Use of molecular typing methods for investigation of *Legionella* Infection

Joint Graduate Seminar

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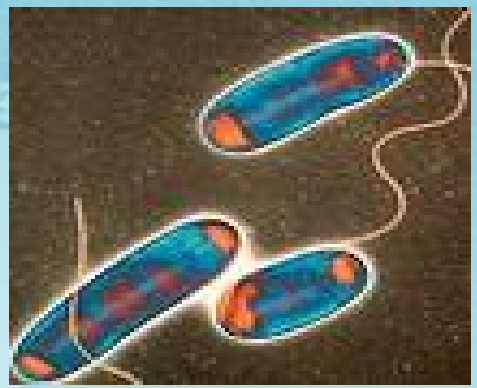
Introduction

- *Legionella pneumophila*, LP
  - Recognized only 35 years, Gram(-)
  - Manmade water supply systems
    Air condition, shower heads, taps, spas



- Legionnaires' disease , LD
  - Aerosols
  - Morbidity and mortality significant
  - Notifiable diseases

Epidemiology

- European Working Group for *Legionella* Infections (EWGLI) Surveillance Net

- world wide
  31 countries in Europe, United States, Japan and Australia

- HK
  2008(13) 2009(37) 2010(20)
  (Dept of Health,The government of HKSAR, http://www.chp.gov.hk/tc/notifiable1/10/26/43.html)

- Mainland China

TABLE 5-1 Nationally Notifiable Diseases

Acquired immunodeficiency syndrome	Hemophilus influenzae (Invasive Disease)
Anthrax	Hansen disease (leprosy)
Botulism*	Hantavirus pulmonary syndrome
Brucellosis	
Chancroid*	Hemolytic uremic syndrome
Chlamydia trachomatis genital infection	post-diarrheal
Cholera	Hepatitis A
Coccidioidomycosis*	Hepatitis B
Congenital rubella syndrome	Hepatitis C/non-A, non-B
Congenital syphilis	HIV infection, pediatric
Cryptosporidiosis	Legionellosis
Diphtheria	Lyme disease
Encephalitis, California	Malaria
Encephalitis, Easte	Measles (Rubeola)
Encephalitis, St. Lo	

Purpose of molecular typing for LP

- Why we must do molecular typing for *Legionella*?

  - 56 species/subspecies , 70 serogroups

(Harrison TG, et al, JCM 2009).

Virulence and Pathogenic very different

- No typical feature of clinical symptom in the infection

- Diagnosis
- Epidemiological tracing
- Improve the control measures
- Outbreak prevention

Purpose of molecular typing for LP

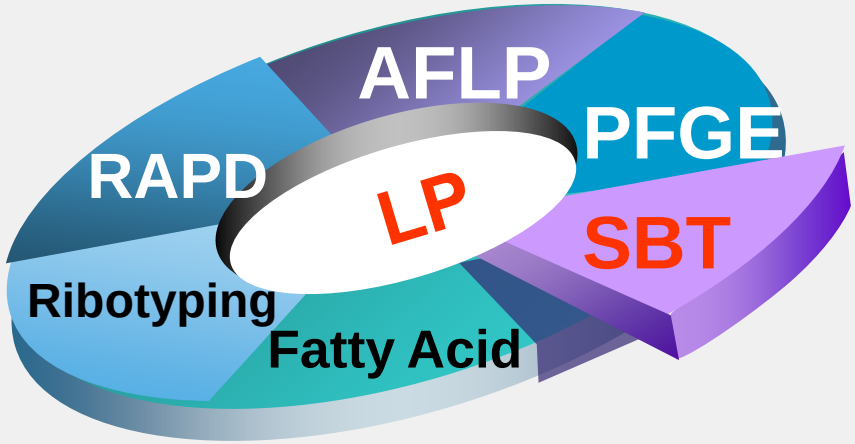
- **The limitations of the traditional sero-typing method**
  - **Cross-reaction**
  - **Only can Identify some of species**
  - **Expensive**

