内で広まる可能性も考えられる。そこで、近隣する3カ国（日本、中国、韓国）の分子疫学を専門とする結核研究者で会議を行い、アジア地域内での結核感染症対策に寄与できるように、共通の方法で分子疫学に関する共同研究を進める。

B. 研究方法

各国での結核に関する状況や対策および最近の研究成果について発表してもらい、今後進める共同研究について議論した。

C. 研究結果

最初に、参加国内で共通の株が広がっているかどうか、挿入配列（IS）*6110* 制限酵素断片長多型（RFLP）分析法などの分子疫学的手法で結核菌遺伝子型の比較検討を行うことでまとまった。しかし、RFLP分析結果の比較は、画像データを共通の解析プログラムで補正する必要があるため、韓国釜山大学と北京疾病予防管理センター結核・胸部腫瘍研究所では難しいということだった。そこで、反復配列多型（VNTR）法で結核菌を型別することになった。この VNTR 分析では、データがデジタルなので、各国間での型別データのやり取りがメール等で容易に行えるという利点がある。

VNTR 分析では、ローカスの選択が非常に重要で、どの locus を何箇所、解析するかで型別法の分解能は大きく左右される。米国疾病予防管理センターでは、Mycobacterial interspersed repetitive units (MIRU)の 12 loci、ヨーロッパ諸国ではフランスパスツール研究所の Supply らが報告した Supply (15)－VNTR が、新しい結核菌の型別法として採用されている。しかし、米国、ヨーロッパ諸国と異なり、北京型結核菌が結核全体の 7~8 割を占める東アジアの国では、散発的に報告があるものの、米国、ヨーロッパで採用されている方法が良いのか等、標準的な分析法はまだ確立されていないという状況である。

昨年、日本全国から集めた結核菌を分析して結核研究所が報告した JATA(12)－VNTR 法が、中国、韓国でも有用な分析法となるか検討を行うことになり、結核研究所として、利用する loci とプライマーの塩基配列、分子量からコピー数への換算表（各 loci おけるコピー数の定義）および精度管理用の DNA の提供を行った。

次回の会議で各国において多数を占める北京型結核菌の型が、蔓延型か祖先型か判明し、JATA(12)－VNTR 分析法が、中国や韓国で利用できるか明らかにすることができる。

D. 考察

日本国内の結核菌を MIRU(12)および Supply(15)－VNTR で分析すると、大きなクラスターが形成することが報告されている。これらの VNTR 分析システムでは、北京型結核菌に対する分解能が低いため大きなクラスター形成する。そのため、北京型結核菌を効率良く型別できる loci が必要であった。JATA(12)－VNTR は、北京型結核菌を効率良く型別できる loci を選択した VNTR システムなので、北京型結核菌が 7 割以上を占める中国、韓国でも、この方法を結核菌型別の標準分析法として取り入れることが出来るものと考えられる。

E. 結論

近年、人の移動が活発になり、感染症が流入する可能性が高まって来ている。共通の方法で結核菌の型別を行いデータベース化することにより、病原性の高い結核菌（スーパースプレッター）などの発生状況や流入を早期に把握するためのシステムの確立が可能となる。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

(1) 前田伸司、菅原勇、加藤誠也：日本、中国、韓国における結核分子疫学担当者会議開催報告. 結核. 2007; 82: 925-927.

2. 学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

Molecular methods for bacterial strain typing

CHANG Chulhun, M.D.
Department of Laboratory Medicine
Pusan National University School of Medicine

1

The goal of this presentation

- **Framework**
  - to facilitate reporting consistancy
  - to assist the laboratories and professionals
    - General approach to data analysis
    - Specific criteria for data interpretation

2

Content

- **Terms and definitions**
- **Biology behind molecular typing**
- **Validation of typing methods**
- **PFGE**
- **Ribotyping**
- **Analysis of electrophoretic data**
- **Analysis of sequence data**
- **Interpretation and report**

3

Content

- **Terms and definitions**
- **Biology behind molecular typing**
- **Validation of typing methods**
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- **Analysis of sequence data**
- **Interpretation and report**

4

Terms and definitions

- **Isolate**
- **Strain**
- **Genotype**

5

Terms and definitions

- **Isolate**
- **Strain**
- **Genotype**
- **Lineage**
- **Indistinguishable / similar / different**

6

Content

- **Terms and definitions**
- **Biology behind molecular typing**
- **Validation of typing methods**
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- **Analysis of electrophoretic data**
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- **Interpretation and report**

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Biology behind molecular typing

- **Sourses of genetic variation**
  - Point mutation
  - Genetic recombination; insertion, deletion
  - PFGE detects
    - point mutation involving restriction sites
    - Recombination events involving larger (>20 kb) DNA sequences
  - Southern blots
    - Assess no. and locations of insertion sequences

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Biology behind molecular typing

- **Population structure of bacteria**
  - Clonal structure; tree structure
    - Strong linkage disequilibrium
    - Interchromosoma recombination is relatively low
    - *E. coli*, Salmonella
  - Panmictic species; mesh or lattice structure
    - functionally sexual population
    - High rates of recombination among isolates
    - *Neisseria gonorrheae*
  - Epidemic structure; mesh with a node structure
    - Recent expansion of a single genotype of panmictic species
    - *S. pneumoniae, N. meningitidis*

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Biology behind molecular typing

