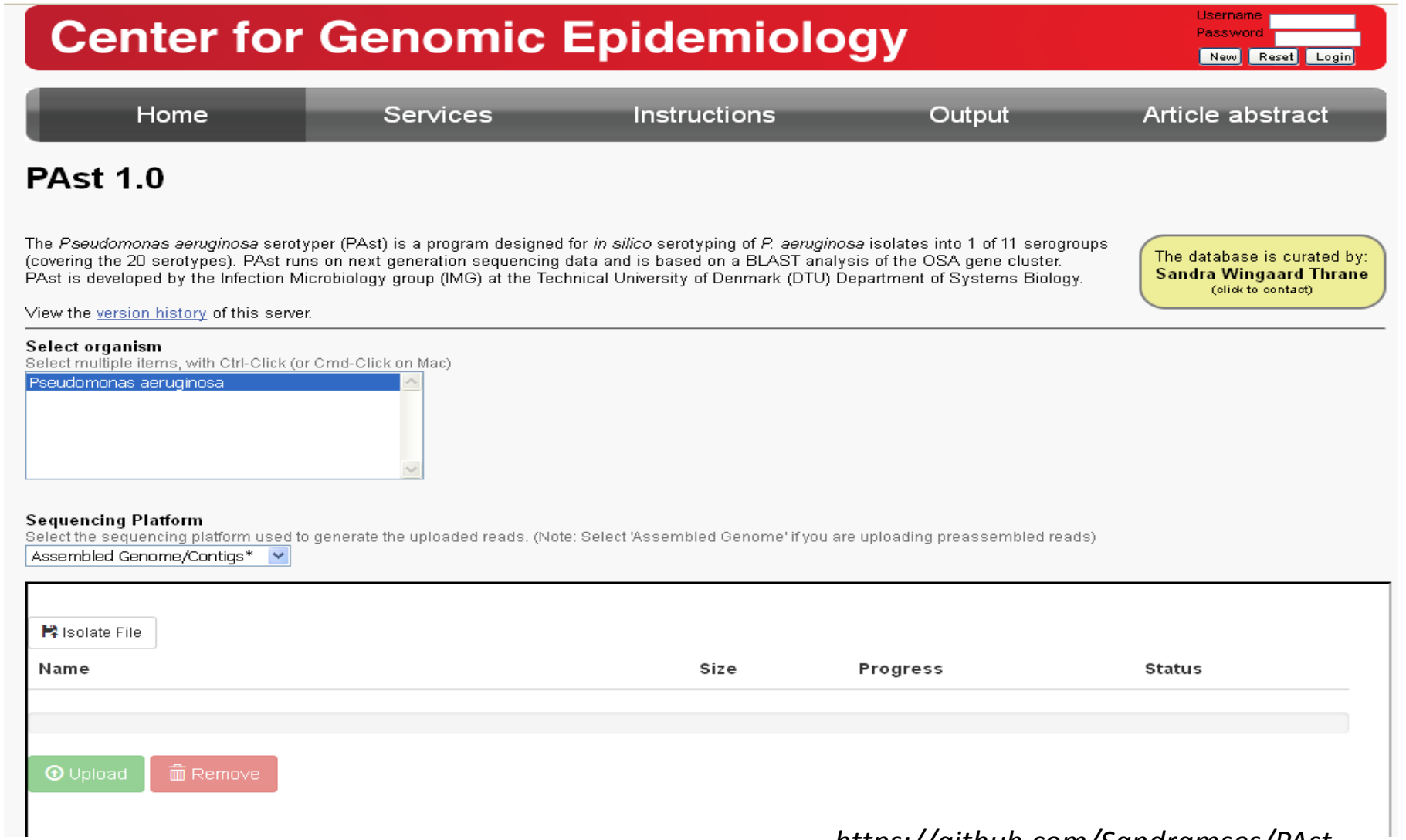
Abstract

Accurate typing methods are required for efficient infection control. The emergence of whole genome sequencing (WGS) technologies has enabled the development of genomics-based methods applicable for routine typing and surveillance of bacterial pathogens. In this study, we developed thePseudomonas aeruginosaserotyper (PAst) program, which enabledin silicoserotyping ofP. aeruginosaisolates using WGS data. PAst has been made publically available as a web-service, and aptly facilitate high-throughput serotyping analysis. The program overcomes critical issues such as the loss ofin vitrotypeability often associated withP. aeruginosaisolates from chronic infections, and quickly determines the serogroup of an isolate based on the sequence of the O-specific antigen (OSA) gene cluster. Here, PAst analysis of 1649 genomes resulted in successful serogroup assignments in 99.27% of the cases. This frequency is rarely achievable by conventional serotyping methods. The limited number of non-typeable isolates found using PAst was the result of either complete absence of OSA genes in the genomes or the artifact of genomic misassembly. With PAst,P. aeruginosaserotype data can be obtained from WGS information alone. PAst is a highly efficient alternative to conventional serotyping methods in relation to outbreak surveillance of serotype O12 and other high-risk clones, while maintaining backward compatibility to historical serotype data.