Molecular typing methods

- Ribotyping

- Random Amplified

  Polymorphic DNA, RAPD



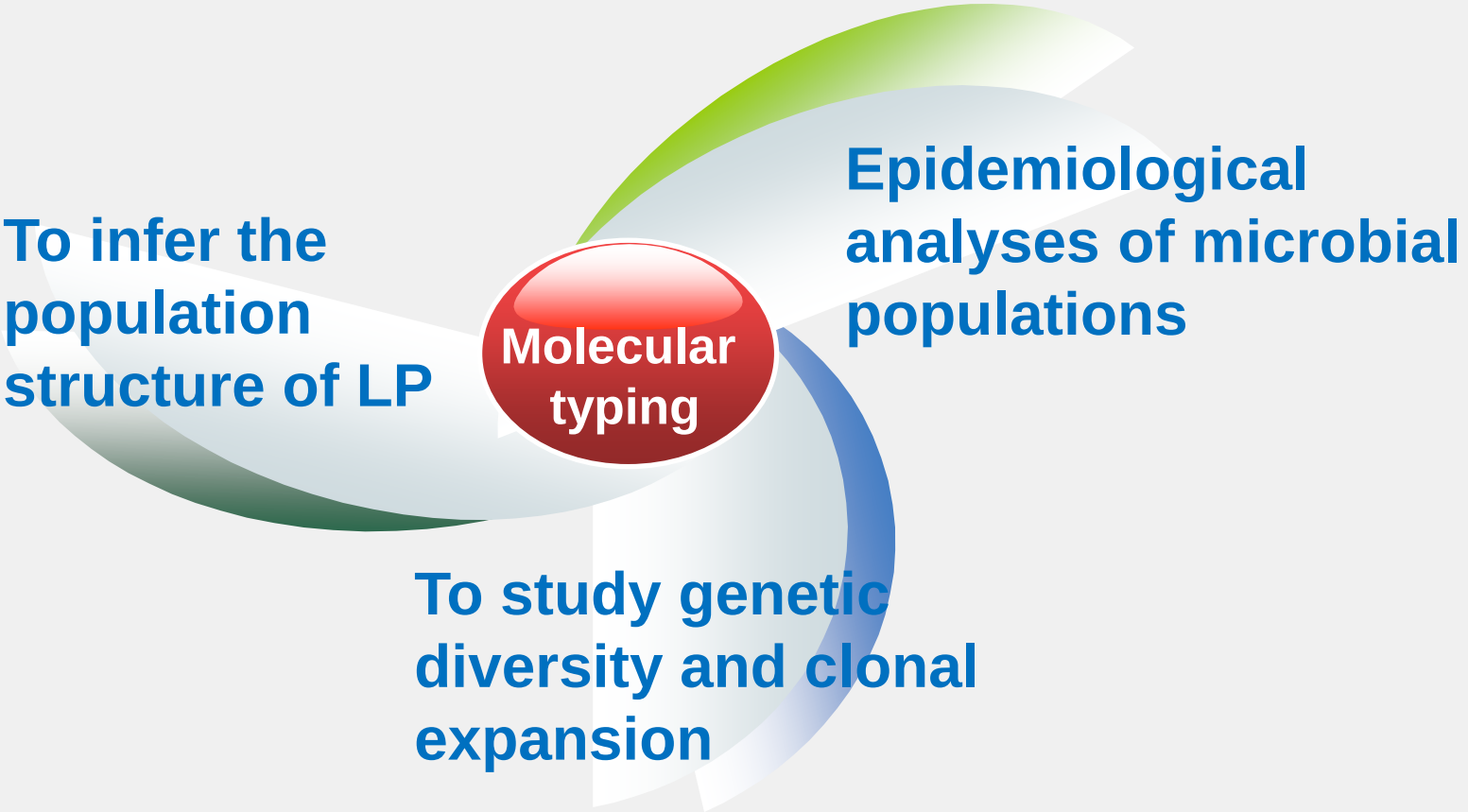
- Amplified Fragment Length Polymorphism, AFLP