- **Selective pressure**
  - Virulence factor
  - Antimicrobial resistance determinant
  - Skin infections from different cities
    - 249/422 (59%): MRSA
    - 218 strains subject to molecular typing
      - 156 (74%); single pattern
      - 212 (97%); closely related to a single PFGE type

        (Moran GJ, et al. *NEJM* 2006;355:666-674)
  - *S. pyogenes, S. pneumoniae, E. coli* O157:H7

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Biology behind molecular typing

- **Application of molecular typing**
  - Episodes of infection within a patient

    ? Reinfection

    ? Relapsing infection

    ? Contamination

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Biology behind molecular typing

- **Application of molecular typing**
  - Episodes of infection within a patient
  - Outbreaks

    ? Outbreaks in hospitals

    ? Food and water-related outbreaks

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Biology behind molecular typing

- **Application of molecular typing**
  - Episodes of infection within a patient
  - Outbreaks
  - Surveillance
    - PulseNet
    - Enter-Net
    - HARMONY

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Biology behind molecular typing

- **Application of molecular typing**
  - Episodes of infection within a patient
  - Outbreaks
  - Surveillance
  - Population genetics

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Content

- **Terms and definitions**
- **Biology behind molecular typing**
- **Validation of typing methods**
- **PFGE**
- **Ribotyping**
- **Analysis of electrophoretic data**
- **Analysis of sequence data**
- **Interpretation and report**

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Validation of typing methods

- **Validation of typing methods**
  - Reproducibility
    - Technical
      - replicate aliquots of a single isolate
      - restriction digests and nucleotide sequences
      - PCR-based approaches

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Validation of typing methods

- **Validation of typing methods**
  - Reproducibility
    - Technical
    - Biologic
      - Isolates representing a bona fide outbreak
      - Isolates of a single episode of infection in one patient
      - Multiple isolates derived from a single specimen (subcultures of independent colonies from a primary culture plate)

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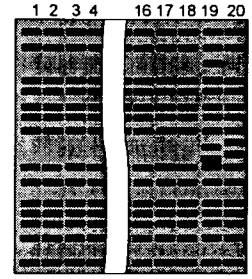
Validation of typing methods

- **Validation of typing methods**
  - Reproducibility
    - Assessment
      - Frequency of variation
        - » 18/20 = 0.90
      - Extent of variation
        - » 3 bands



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Validation of typing methods

■ **Validation of typing methods**

– Discriminatory power

$$D = 1 - \frac{1}{N(N-1)} \sum_{i=1}^{K} n_i(n_i - 1)$$

K, No. of distinct types; N, total No. of isolates

n_i, No. of isolates of the ith type

D, Simpson's index of diversity (0 - 1)

D >0.90: effective discriminatory power

cf) Simpson's index, Simpson's Reciprocal Index

Simpson's index of diversity

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Validation of typing methods

■ **Validation of typing methods**

– Discriminatory power

$$D = 1 - \frac{1}{N(N-1)} \sum_{i=1}^{K} n_i(n_i - 1)$$

reproducibility and discriminatory power

- As reproducibility decreases, discriminatory power is also likely to decrease.

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Validation of typing methods

■ **Validation of typing methods**

– Characterizing Reproducibility

- Technical
  - two independent laboratories
  - at least ten replicate aliquots of a pure subculture
  - 100% concordance

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Validation of typing methods

■ **Validation of typing methods**

– Characterizing Reproducibility

- Biologic
  - at least ten sets of isolates
  - each set comprises at least five independent isolates recently derived *in vivo* from a common precursor
  - <100% concordance

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Validation of typing methods

■ **Validation of typing methods**

– Characterizing Discriminatory Power

- Biologic
  - 100 epidemiologically unrelated isolates cultured from geographically and temporally diverse sources
  - statistically useful when the discriminatory power exceeds 0.90

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Validation of typing methods

■ **Validation of typing methods**

– Competency for Molecular Strain Typing

- a panel of 20 isolates
  - 10 different isolates
  - 5 isolates identical to one of 10
  - 5 isolates similar to one of 10

. ♨ ♣ ♥ ● ☎ ◐ ■ .

. . . . . ▶▶ . ◀◀

→ Results: 6 outbreaks, 5 related to outbreaks, 9 different

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Content

- Terms and definitions
- Biology behind molecular typing
- Validation of typing methods
- PFGE, Ribotyping
- Analysis of electrophoretic data
- Analysis of sequence data
- Interpretation and report

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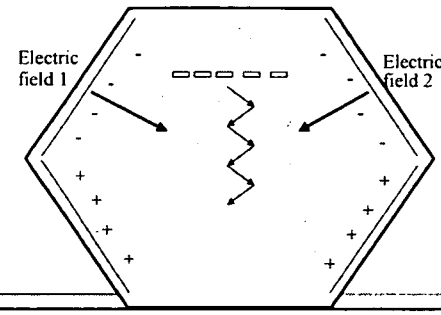
PFGE

- Pulsed field increases discrimination



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PFGE

- Macrorestriction enzyme cuts the whole chromosome into ~30 fragments

Organism	Enzyme*	Typical number of restriction fragments	Typical fragment size range (kb)
Staphylococcus aureus	*Sma*I	15-20	10-700
Staphylococcus epidermidis	*Sma*I	15-20	5-400
Stenotrophomonas maltophilia	*Xba*I	7-15	10->1000
Streptococcus pneumoniae	*Sma*I	10-19	20-300
Streptococcus pyogenes	*Sma*I	15-20	5-500
Vibrio cholerae	*Sfi*I	20-25	5-500
Pseudomonas aeruginosa	*Spe*I	20-25	10-700
Salmonella spp.	*Xba*I	10-24	30-700
Serratia marcescens	*Xba*I	~20	10-700
Shigella spp.	*Xba*I	15-23	10-700