the Pseudomonas aeruginosa serotyper (PAst) program, which enabled in silico serotyping of P. aeruginosa isolates using WGS data



the Pseudomonas aeruginosa serotyper (PAst) program



https://github.com/Sandramses/PAst

frontiers in Cellular and Infection Microbiology

REVIEW
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Are *Escherichia coli* Pathotypes Still Relevant in the Era of Whole-Genome Sequencing?

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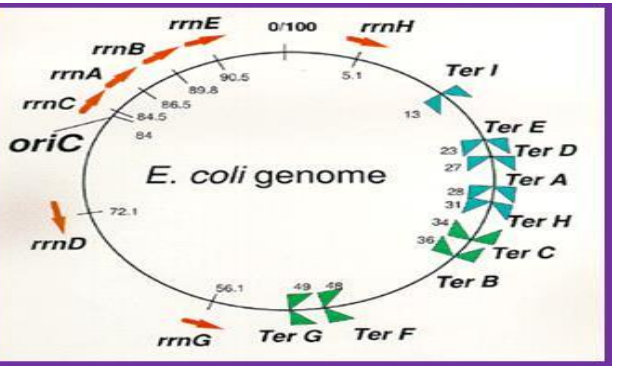

The ability to divide *E. coli* into subtypes is essential to understand the epidemiology and pathogenesis of particular clones. The use of sequence typing, biotyping, serotyping, and pathotyping to group similar bacteria together while separating them from others is helpful in many circumstances, such as when tracing outbreaks, but can be misleading when serotypes change or classification systems struggle to accommodate novel strains.

The subdivision of DEC into pathotypes is critical for understanding how these bacteria cause disease. The identification of pathotypes is also invaluable clinically (to determining prognosis and guide clinical management) and epidemiologically to detect outbreaks and estimate the contribution of different types of DEC to the overall burden of disease, as well as for the control of these diseases by public health interventions and immunisation (Levine et al., 1983; Sjöling et al., 2015).

Whole genome sequencing of *E. coli* strains has vastly enhanced our understanding of the evolution and pathobiology of this highly adaptable and versatile species. A major

Whole Genome Sequencing (WGS)

ΜΕΙΟΝΕΚΤΗΜΑΤΑ

• Η λειτουργία του μεγαλύτερου αριθμού των γονιδίων του ανθρώπινου γονιδιώματος είναι ακόμη άγνωστη

• Απαιτείται ιδιαίτερα εξειδικευμένο προσωπικό

• Το κόστος είναι πολύ υψηλό

• Το γονιδίωμα ενός ατόμου μπορεί να περιέχει πληροφορίες τις οποίες δε θέλει να γνωρίζει!!!!

• Ο όγκος της πληροφορίας που λαμβάνεται είναι τεράστιος! **Θέματα ηθικά, ασφάλειας και προστασίας προσωπικών δεδομένων δεν έχουν ακόμη διαχειριστεί.**

Methods Mol Biol. 2010;628:215-26. doi: 10.1007/978-1-60327-367-1_12.

Whole genome sequencing.

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Abstract

Whole genome sequencing provides the most comprehensive collection of an individual's genetic variation. With the falling costs of sequencing technology, we envision paradigm shift from microarray-based genotyping studies to whole genome sequencing. We review methodologies for whole genome sequencing. There are two approaches for assembling short shotgun sequence reads into longer contiguous genomic sequences. In the de novo assembly approach, sequence reads are compared to each other, and then overlapped to build longer contiguous sequences. The reference-based assembly approach involves mapping each read to a reference genome sequence. We discuss methods for identifying genetic variation (single nucleotide polymorphisms, small indels, and copy number variants) and building haplotypes from genome assemblies, and discuss potential pitfalls. We expect methodologies to evolve rapidly as sequencing technologies improve and more human genomes are sequenced.