- Pulsed Field Gel Electrophoresis, PFGE

- Cellular fatty acid analysis, CFAs

- Sequence-based typing, SBT

Typing methods also allow:



Typing methods of *Legionella*

- **Cellular fatty acid analysis, CFAs**

  - principle: gas chromatograph, **GC**

  pathogens has different Cellular fatty acid spectrum

  Legionella –specific Fatty acid spectrum

  - MIS Sherlock system

  2005 MIDI. company (U.S)

  saponification, esterification,

  extraction, salt extraction, Agilent 7890N

  CLIN6 Database

  Similarity Index (SI) criteria

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CFAs

- Benefits

Batch identification

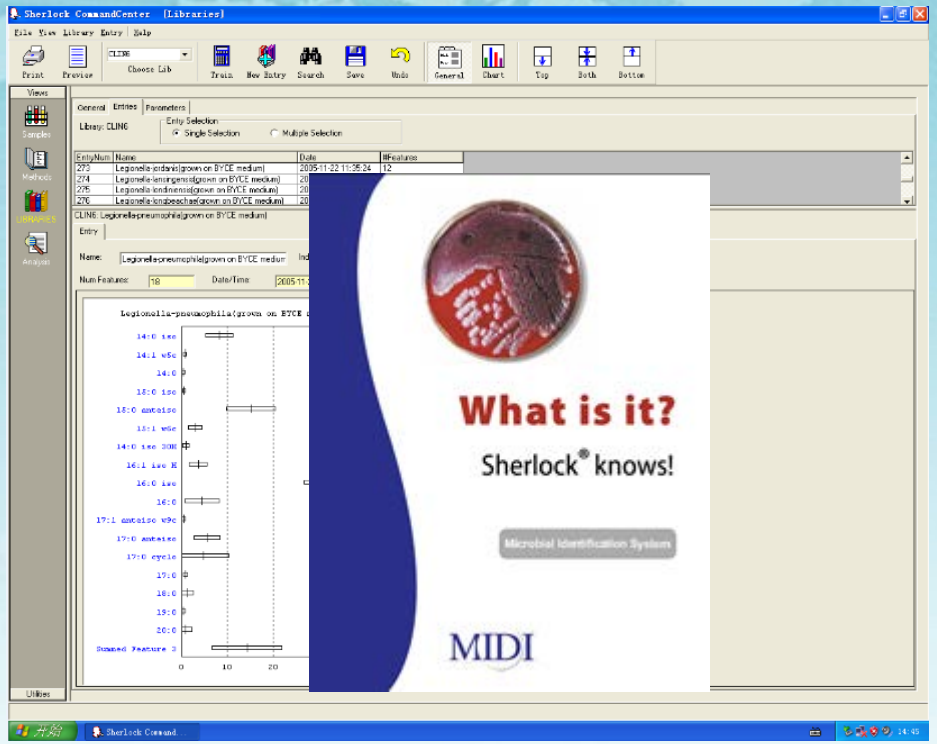
Low cost

Automated

Easy to compare

- Drawbacks

Species level



www.MIDI Inc.net

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Typing methods of *Legionella*