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PFGE

- Prevention of mechanical shearing of long chromosome
  - Incorporation of bacteria into agarose plugs and then extracting the DNA *in situ*.
- Highly reproducible and discriminatory
- Assesses both sources of genetic variation
  - restriction site (0.01-0.05%); sensitive to point mutation
  - Fragment profile (>90%); vulnerable to recombination events (rearrangements, insertions, deletions)

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PFGE

- How many differences in restriction fragment position constitute a "true" strain difference?
  - Two isolates of the same "strain" can be different by a single genetic event.

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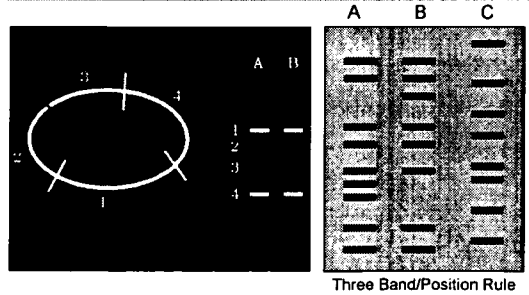
PFGE



Three Band/Position Rule

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Ribotyping / *IS6110*-RFLP

- Southern blot analysis of RFLP
- rRNA genes have been highly conserved during evolution
- applicable to a wide range of bacterial species
- *Mycobacterium* spp. typically have only a single *rrn* operon in their genome

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Content

- **Terms and definitions**
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- **Analysis of electrophoretic data**
- **Analysis of sequence data**
- **Interpretation and report**

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Analysis of electrophoretic data

- Cluster analysis
  - Quantitate the relatedness within and between sets ("clusters") of isolates
  - Repeated process
    - each isolate represents a separate cluster at first.

    → Finally, all isolates were rearranged into a sequential (hierarchical) union of clusters (dendrogram)

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Analysis of electrophoretic data

- Cluster analysis
  - Similarity coefficient (SC)
    - Quantitative description of the relatedness between two genotypes
    - 1: indistinguishable
    - 0: no relatedness at all

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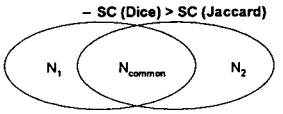
Analysis of electrophoretic data

- Cluster analysis
  - Similarity coefficient (SC)
    - Quantitative description of the relatedness between two genotypes
  - Dice coefficient vs. Jaccard coefficient

$$\text{Dice} = \frac{2 \times N_{common}}{(N_1 + N_2)} \qquad \text{Jaccard} = \frac{N_{common}}{N_{total}}$$

– SC (Dice) > SC (Jaccard)



$N_{total} = N_1 + N_2 - N_{common}$

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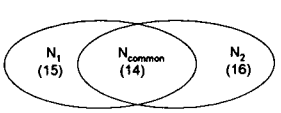
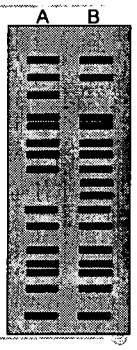
Analysis of electrophoretic data

- Cluster analysis
  - (ex) PFGE profiles
    - 15 fragments
    - Single mutation involving restriction sites
      - 3 band differences
    - Dice coefficient = 0.9 [2 • 14/(15+16)]





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Content

- **Terms and definitions**
- **Biology behind molecular typing**
- **Validation of typing methods**
- **PFGE**
- **Ribotyping**
- **Analysis of electrophoretic data**
- **Analysis of sequence data**
- **Interpretation and report**

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Interpretation and report

- **Categories of genotypic relatedness**
  - Indistinguishable
    - No variation or difference
    - Visual; subjective
    - Image-analysis system; depending on reproducibility

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Interpretation and report

- **Categories of genotypic relatedness**
  - Indistinguishable
  - Different
    - inconsistent with clinical or epidemiologic relatedness

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Interpretation and report

- **Categories of genotypic relatedness**
  - Indistinguishable
  - Different
  - Similar
    - defined by exclusion

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Interpretation and report

- **Three steps of interpretation**

  Step 1: Identify the "reference" isolate
  - the first isolate in the putative outbreak
  - earliest isolate from a sterile site
  - the first isolate of the modal (most common) strain type

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Interpretation and report

- **Three steps of interpretation**

  Step 1: Identify the "reference" isolate

  Step 2: Compare each isolate to the Ref. isolate

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Interpretation and report

- **Three steps of interpretation**

  Step 3: Translation of genotypic relatedness into epidemiologic and clinical relatedness

Table 2. Interpretation of Typing Results Within Different Contexts

Context	Category of Genotypic Relatedness		
	Indistinguishable	Similar	Different
Clinical – multiple isolates from one individual	Consistent with a single (monoclonal) infection	Consistent with variation during a single (monoclonal) infection	Indicates isolates represent >1 infecting strain
Epidemiologic – multiple isolates representing putative outbreak	Consistent with epidemiologically related isolates representing an outbreak strain	Consistent with variation during an outbreak; additional microbiologic and epidemiologic correlation required	Indicates isolates represent epidemiologically unrelated strains

