Taiwan Epidemiology Bulletin

July 5 , 2011/ Vol.27 / No.13

Outbreak Investigation Express

Investigation of a diarrhea outbreak in a high school in Pingtung county, April 2011

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Abstract

Taiwan CDC received a citizen's report of suspected diarrhea cluster in a high school in Pingtung County from the 1922 Hotline on April 30, 2011. Thus the Fifth Branch of Taiwan CDC, the Field Epidemiology Training Program (FETP) and Public Health Bureau of Pingtung County launched investigation and found that several students of the school developed symptoms since April 11. As of May 20 there were 665 probable cases (attack rate 38%), affecting students of 10th to 12th grades and some staff. The peak case number occurred on both April 25 and April 29. The Fifth Branch recommended the school to take related prevention immediately, including health education to teachers and students, staying home if ill, proper hand washing procedures, reinforce the cleaning and disinfection of campus environment, and daily health surveillance of teachers and students. Reported numbers have declined since May 6 to five cases per day. Both rotavirus and norovirus were detected from samples. The cause of the large scale campus cluster of viral gastroenteritis was suggested to be associated with ill persons failed to implement good hand-washing habits and duly environmental cleaning and disinfection that spread the outbreak through human to human transmission.

Keywords: diarrhea outbreak, rotavirus, norovirus

The Taiwan Epidemiology Bulletin series of publications is published by Centers for Disease Control, Department of Health, Taiwan (R.O.C.) since Dec 15, 1984.
Publisher : Feng-Yee Chang
Editor-in-Chief : Yi-Chun Wu
Executive Editor : Li-Gin Wu, Hsiu-Lan Liu
Telephone No : (02) 2395-9825
Address : No.6, Linshen S. Road, Taipei, Taiwan 100 (R.O.C.)
Website : http://teb.cdc.gov.tw/
Suggested Citation :
[Author].[Article title].Taiwan Epidemiol Bull 2011;27:[inclusive page numbers].

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Original Article

Evaluation on molecular characterization of BCG strains for vaccine quality control

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Abstract

The risk of extra-pulmonary tuberculosis infection among infants and children under 2 years old was 2.88 times higher than children older than 2 years old in Taiwan. To prevent tuberculosis (TB) infection in infants and young children, 98% of neonates in Taiwan have received Bacillus Calmette - Guérin (BCG) vaccination at birth. Because the number of viable bacilli in BCG vaccine exerts a great influence on its efficacy, the vaccines manufactured by Taiwan Centers for Diseases Control (Taiwan CDC) were stored in batches for 1, 3, and 6 months at 4, 25, and 37 degrees Celsius for stability tests, which was evaluated by quantification of the viable organism. Under the worst case scenario, the vaccines stored at 37℃ for 6 months still retained 45% of their potency, which was above the standard set by World Health Organization (WHO), which requires that vaccines to retain 20% of their potency when stored at 37℃ for 28 days.

There are two different colony morphologies of mycobacteria isolated from BCG vaccines. Type I is a smooth form and Type II is a rough form. Using molecular biological methods to differentiate their genotypes, most mycobacteria colonies isolated from BCG vaccine strain are Type I. The proportion of Type I colony morphology of mycobacteria stored for 1, 3, and 6 months was 85%, 70% and > 60% respectively. Using PCR and DNA sequencing to detect RD 16 gene, the nucleotide number of Type I colony was 22 base pairs shorter than that of Type II colony. Using multiplex PCR to identify RD2, RD8, RD 14, and RD 16 genes, the only mutated gene of the BCG-vaccine strain in RD segments was RD 16. Among the vaccines stored at different temperatures, there was no significant difference in their genotypes. Therefore, the quality of BCG vaccines manufactured by Taiwan CDC fully met the WHO standards, and the molecular genotyping method could be helpful in quality control.

Keywords: Bacillus Calmette- Guérin, BCG; potency test; multiplex PCR

Introduction

BCG (Bcillus Calmette Guérin) vaccine was a live-attenuated vaccine invented by French scientists Calmette and Guérin in 1908 using *Mycobacterium bovis* subcultured for 230 generations. It is one of the most longest and widely used vaccines in human history, and it could prevent infants and young children from getting progressive primary tuberculosis, including TB meningitis [1].