Sequence-Based Typing , SBT

- principle: 7 genes those under diversifying pressure.
  e.g. virulence genes

evolved from MLST house keeping (non-selective)genes

- EWGLI Online database

http://www.ewgli.org

Gene	Primer name	Position	Primer sequence (5'-3')	Annealing temperature
flaA	flaA-587F	568-587	GCG TAT TGC TCA AAA TAC TG	55 °C
	flaA-960R	981-960	CCA TTA ATC GTT AAG TTG TAG G	
pilE	pilE-35F	12-35	CAC AAT CGG ATG GAA CAC AAA CTA	55 °C
	pilE-453R	471-453	GCT GGC GCA CTC GGT ATC T	
asd	asd-511F	487-511	CCC TAA TTG CTC TAC CAT TCA GAT G	55 °C
	asd-1039R	1062-1039	CGA ATG TTA TCT GCG ACT ATC CAC	
mip	mip-74F	58-74	GCT GCA ACC GAT GCC AC	55 °C
	mip-595R	616-595	CAT ATG CAA GAC CTG AGG GAA C	
mompS	mompS-450F	430-450	TTG ACC ATG AGT GGG ATT GG	55 °C
	momp-1126R	1140-1126	TGG ATA AAT TAT CCA GCC GGA CTT C	
proA	proA-1107F	1090-1107	GAT CGC CAA TGC AAT TAG	55 °C
	proA-1553R	1570-1553	ACC ATA ACA TCA AAA GCC	
neuA	neuA-196F	176-196	CCG TTC AAT ATG GGG CTT CAG	55 °C
	neuA-611R	634-611	CGA TGT CGA TGG ATT CAC TAA TAC	

SBT

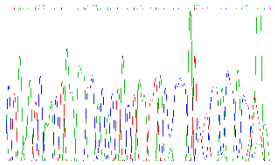
Identification

BCYEculture

PCR amplification

DNA Sequencing

Sequence analysis

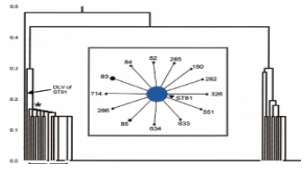


Reliable sequence

```
                10        20        30
CATGCCGAGG ATCTAAAGTT ACTGATGCGT
TGATTAAATA TTATAAAGAA TATGTTAACG
GAAAAGATGT GACTGCCAGT CAAGAATGGA
TCATCAACCG TTATCAAGCA ATGGTTGACG
AACTTGGTTT CACAGCTAAC AGCATGAAGA
AAATCGCAGA CAGCATCATC TCTTTGGCCA
GCCTACCAAT TGATGATAAC GAATTCCTTT
ACGACGCATT CTTAGCAGCT GGTGAAGATA
```

Online database

Split tree



Conclusion

Typing methods of *Legionella*

- Benefits

Standardization

Greater reproducibility

Results can be shared and compared between laboratories

- Drawbacks

Expensive

Time consuming

Example 1 (Rosalyn E, et al, BMCID 2007)

From June to November 2005, 18 cases of community-acquired LD were reported in Rapid City (1 death) .

- Whether the disease is **sporadic or outbreak?**
- How to identify the **source** of the outbreak?
- How to control it?

- **Molecular typing** can be a great help to answer these questions

Whether the disease is sporadic or outbreak?

- Use SBT to analyze Clinical isolates. Most of them have the same SBT pattern. They are all *Benidorm* strain (SBT pattern 4,7,11,3,11,12)
- Combine the epidemiologic information(disease onset time, areas, etc)
- We can conclude it is an outbreak

(Rosalyn E, et al, BMCID 2007)