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Interpretation and report

- **Report**
  - A statement of the question
    - communication between the epidemiologist and the laboratory

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Interpretation and report

- **Report**
  - A statement of the question
  - Summary of each isolate's information
    - the identifying number
    - collection date of the specimen
    - type of specimen
    - source of specimen
    - location of source

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Interpretation and report

- **Report**
  - A statement of the question
  - Summary of each isolate's information
  - Description of the method
    - Technique
    - Performance characteristics of the test, i.e., the discriminatory power and the reproducibility
    - Criteria for the interpretative categories

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Interpretation and report

- **Report**
  - A statement of the question
  - Summary of each isolate's information
  - Description of the method
  - Primary data
    - a copy of the image of the gel
    - a table of the relevant nucleotide sequences

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Interpretation and report

- **Report**
  - A statement of the question
  - Summary of each isolate's information
  - Description of the method
  - Primary data
  - Summary of the typing results
    - strain type assigned to each isolate
    - interpretation of the relatedness among the isolates

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Interpretation and report

- **Report**
  - A statement of the question
  - Summary of each isolate's information
  - Description of the method
  - Primary data
  - Summary of the typing results
  - Overall interpretation
    - Answer to the primary question

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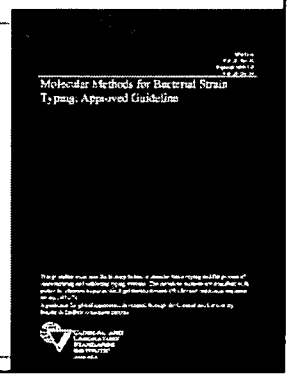
Acknowledgement

This presentation was based on the CLSI Guideline MM11-A.



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Korean Institute of Tuberculosis

Abstract

The proportion of recurrent tuberculosis (TB) cases caused by re-infection has varied widely in previous studies.

The aim of the present study was to determine the relative frequency of relapse and exogenous re-infection in patients with second episodes of TB, using DNA fingerprinting.

A population-based retrospective descriptive study was conducted in Gyunggi Province (Korea) during 2004–2006.

The study consisted of 2,067 patients with culture-confirmed TB.

Of these, 67 (3.2%) were retained because they presented with a second isolate of Mycobacterium tuberculosis.

All strains were typed by restriction fragment length polymorphism analysis and some by RFLP.

The patients genotyping patterns were compared with each other.

For 65 out of 67 patients, the restriction fragment length polymorphism patterns of the *Mycobacterium tuberculosis* strains from the episodes of recurrent disease showed identical initial and final genotypes, indicating relapse; 2 out of 67 patients showed different genotypes, suggesting exogenous re-infection.

Re-infection is possible among people in developed countries, but the rates are lower than those occurring in high-risk areas.

The risk factors for recurrent tuberculosis should be taken into account in the follow-up of treatment and tuberculosis control strategies.

Introduction

The role of re-infection compared to relapse in the recurrence of tuberculosis (TB) in general is still unclear and has potential implications for public health.

The relative contributions of re-infection and relapse are likely to depend upon the epidemiological context.

In populations at high risk of infection, there is a substantial chance of repeated infection, and hence re-infection may be a major contributor to the overall rate of TB in adults.

However, in populations with a low risk of infection, there is little probability of repeat infection, and thus most cases of second episodes of TB in adults are probably the result of relapse.

(Fine PEM, Small PM. Exogenous reinfection in tuberculosis. N Engl J Med 1999; 341: 1226–1227.)

With the introduction of short-course combination therapy, the relapse rate has dropped from 21 to 1–2%,

(van Rie A, Warren R, Richardson M, et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med 1999; 341: 1174–1179.)

calling into question, in an era of effective treatment regimens, the notion that multiple episodes of TB in one patient are almost always caused by endogenous reactivation.

The *Mycobacterium tuberculosis* genotype can now be characterized by DNA fingerprinting, which can reveal whether a new episode of the disease was caused by infection with the same strain that caused a previous episode or a different strain.

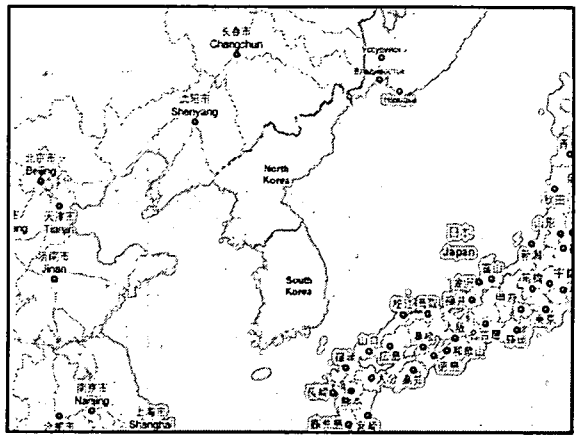
In the present study, DNA fingerprinting was used to determine the relative frequency of relapse and exogenous re-infection in patients with second episodes of TB.

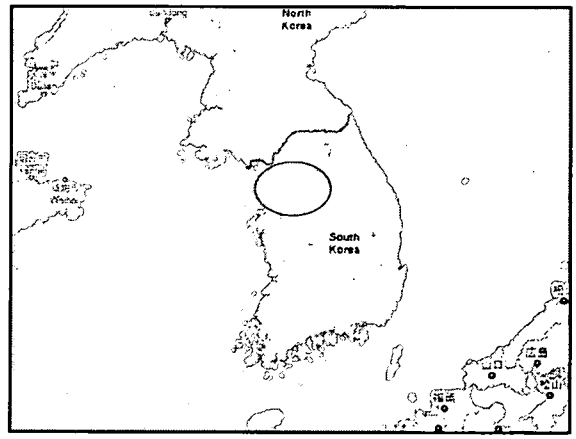
Study population and data collection

The present investigation was a population-based retrospective three years descriptive study.