To prevent infants and young children from TB infection, 98% neonates in Taiwan have had BCG vaccination. According to a retrospective study reviewing the records of 1996-2003, the TB overall incidence in persons younger than 20 years old was 9.6/100,000 person-years. In a retrospective study revealed the highest incidence of extra-pulmonary TB was among children aged 1-2 years old (3.29/100,000 person-year). The risk of TB infection in children younger than 2 years was 2.88 times higher for children older than 2-year-old [2]. The BCG vaccine strain used by Vaccine Center at Taiwan CDC since 1979 was a subculture of Tokyo strain 172, which was safe and with few adverse events. Between 2002 and 2006, the incidence of BCG-related osteomyelitis was 3.68/1,000,000 and the incidence of disseminated BCG-strain infection was 0.92/1,000,000. Both incidences met the WHO standards [3].

In Taiwan, the BCG vaccines were manufactured by Vaccine Center at Taiwan CDC with a standardized quality control process. The number of viable organisms of BCG vaccine is a major determinant of effectiveness in vaccination, and quantification of mycobacteria in a BCG vaccine is an important indicator of potency test. In potency test, a BCG vaccine was inoculated onto Lowenstein-Jensen culture media. The colony numbers after 4-5 weeks later were counted to calculate the potency. In this process, two different colony morphologies (smooth and rough form) were observed which was consistent to the finding of Yamamoto T. in 2006 [4]. He then used real-time PCR to differentiate and quantify the mycobacteria with different colony morphologies [5]. To ensure the vaccine quality and to achieve the best effectiveness, monitoring the genotypes of BCG vaccine strains and its association with the potency are of great importance. To evaluate the relationship between the genotypes of BCG-vaccine strain and its potency, we stored the vaccines in batches for 1, 3, and 6 months at 4, 25, and 37 degrees Celsius and calculated the number and proportion of isolated mycobacteria with different colony morphologies. PCR was used to detect mutations in the gene RD16 and to assess the association between mutations and potency. For vaccines stored at different temperatures for various durations, the multiplex PCR assay was used to detect mutations in several genes.

Materials and Methods

A. BCG vaccines and storage

Five different batches of vaccine were sampled and stored at 4, 23, and 37 degree Celsius for 1, 3, and 6 months.

B. Evaluation the proportion and colony morphology of mycobacteria isolated from BCG vaccines

Sampled 5 ampules of BCG vaccines and mixed them with normal saline (NaCl) homogenously until the concentration reached 0.5mg/mL. Diluted the mixtures with sterile distilled water to three different concentrations: 2×10^{-5} 、1×10^{-5} 、5×10^{-6}, and inoculated 0.1mL to Lowenstein-Jensen slant culture media. The former two diluted solutions were inoculated on 5 tubes of culture media respectively and the later

one was inoculated 10 tubes. Positioning in an incubator at 37 degree Celsius, 80% humidity, for 4-5 weeks, the colony morphologies (smooth or rough), the number and proportion of different colonies, and the potency were documented and calculated.

C. Evaluation of the potency according to Chinese Pharmacopoeia (6^{th} version) [6]

The standard deviation of the colony number of mycobacteria isolated from culture media with different concentrations was calculated using the following formulas:

$\chi^2 = (nSx^2/Sx) - Sx$

Use colony numbers to calculate χ^2

N: the number of inoculation

Sx: the colony number obtained from a diluted solution

Sx^2: the summation of the square of the colony number

The P-value (Probability) was 0.05, n was 5, χ^2 should not exceed 9.5; if n was 10, χ^2 should not exceed 16.9. If any χ^2 exceeded the default value, reassessment is needed.

X_1, X_2, and X_3 represented the colony numbers of each diluted solution respectively. Fitness (ω) was set at 40.

If $X_1 + X_2 + 2X_3 \leqq 2\omega$, the colony number = $1/2\ (X_1 + X_2 + 2X_3)$

If $X_1 + X_2 + 2X_3 \geqq 2\omega \geqq X_2 + X_3$, the colony number = $(\omega \cdot X_1)/[2\omega + X_1 - (X_2 + 2X_3)]$

If $X_2 + 2X_3 \geqq 2\omega \geqq 2X_3$, the colony number = $(\omega \cdot X_2)/(2\omega + X_2 + 2X_3)$

If $2X_3 \geqq 2\omega$, the colony number = X_3

The colony number that could be isolated from each milliliter (mL) of vaccine could be calculated by multiplying the colony number and the dilution times.