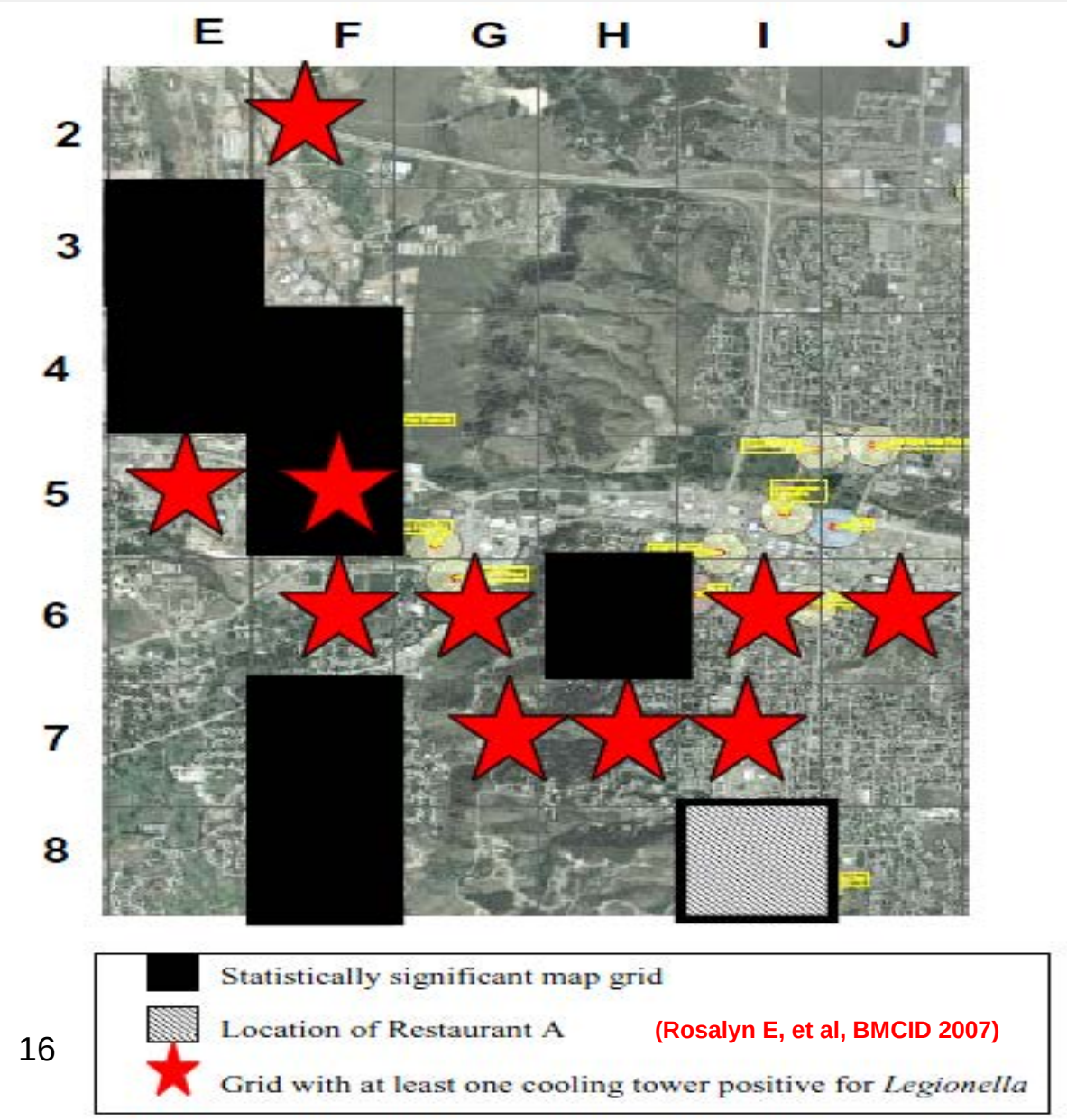
How to identify the source of the outbreak?

- We collected information about activities of patients during 14 days before symptom onset.