The cohort of TB patients included those whose diagnosis was confirmed between January 1, 2004 and December 31, 2006 in the Gyunggi Province (Korea).

Patients who met the criteria was included in the analysis: patients suffering from an episode of TB with a positive culture for *M. tuberculosis*.





The treatment regimens used included: 1) 2 months of isoniazid (H), rifampicin (R) and pyrazinamide (Z) followed by 4 months of H and R; and

2) 2 months of H, R, Z and ethambutol (E) followed by 4 months of H and R.

Patient information was obtained from the KTBS (Korean Tuberculosis Surveillance System (Korean Institute of Tuberculosis and Korea CDC), which contains information on demographics, treatment (?), bacteriology and outcome for all suspected and confirmed cases of TB.

Procedures

All *M. tuberculosis* strains were sent to the laboratory of the Korean Institute of Tuberculosis (Supra National Reference Laboratory, Seoul, Korea) and subjected to standardized insertion sequence (IS) 6110-based restriction fragment length polymorphism (RFLP) typing.

(Van Embden JDA, Cave MD, Crawford JT, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993; 31: 406–409.)

Isolates taken from 67 out of the 2,067 patients that belong to the recurrent TB group could be typed by means of the spoligotyping analysis.

(Kremer K, van Soolinger D, Frothingham R, et al. Comparison of methods based on different molecular epidemiological markers for typing of Mycobacterium tuberculosis complex strains: interlaboratory study of discriminatory power and reproducibility. J Clin Microbiol 1999; 37: 2607–2618. 7 Supply P, Allix C, Lesjean S, et)

Similarity among strains was compared using GelCompar version 3.0 software (Applied Maths, Kortrijk, Belgium).

Drug susceptibility testing for Isoniazid, Rifampicin, Ethambutol, Streptomycin, Kanamycin, Amikacin, Capreomycin, Ofloxacin, Moxifloxacin, Protionamide, Cycloserine, *P*-aminosalicylic acid, Rifabutin, Pyrazinamide was performed by the L-J Media based proportional method.

Definition of relapse and re-infection

A patient whose isolates of *M. tuberculosis* from the first and second episodes of TB were identical on RFLP analysis with each DNA sample was considered to have TB due to relapse.

A patient whose isolates from the first and second episodes of TB were different was considered to have TB due to a new exogenous infection.

RESULTS

Out of the total 2,067 patients with positive cultures assessed during the study period, 67 (3.2%) were studied because they yielded a second isolate of *M. tuberculosis* after receiving treatment.

Out of the 67 patients, 65 (97%) showed same RFLP patterns.

Out of the 65 relapsed patients, 9 (0.14%) reported different drug resistance.

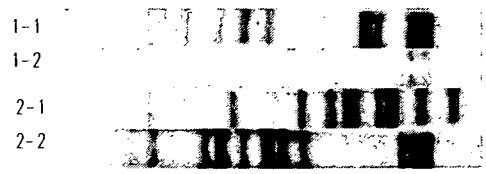


FIGURE 1. Restriction fragment length polymorphism patterns of bacterial isolates from first and second episodes of tuberculosis in nine patients.

Table 1. Demographic and diagnostic characteristics of nine relapsed tuberculosis patients.

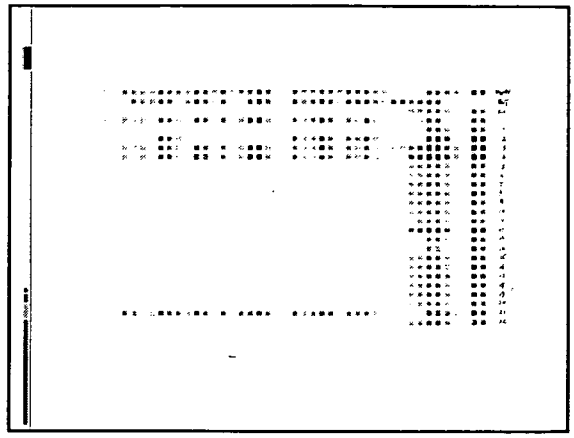
[redacted]

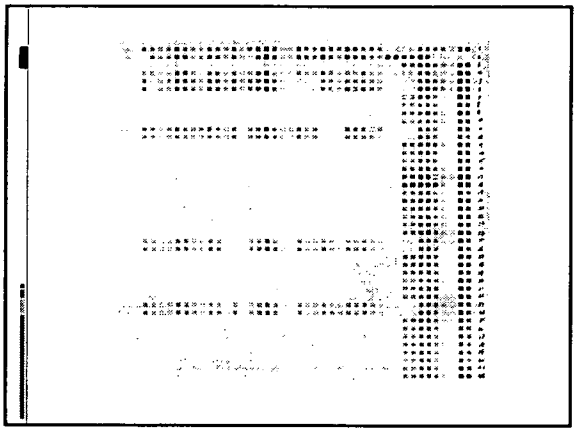
During the study period (2004 to 2006), DNA from cultures of *M. tuberculosis* was available for at least one RFLP analysis for all of 2,067 patients.