D. Extraction and purification of the genomic DNA

The mycobacteria isolates derived from BCG vaccine was dissolved in a buffer solution TEST (10 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1M Na Cl, Triton X-100 (1/200 v/v). Lysozyme 2mg/mL was added and held at 37℃ for 30 minutes, then 4% sodium dodecyl sulphate (SDS) and 2 mg/mL proteinase K were added and held at 50% overnight for lysis. In the end, phenolchloroform- isoamylalcohol (25:24:1) was added for purification and extraction, and DNA was reconstituted in sterile distilled water with potassium acetate (final concentration 0.3M).

E. Detection of RD gene segments (RD2, RD8, RD14, RD 16) using PCR and multiplex PCR [7-9].

Primers to identify RD16 gene of BCG-strain mycobacteria were designed. The sequence of the forward primer (RD16l) was ATCGTTCACGGACAGC CGTAGT and the sequence of the reverse primer (RD16r) was CTCGATCC AAGTTCAACCACG. In a 20 uL PCR reactants, containing 1mM of forward primer and reverse primer of RD16 gene, 1 mM of probe, and 20 ng of DNA) into the PCR machine (TaqMan Unversal, Applied Biosystems, cat. 4304437). The thermal cycling conditions for amplifying the targeted gene were 2 minutes of incubation at 50℃, 10 minutes of activation at 95℃, 15 seconds at 95℃ and 1 minute at 60℃ for 40 PCR cycles.

Four primers were used in multiplex PCR, including RD2l: CCAGATTCAAATGTCCGACC / RD 2r: GTGTCATAGGTGATTGGCTT, RD8l:ACTCCTAGCTTTGCTGTGCG CT / RD8r:GTACTGCGGGATTTGC AGGTTC, RD14l:CAGGGTTGAAGG AATGCGTGTC / RD14r: CTGGTAC ACCTGGGGAATCTGG, and RD16l: ATCGTTCACGGACAGCCGTAGT / RD16r:CGATCCAAGTTCAACCACG. Adding 100 ng of template DNA, 0.2 mM dNTP mixture, and 1.5U Taq DNA polymerase into 0.4 mM of all primers, the thermal cycling conditions were 2 minutes of incubation at 50℃, 10 minutes of enzyme activation at 95℃, 1 minute and 5 seconds at 94℃, 1 minute at 55℃ and 2 minutes at 72℃ for 30 PCR cycles, and 10 minutes at 72℃ at the end. The amplified products were stored in 2% agarose gel.

F. DNA sequencing

Use automatic sequencer (model 3730, Applied Biosystem) to sequence the RD 16 gene and identify the mutant sequences.

Results

BCG vaccines were stored in batches at 4℃、23℃ and 37℃; and potency tests were performed after 1, 3, and 6 months. The potency decreased in all batches after stored for 1 month. The potency of vaccines stored at 23℃ was less than that at 4℃, but was above 70%. The potency of batches stored at 37℃ was less than at 4℃ as well, but was above 50%. The potency of those stored for 3 months was similar to that stored for 1 month, while the potency of batches stored for 6 months was significantly lower. Among those stored for 6 months, potency of batches stored at 23℃ decreased but remained above 50%; those stored at 37℃ also decreased but remained 41.5% (Table1).

Batches of BCG vaccine were stored at 4℃、23℃ and 37℃; the specimens were inoculated on Lowenstein-Jensen slant culture media after 1, 3, and 6 months. The morphology and number of Type I smooth colony and Type II rough colony were recorded. Most of colonies from stored at 23℃ and 37℃, at all storage durations, were smooth form. For those stored for 1 month, the proportion of Type I colony was above 80%; and for those stored for 3 months, the proportion exceeded 70%. For those stored for 6 months, the proportion was more than 60% (Table 2).

Two batches of BCG vaccines were randomly selected for RD16 gene analysis by using PCR (batch number BG039701 and BG039703). Under all storage conditions (4℃、23℃ and 37℃), the length of RD16 gene in Type I colony was shorter than that in Type II colony (Figure1). The findings were consistent with Honda's study results [4].