  Environmental samples (n = 291) were cultured for *Legionella*

- **43** potential environmental sources **culture(+)**

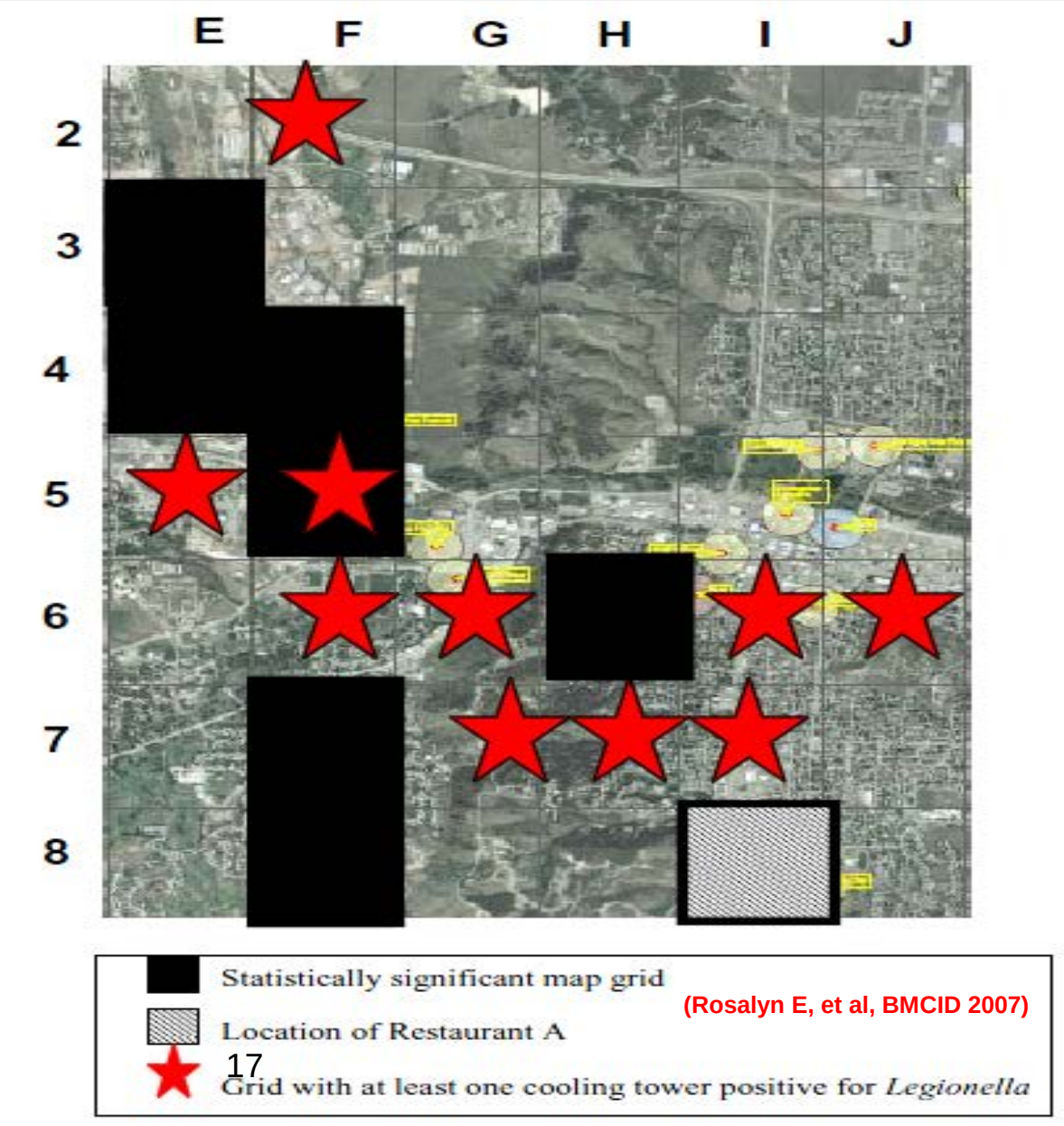
- Clinical and environmental isolates were compared using SBT



(Rosalyn E, et al, BMCID 2007)

How to identify the source of the outbreak?

- The outbreak strain, *Benidorm* strain (SBT pattern4,7,11,3,11,12) was isolated **from only one source**
  a decorative fountain in Restaurant A



(Rosalyn E, et al, BMCID 2007)

How to control it?

- The decorative fountain had a water Legionella colony count of 3000 cfu/ml

- No cases of LD with an onset date of more than five days after the date the fountain ceased operation were reported in Rapid City

Figure 3
Decorative fountain situated in the lobby of Restaurant A.