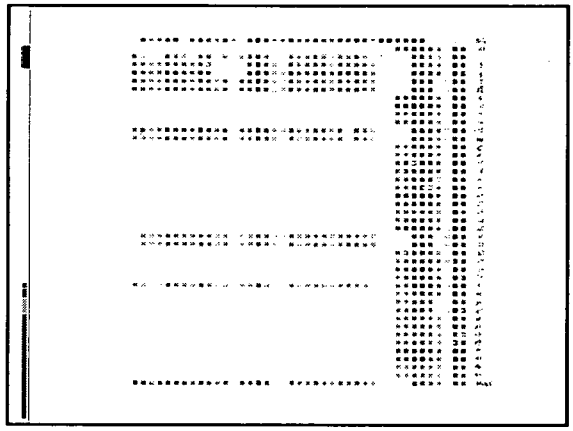
Fingerprinting results were available for all 67 patients with at least two cultures positive by RFLP analysis and for all of the 67 patients by Spoligotyping analysis.

All isolates from these patients showed five or more copies of IS6110.

FIGURE 2. Spoligotyping patterns of bacterial isolates from first and second episodes of tuberculosis in sixty seven patients.







Discussion

The possibility of persons previously infected with *M. tuberculosis* being exogenously re-infected has been debated since the middle of the twentieth century.

However, it was supposed that this rarely occurred given the immunity conferred by the initial infection.

In the present study, only 2 patients showed re-infected TB episode.

The recurrent percentage was 1-7 % in previous studies in areas with a low and moderate incidence of TB, e.g. 1.5% in Northern Italy,

(Bandera A, Gori A, Catozzi L, et al. Molecular epidemiology study of exogenous reinfection in an area with a low incidence of tuberculosis. J Clin Microbiol 2001; 39: 2213–2218.)

and 2.4% in Gran Canaria,

(Caminero JA, Pena MJ, Campos-Herrero MI, et al. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. Am J Respir Crit Care Med 2001; 163: 717–720.)

and in studies from the USA and Canada (6.8%)

Jasmer RM, Bozeman L, Schwartzman K, et al. Recurrent tuberculosis in the United States and Canada. Relapse or reinfection? Am J Respir Crit Care Med 2004; 170: 1360–1366.)

and Madrid (7%).

Garcı´a de Viedma D, Marı´n M, Hernango´mez S, et al. Tuberculosis recurrences. Reinfection plays a role in a population whose clinical/epidemiological haracteristics do not favor reinfection. Arch Intern Med 2002; 162: 1873–1879. 12 Das S, Chan SL, Allen)

In conclusion, the present data tried to confirm the fact that reinfection is possible among people in Korea, but the rate was very limited.

Relapse of a previous infection remains the more probable cause of recurrence.

However, this scenario could change in the future, on the basis of social, microbiological and epidemiological factors.

Re-infection may be a major contributor to the overall rate of TB in adults in immigrant populations from high-risk areas in particular, especially those living in poor socioeconomic conditions.

These events should be considered when planning clinical trials and national tuberculosis control programmes.

Highly polymorphic VNTR loci for differentiating Beijing genotype strains of *M. tuberculosis* in Shanghai, China

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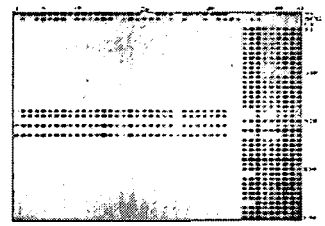
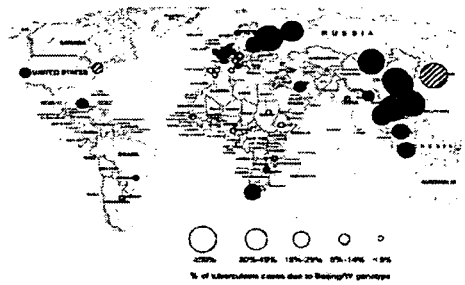
Fudan University
02.13.08

Definition of Beijing Genotype Strains

- BGS are a family of strains that are genetically closely related, have a characteristic spoligotype pattern



¼ Cases Worldwide are Caused by BGS



EID, 2006, 12: 736-743

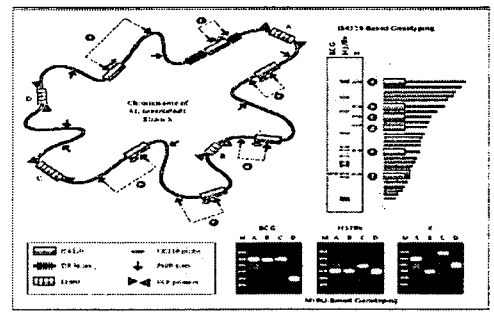
BGS in China

- 80%-90% of the strains of *M. tuberculosis* in the Beijing area are BGS since the 1950s.
- BGS are prevalent in other parts of China, such as Ningxia (67%) , Shanghai (89%) , Shandong (80%) and Guangdong (25%) .

Why Study the BGS?

- Association with multiple drug resistance
- Association with treatment failure, HIV status
- Their ability to multiply rapidly in human macrophages
- More virulence or easier become to drug resistant?
- To study the transmission of BGS, a good genotyping method is important.

Genotyping of BGS



Barnes, P. F. and Cave, M.. D. 2003 *N Eng J Med*; 349: 1149-56.