The length of RD16 gene was different in Type I and Type II colonies, and the recognition site specific for HpyCH4III restriction enzyme only located at the excessive segment of RD16 gene in Type II colonies, no matter how long the specimens were stored. There was no mutation found in the recognition site specific for HpyCH4 III in mycobacteria isolates stored at different temperatures and different durations (Figure 2). This finding supported the assumption that RD16 gene was different in colonies of different morphologies.

Sequence of the RD16 gene in Type I colonies (smooth form) was 22 base pairs shorter than that in Type II colonies (rough form), no matter the specimens were stored at 4℃、23℃ or 37℃ (Figure 3).

Table 1. Results of potency tests of BCG vaccines stored at 4℃, 23℃, and 37℃ for1, 3, and 6 months

Potency of the BCG stored for a month			
Storage	4℃	23℃	37℃
BG039701	2.25×10^7	1.5×10^7	1.05×10^7
BG039702	1.8×10^7	1.25×10^7	0.95×10^7
BG039703	2.8×10^7	2×10^7	1.5×10^7
BG039704	2.3×10^7	1.55×10^7	1.1×10^7
BG039705	2.05×10^7	1.45×10^7	1.05×10^7
BG039801	2.15×10^7	1.55×10^7	1.1×10^7
BG039802	1.8×10^7	1.3×10^7	1×10^7
average percentage （%, compare with 4℃）	--	70.0%	51.2%
Potency of the BCG stored for 3 month			
Storage	4℃	23℃	37℃
BG039701	1.8×10^7	1.2×10^7	0.8×10^7
BG039702	1.5×10^7	1.0×10^7	0.5×10^7
BG039703	1.6×10^7	1.6×10^7	0.6×10^7
BG039704	1.9×10^7	1.6×10^7	0.8×10^7
BG039705	1.5×10^7	1.2×10^7	1.0×10^7
BG039801	1.6×10^7	1.1×10^7	1.2×10^7
BG039802	1.5×10^7	0.6×10^7	1.1×10^7
average percentage （%, compare with 4℃）	--	72.8%	52.6%
Potency of the BCG stored for 6 month			
Storage	4℃	23℃	37℃
BG039701	1.1×10^7	0.6×10^7	0.3×10^7
BG039702	0.6×10^7	0.3×10^7	0.2×10^7
BG039703	0.7×10^7	0.3×10^7	0.3×10^7
BG039704	0.9×10^7	0.6×10^7	0.4×10^7
BG039705	0.6×10^7	0.3×10^7	0.2×10^7
BG039801	0.8×10^7	0.5×10^7	0.5×10^7
BG039802	0.6×10^7	0.4×10^7	0.3×10^7
average percentage （%, compare with 4℃）	--	56.6%	41.5%

Table 2. Colony morphology of mycobacteria isolates of BCG-vaccine

Colony morphology of the BCG stored for 1 month						
Morphology	S-colonies(%)			R-colonies(%)		
Storage	4℃	23℃	37℃	4℃	23℃	37℃
BG039701	100	86.96	100	0	13.04	0
BG039702	89	100	100	11	0	0
BG039703	98	91	93	2	9	7
BG039704	99	100	100	1	0	0
BG039705	98	100	100	2	0	0
BG039801	96	83	95	4	17	5
BG039802	99	87	82	1	13	18
Colony morphology of the BCG stored for 3 month						
Morphology	S-colonies(%)			R-colonies(%)		
Storage	4℃	23℃	37℃	4℃	23℃	37℃
BG039701	86	100	85	14	0	15
BG039702	73	80	92	27	20	8
BG039703	75	85	84	25	15	16
BG039704	70	82	100	30	18	0
BG039705	83	81	100	17	19	0
BG039801	95	75	82	5	25	18
BG039802	93	98	92	7	2	8
Colony morphology of the BCG stored for 6 month						
Morphology	S-colonies(%)			R-colonies(%)		
Storage	4℃	23℃	37℃	4℃	23℃	37℃
BG039701	90.6	84	100	9.4	16	0
BG039702	87	100	100	13	0	0
BG039703	89	88	92	11	12	8
BG039704	89	91	92	11	9	8
BG039705	100	71	80	0	29	20
BG039801	90.6	64	100	9.4	36	0
BG039802	87	100	100	13	0	0