(Rosalyn E, et al, BMCID 2007)

Example 2 (M. Scaturro, et al, JCM 2005)

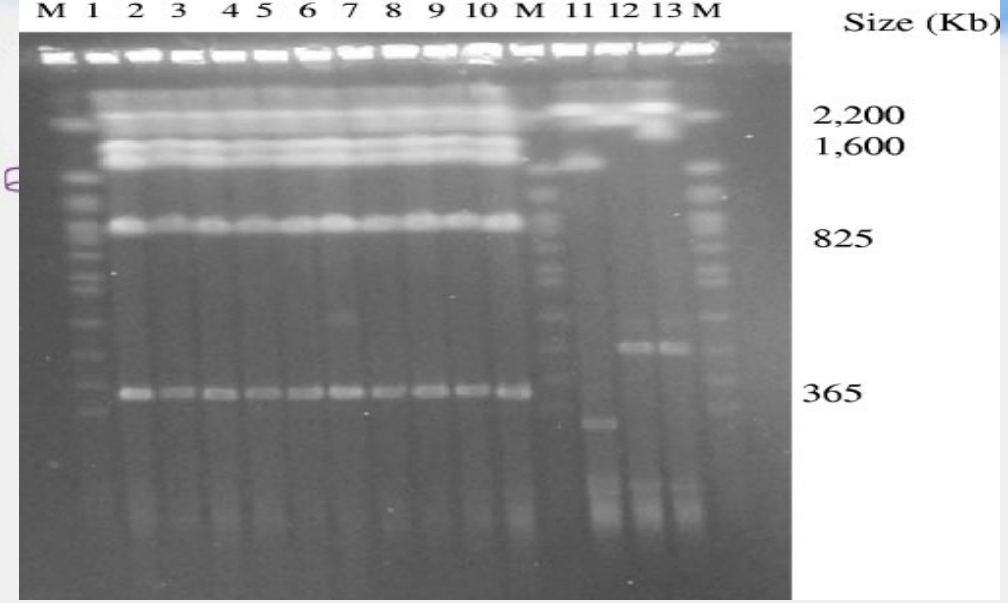
- The outbreak occurred 2003 in Rome, 15 notified cases, 1 death
- serotyping is insufficient as a means of ascertaining the actual source of contamination

- 3 Methods were compared in the epidemiological investigations

  - PFGE: reliable, but :time-consuming, technically difficult, need to prepare plugs with fresh bacterial cultures

  - AFLP: "gold standard" BEFORE. easier and faster .Banding patterns by visual, band discrimination is subjective