Genotyping of BGS

- Spoligotyping
  - low discriminatory power
- IS6110-RFLP
  - Time consuming and technically demanding
  - high IS*6110* copy numbers and similar RFLP patterns
- VNTR
  - Promising, relatively easy and rapid real-time genotyping method
  - Depend on the loci

VNTR Genotyping Method

- Varied of the discriminatory power of the VNTR loci used for BGS
- One VNTR locus exhibited different discriminatory power among BGS from geographically distant areas

Discriminatory Power of VNTR Loci

VNTR locus	South Africa	Russia	Hong Kong	Hong Kong	Thailand	Japan	Japan
QUB 26			0.299	0.314	0.449	0.7409	0.215
MIRU 26	0.25	0.445	0.200			0.3830	0.283
Mtub 21		0.105			0.694	0.3927	0.537
QUB-11a		0.177	0.384	0.514		0.6854	0.535
QUB 1895			0.229		0.529	0.3637	0.468
MIRU 10	0.52		0.377			0.4189	0.291
Mtub 24					0.308		0.614
MIRU 39	0.44		0.320		0.346	0.2212	0.160

$$HGI = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j-1)$$

Hunter P. R. and M. A. Gaston. J Clin Microbiol, 1998

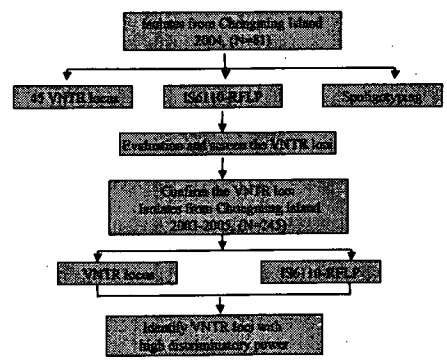
The Purpose of the Study

Evaluate the VNTR loci used in previously published studies and to develop a set of VNTR loci with high discriminatory power for the Beijing genotype strains that occur in Shanghai, China.

Hypothesis

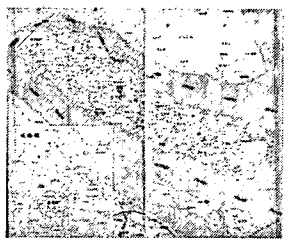
- We assumed that the VNTR loci that were highly polymorphic among BGS from a small area, should also be highly polymorphic and help discriminate *M. tuberculosis* GGS in Shanghai and other provinces of China.

Strategy



Clinical Isolates

- Chongming Island is the third largest island
- The island has a population of 635,000 people, mostly farmers and some migrants.
- 2003-2005, 224 clinical isolates were available



Methods

- By Internet search, 49 VNTR loci, including 12 MIRU loci, a repeat unit that was not less than 45 bp long
- Hunter-Gaston discriminatory index (HGI) values was used for evaluation each VNTR locus
- PCR product was analyzed by electrophoresis in 0.7%-1.5% agarose gels
- Genotyping results were analyzed using Bionumerics software

Identification of BGS

- Finally, 45 VNTR loci were used, 4 VNTR loci (ETR-C, Mtub-16, QUB-3232, QUB-3336) were excluded.
- Based on the characteristic spoligotype pattern, 189 of the total 224 isolates from 2003-2005 (189/224, 84.4%), including 65 (65/81, 80.3%) *M. tuberculosis* isolates from 2004 were BGS.

Selection of top 20 Highly Polymorphic VNTR loci

Order	VNTR locus	Beijing genotype strains (n=65)		All isolates (n=81)	
		HGI (individual)	HGI (cumulative)	HGI (individual)	HGI (cumulative)
1	VNTR 3820	0.8500	0.8500	0.8938	0.8938
2	QUB-11b	0.6548	0.9418	0.7359	0.9617
3	QUB-18	0.6534	0.9601	0.7415	0.9735
4	MIRU26	0.6120	0.9832	0.7082	0.9883
5	QUB-11a	0.6082	0.9870	0.6927	0.9907
6	QUB-26	0.5952	0.9875	0.6179	0.9910
7	Mtub21	0.5231	0.9899	0.6485	0.9926
8	QUB-4156	0.4923	0.9899	0.4562	0.9926
9	QUB-1895	0.4442	0.9899	0.4111	0.9926
10	Mtub04	0.2971	0.9918	0.4415	0.9938
11	MIRU39	0.2856	0.9928	0.4802	0.9947
12	Mtub24	0.2755	0.9928	0.2664	0.9947
13	MIRU31	0.2461	0.9947	0.4466	0.9960
14	MIRU16	0.2423	0.9952	0.3436	0.9963
15	ETR-F	0.2005	0.9952	0.3435	0.9963
16	MIRU10	0.1952	0.9961	0.3636	0.9969
17	VNTR 2372	0.1771	0.9961	0.382	0.9969
18	MIRU40	0.1471	0.9961	0.3488	0.9969
19	VNTR 2703	0.0936	0.9961	0.0749	0.9969
20	VNTR4120	0.0918	0.9961	0.3139	0.9969