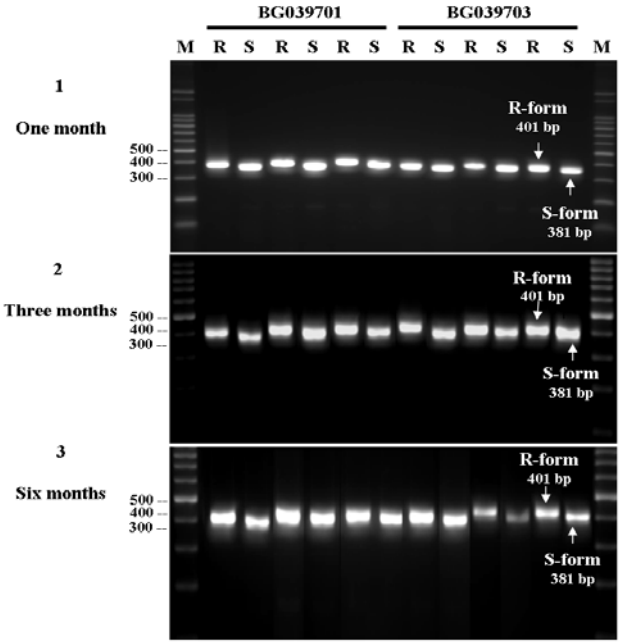

Figure 1. No matter the vaccines were stored at 4℃、23℃ or 37℃, the length of RD16 gene in Type I colony was shorter than that in Type II colony

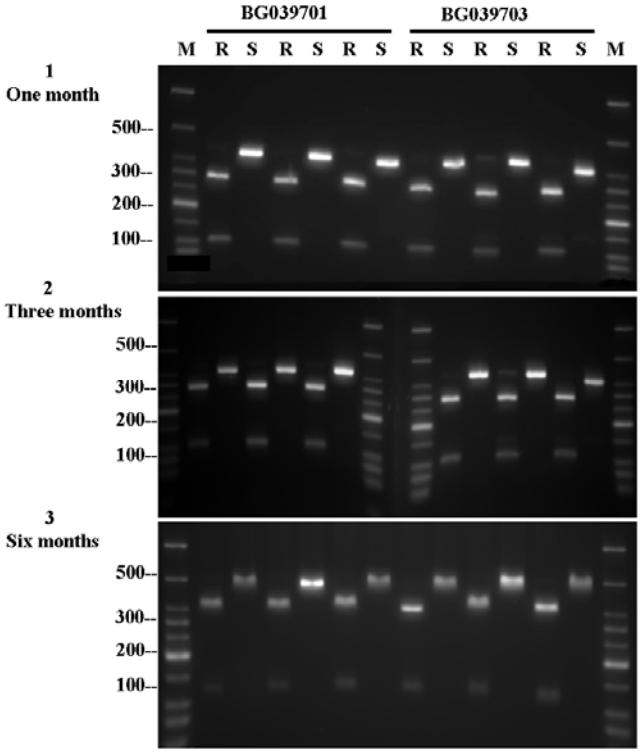

Figure 2. The recognition site specific for a restriction enzyme (HpyCH4III) could be found at the excessive segment of RD16 gene in Type II colonies (rough form), no matter how long and at what temperatures the specimens were stored. The recognition site could not be found in Type I colonies (smooth form). This finding supported the assumption that RD16 gene was different in colonies of different morphologies.

BG039701-4R HpyCH4 III

TTGGGGCCGTGCTCGATCACCTGGGCACGAAGCTGACCAGACTGTTGCACTCCGAGGCGC

BG039701-4S

TTGGGGCCGTGCTCGATCACCTGGGCAC----------TCCGAGGCGC

BG039701-23R

TTGGGGCCGTGCTCGATCACCTGGGCACGAAGCTGACCAGACTGTTGCACTCCGAGGCGC

BG039701-23S

TTGGGGCCGTGCTCGATCACCTGGGCAC----------TCCGAGGCGC

BG039701-37R

TTGGGGCCGTGCTCGATCACCTGGGCACGAAGCTGACCAGACTGTTGCACTCCGAGGCGC

BG039701-37S

TTGGGGCCGTGCTCGATCACCTGGGCAC----------TCCGAGGCGC

Figure 3. RD16 in Type I colonies (smooth form) was 22 base pairs shorter than that in Type II colonies (rough form).