  - SBT: "gold standard" NOW .high discriminatory power; expensive

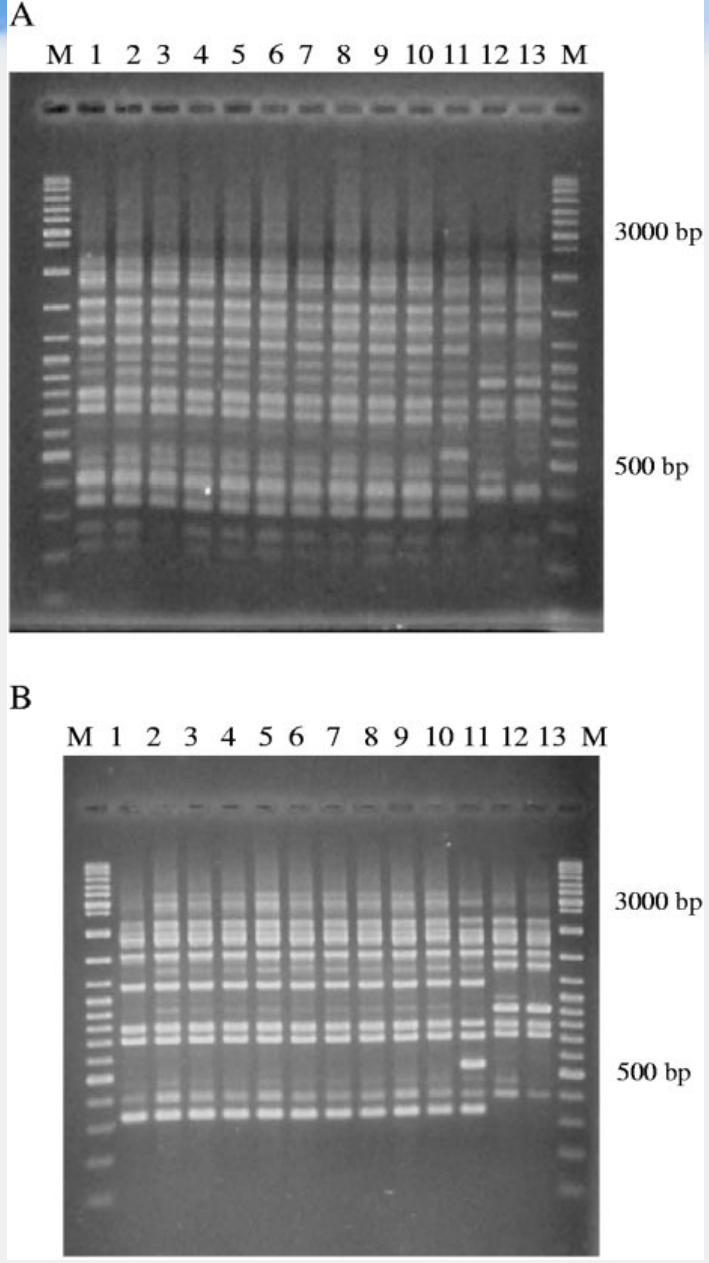


PFGE

TABLE 1. Allelic profiles of the *L. pneumophila* serogroup 1 strains used in this study

Strain[a]	Allele no.				
	flaA	*pilE*	*asd*	*mip*	*proA*
1	2	3	9	10	1
2	2	3	9	10	1
3	2	3	9	10	1
4	2	3	9	10	1
5	2	3	9	10	1
6	2	3	9	10	1
7	2	3	9	10	1
8	2	3	9	10	1
9	2	3	9	10	1
10	2	3	9	10	1
11	1	4	3	1	1
12	3	4	1	1	1
13	3	4	1	1	9

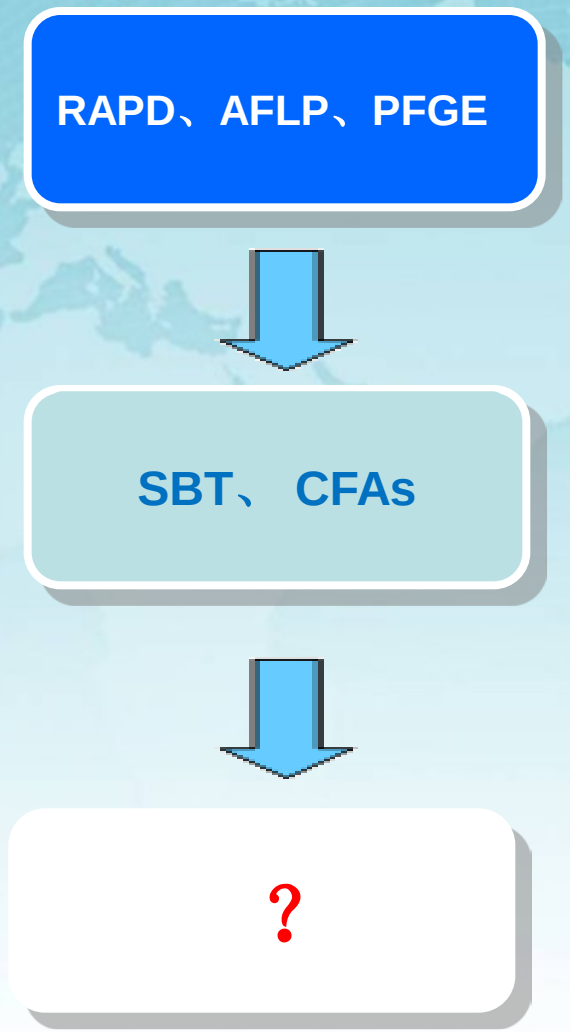
SBT



AFLP

 (M. Scaturro, et al, JCM 2005)

Trends



Electrophoresis of digested genomic fragments
subjective factor in the results interpretation

Hard to make a standardized protocol
difficult to compare the results among different laboratories

Digital results
Online comparison
High-throughput
Reproducibility

Non-culture-based method
Time Saving
Automation
Cost saving

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Thank you very much !

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