Optimization of Sets of 7 VNTR loci and 16 VNTR loci

	VNTR loci	Beijing genotype strains (n=189)				All isolates (n=224)			
		HGI individual	HGI cumulative	No. of types	% of Clustering	HGI individual	HGI cumulative	No. of types	% of Clustering
1	VNTR3820	0.8674	0.8674	34	82.0	0.8700	0.8700	28	87.5
2	QUB-11b	0.6888	0.9270	51	73.0	0.7431	0.9469	69	69.2
3	QUB26	0.6295	0.9587	84	55.6	0.6689	0.9701	109	51.3
4	MIRU26	0.6139	0.9827	111	41.3	0.7005	0.9881	143	36.2
5	QUB-18	0.6072	0.9889	126	33.3	0.6975	0.9918	153	31.7
6	Mtub21	0.5444	0.9912	132	30.2	0.6543	0.9935	162	27.7
7	QUB-11a	0.5383	0.9944	140	25.9	0.6355	0.9957	168	25.0
8	QUB4156c	0.4691	0.9944	141	25.4	0.4587	0.9957	168	25.0
9	QUB1895	0.3650	0.9950	143	24.3	0.3556	0.9962	171	23.7
10	MIRU31	0.3280	0.9960	147	22.2	0.4833	0.9968	174	22.3
11	ETR-F	0.2897	0.9961	148	21.7	0.3757	0.9969	175	21.9
12	Mtub04	0.2658	0.9970	149	21.2	0.4207	0.9975	176	21.4
13	MIRU10	0.2388	0.9974	154	18.5	0.3965	0.9980	183	18.3
14	Mtub24	0.2232	0.9976	156	17.5	0.2369	0.9981	186	17.0
15	MIRU39	0.1406	0.9977	158	16.4	0.3533	0.9981	186	17.0
16	MIRU16	0.1308	0.9979	159	15.9	0.2185	0.9982	188	16.1

Comparison of Different Genotyping Methods

Typing methods	Beijing genotype strains (n=181)			All isolates (n=215)		
	HGI	No. of types	% of Clustering	HGI	No. of types	% of Clustering
IS*6110* RFLP	0.9977	153	15.5	0.9980	179	16.7
VNTR(7 loci)	0.9938	135	25.4	0.9953	162	24.7
VNTR(16 loci)	0.9979	155	14.4	0.9983	183	14.9

Comparison of Different Genotyping Methods

Discriminatory parameter	Beijing genotype strains (n=181)	All isolates (n=215)
IS*6110* RFLP		
No. of clusters	24	32
No. (%) of clusters differentiated by VNTR-7	3 (12.5 %)	3 (9.4 %)
No. (%) of cluster differentiated by VNTR-16	9 (37.5 %)	10 (31.3 %)
VNTR-7		
No. of clusters	28	35
No. (%) of clusters differentiated by VNTR-16	10 (35.7 %)	11 (31.4 %)
No. (%) of clusters differentiated by RFLP	12 (42.9 %)	12 (34.3 %)
VNTR-16		
No. of clusters	21	27
No. (%) of clusters differentiated by RFLP	7 (33.3 %)	7 (25.9 %)

Results

- Two optimized sets of loci, VNTR-7 and VNTR-16, were the most parsimonious and discriminatory sets of loci among BGS.
- Some IS*6110* RFLP clusters were further subdivided by VNTR-7 or VNTR-16.
- In contrast, VNTR-7 an VNTR-16 clusters were further subdivided by IS*6110* RFLP typing.

Discussion

- Many previously described VNTR loci showed variations in their ability to discriminate BGS from geographically distant areas.
- Highly polymorphic VNTR loci from Hong Kong, Thailand, Japan and Russia may not be able to discriminate between BGS in our study
- The differences in the discriminatory power of different loci can be attributed to differences in the *M. tuberculosis* strains from different populations in distinct geographic areas.

VNTR loci for Differentiating BGS

VNTR locus	South Africa	Russia	Hong Kong	Hong Kong	Thailand	Japan	Japan	Chongming
VNTR3820					0.642	0.8000	0.817	0.8205
QUB-11b			0.618	0.669		0.7716	0.763	0.6888
QUB 26			0.299	0.314	0.449	0.7409	0.215	0.6295
MIRU 26	0.25	0.445	0.200			0.3830	0.283	0.4139
QUB 18		0.489	0.74	0.488			0.629	0.6072
Mtub 21		0.105			0.694	0.3927	0.537	0.5444
QUB-11a		0.177	0.384	0.514		0.6854	0.535	0.5282
QUB 4156					0.472	0.6106	0.603	0.4691
QUB 1895			0.229		0.529	0.3637	0.468	0.3650
MIRU 31		0.176				0.3215	0.379	0.3280
ETR-F					0.331		0.499	0.2897
Mtub 04						0.4587	0.581	0.2658
MIRU 10	0.52		0.377			0.4189	0.291	0.2388
Mtub 24					0.308		0.614	0.2232
MIRU 39	0.44		0.320		0.346	0.2212	0.160	0.1406
MIRU 16						0.3104	0.421	0.1309
QUB 3232		0.621		0.804	0.844	0.8799	0.813	
VNTR2372					0.463		0.345	
VNTR4120					0.580	0.9022	0.882	
Mtub 30						0.4034	0.210	
QUB 1336						0.4870	0.482	
MIRU 40						0.3268	0.473	
Mtub 39							0.271	
QUB15							0.629	

Conclusion

- VNTR-7 and VNTR-16 typing are reliable method for genotyping BGS of *M. tuberculosis*.
- Due to the slightly lower discriminatory power of VNTR-7 typing, VNTR-7 could be used as a first-line typing method followed by IS*6110* RFLP and VNTR-16 to efficiently differentiate Beijing genotype strains.

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