In addition to RD16, the presence of mutation of other RD genes, such as RD2, RD8, and RD14, stored at different temperatures for different durations was also analyzed using multiplex PCR [7-9]. The length of RD2, RD8, and RD14 genes were 315bp, 472bp, and 252bp respectively in both smooth colonies and rough colonies. There was no difference between smooth colonies and rough colonies except in RD16 (Figure 4).

Discussion

In this study, we found two different mycobacterial colonies that could be isolated from BCG vaccines, and Type I smooth colonies were more significant. We also found that RD16 gene of smooth colonies was 22bp shorter than that of rough colonies. The differences in colony morphology and genotype might be attributed to serial subcultures, and the results were consistent with the findings in previous studies [4] though the exact mechanism was uncertain. The lost of 22bp in smooth colonies was located at a gene encoding a transcriptional regulatory protein. Because smooth colonies lack these 22bp, the transcription of this protein would be terminated earlier and the regulatory function of this protein would be damaged [5]. The function and impact of this transcriptional regulatory protein remained to be determined, but it was not a factor that might affect the survival of mycobacteria. The 22bp in RD 16 gene was absent in BCG vaccines manufactured in most countries [8]. Type I colonies were found to grow better than Type II colonies, both in vitro and in vivo (mice) [10], so the absence of the gene segment might be helpful for mycobacterial growth or there might be other mutations in Type I colonies that could improve their growth.

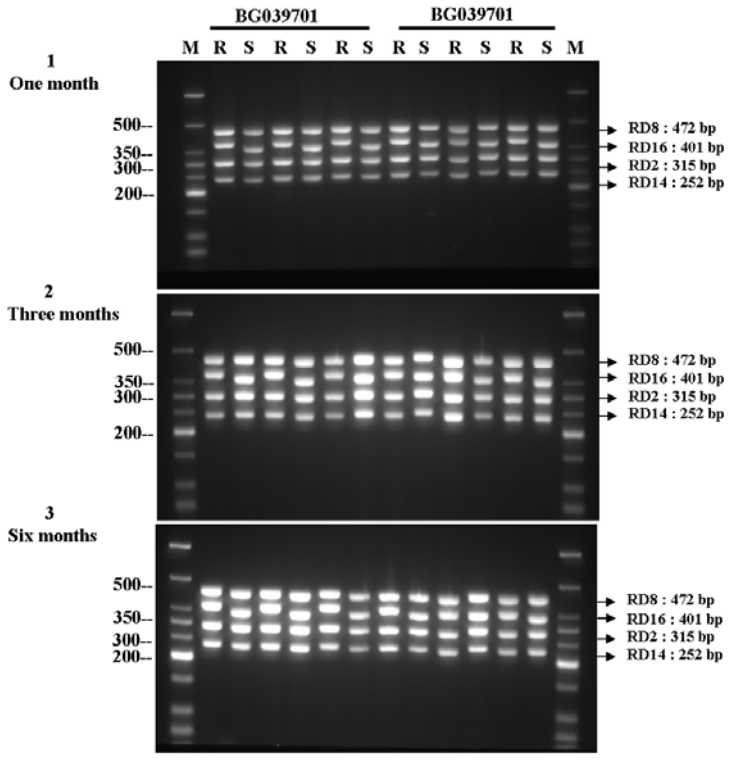

Figure 4. After storage for 1, 3, and 6 months, there was no significant difference between smooth colonies and rough colonies except in RD16.

BCG vaccines were stored at 4℃、23℃and 37℃ for 1, 3, and 6 months, and most isolated mycobacterial colonies were smooth form (64%). Compared the DNA sequences, RD16 gene was found to be 22bp shorter in smooth colonies than in rough colonies and HpyCH4 was used to confirm this result. Using multiplex PCR to detect other RD genes, including RD2, RD8, and RD14, difference between two forms of colonies could not be found except in RD16. These findings were consistent with previous studies [4-5, 7, 9]. Compared the BCG vaccine strains worldwide, RD16 gene in Japanese strain was 22bp shorter than strains from Russia (Russia strain), France (Pasteur strain), British Glaxo strain (derived from Danish strain), Swiss (Tice strain), and Ireland (Connaught strain).

According to the potency test results, Japanese Tokyo 172 strain used in Taiwan exhibited satisfactory protection against mycobacterial infection. The potency remained 41.5% even under the worst case scenario (stored at 37℃ for 6 months), which still met the WHO standards, requiring that potency should be at least 20% after storage at 37℃ for 28 days [11-14]. Using molecular biological methods to detect genetic differences, genotypes were found to be similar in isolates from storage at all temperatures for various durations. The quality and stability of our BCG vaccines were reassured.

WHO has not yet established regulations and standards on detection of genetic differences in BCG vaccines. So a standardized, convenient, and efficient method to identify the difference and assure the stability might be helpful in BCG vaccine quality control. The multiplex PCR assay system established in this study was useful in genotypic identification, but further studies would be needed to evaluate the biological characteristics and the influence on vaccine effectiveness of the two different BCG vaccine strains.

Acknowledgements

We deeply appreciate the assistance from colleagues of Vaccine Center, Taiwan CDC, and proofreading by Dr. Ping-Fuai Wu.

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Biosafety and Biosecurity

A note from the Editor-in-Chief

The occurrence of a laboratory-acquired SARS (Severe Acute Respiratory Syndrome) case in Taiwan in December 2003 has evoked many concerns from public about the issue of laboratory biosafety. As the national competent authority in charge of communicable disease control, the Taiwan Centers for Disease Control (Taiwan CDC) bears the primary responsibilities in preventing the occurrence of laboratory-acquired infection and, therefore, has instituted the Regulations on Management of Infectious Biomaterials and Specimen Sampling from Communicable Disease Cases in September 2005 that became enforced in March 2006. The government expects, through regulation implementation and administrative procedures, to strengthen the internal self-management ability of the install units, to elevate safety protection perception of the laboratory personnel, and to gradually establish and fulfill laboratory biosafety management system in this country. However, the issues of lacking of a channel from which the information on the software/hardware and management activity relevant to laboratory biosafety can be obtained have repeatedly been raised in recent years by laboratory personnel when they were receiving laboratory biosafety audit as well as by trainees when they were attending laboratory biosafety training program. In order to meet their needs and to full-dimensionally elevate knowledge and ability in laboratory biosafety practices, the Taiwan Epidemiology Bulletin, starting from this issue, has created a special

session to gradually and systematically provide a series of reports regarding concepts and information relevant to laboratory biosafety and biosecurity. Especially, we expect that this session can be used as a platform for interaction and communication between Taiwan CDC and the readers to build up a culture of excellent laboratory biosafety practices.

Overview on laboratory biosafety

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Microbiology laboratory plays a very important role in communicable disease control practices, besides its important mission in clinical diagnosis and scientific research. An efficient and safe laboratory operation will be helpful for clinical treatment and the implementation of communicable disease control strategy.

Laboratory-acquired infections have occurred continually in history in international communities. Some of those frequently occurred during 1979-1999 were infections caused by agents of Brucella spp., *Coxiella burnetti* (Q fever), hepatitis virus, *Salmonella enterica* serovar Typhi, Francisella tularensis, Mycobacterium tuberculosis complex and many others. Although infections caused by Salmonella spp. have occurred rarely since 2000 [1], the bioterrorism attack that usually has profound effect on our society and the laboratory biosafety incidents resulted from emerging infectious agents have instead become remarkable. For example, the bioterrorism attack that delivers *Bacillus anthracis* through postal mail occurred in USA in 2001. Subsequently, the world-shocking, laboratory-acquired infections of SARS (Severe Acute Respiratory Syndrome) were continually identified in Singapore, Taiwan, and Beijing, China during 2003-2004 [2]. In 2010, a laboratory-acquired infection caused by unknown species of adenoviridae from monkey happened in California, USA. In addition, dengue fever and shigellosis infections have occurred in laboratories operated for research purpose on the university campus in Taiwan during 2005-2006, these laboratory incidents could be associated with several factors, such as inadequate management of biomaterials, unsuitable personal protective equipments, inferior laboratory skills, and insufficient laboratory facilities.

In general, there are four major components that may affect the quality of laboratory biosafety practices. These are biomaterials, personnel, facility or space allocation, and self-management, and all of these involve coordination of multidisciplinary knowledge and communication of inter-authorities. Among the four components, biomaterials would be the center in terms of laboratory biosafety practices. All personnel working in the laboratory should have a certain

level of knowledge about the risk of biomaterials, and should follow the established procedures, rules, and guidelines to perform laboratory operations. In addition, laboratory facility and space allocation should be designed and constructed in observing relevant regulations and standards to ensure the health and safety of personnel. However, these regulations or guidelines involve various professional disciplines, including microbiology, risk assessment, architecture design, air conditioning and ventilation, electricity and water lines, and infection control, and are enforced by different departments of government. Therefore, it is very important for an institute having a laboratory to fulfill internal biosafety self-management. This can be done through the establishment of laboratory operation rules, guidelines, and checklists (for personnel training, personnel ability evaluation, and biomaterial transport); continuous record keeping, analysis, and management of relevant data; the development of relevant emergency response plan; and finally undergoing internal and external audit, so that deficiency concerning biosafety can be timely detected and be immediately improved to decrease the risk of biosafety incidents [3-5].

In recent years, because of the emerging infectious diseases occurred opportunistically due to global warming, and the drug-resistant bacterial strain induced by abuse of antibiotics, the opportunity of exposure to potential or unknown agents increased when laboratory personnel was conducting examination or research. Therefore, continuous education and training are very important for personnel working in laboratory. Moreover, for maintaining personal and community residents' health, ensuring environment security, and achieving the goal of "zero laboratory-acquired infection", personnel should take precaution even during non-epidemic period, follow relevant procedures and rules, *strengthen* emergency response ability, and adhere to government's policy and regulations.

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Laboratory biosafety management in Taiwan

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Since the outbreak of severe acute respiratory syndrome (SARS) in 2003, three SARS laboratory infection incidents have occurred in Singapore, Taiwan, and mainland China, raising worldwide concerns over laboratory biosafety. In response, legislation or regulations were enacted to protect the personnel safety.

The *Communicable Disease Control Act* (of 75 articles) in Taiwan has been amended on January 20, 2004. The amended articles 32 and 45 provided legal basis for the management of infectious biological materials and laboratory safety. Next year, *Regulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases* (hereinafter referred to as "the regulations") was promulgated by the Department of Health, the Executive Yuan, on September 26, 2005 (under the order of Shu-Shou-Chi No. 0940000614). The regulations of 19 articles, implemented as of March 26, 2006, are the legislative cornerstone of laboratory safety management in Taiwan.

In April 2004, to reinforce the laboratory safety management system, Taiwan Centers for Disease Control (TCDC) had established the Biosafety Committee of the Department of Health, the Executive Yuan. It was restructured and renamed to Communicable Disease Control Advisory Committee on Biosafety in 2006. The committee provides professional advocacy on biological safety policy. In accordance with the regulations to ensure the self-management and processing of the biosafety issues in laboratories, and to keep accurate records of infectious biological materials, including changes, register, and transfer. Review and supervise the establishment and certification of Biological Safety Level 3 (BSL-3) and above laboratories, plus the inspection on the laboratories which manipulate the infectious biological materials of high risk groups and follow up on correction of any deficiency in these laboratories.

According to law, any institution that possesses, stores, or uses the infectious biological materials of BSL-2 and above and has more than 4 staff members shall set up a biosafety committee for management; if the number of staff is under 5, a designated personnel shall be in charge of the management. There are 499 institutions registered to have a biosafety committee organized as March 2011. New additions, disposal, deposit or sharing of the Level 2 infectious biological materials shall not be made without the consent of biosafety committee or the designated personnel in charge, while any changes of Level 3 and above infectious biological materials shall be submitted to the central authority for approval before their use. By 2010, TCDC received over 270 notifications for the record, including 164 kinds of Level 2 and 26 kinds of Level 3 infectious biological materials in Taiwan.

Given the SARS crisis of 2003 and a variety of emerging infectious diseases, to enhance the ability of diagnosing highly infectious or unknown pathogens, TCDC used the Prevention and Relief of SARS Special Budget approved by the Executive Yuan on subsidizing 9 contracted laboratories to build BSL-3 laboratories based on the facilities standards of *Safety Guidelines for Biosafety Level 3 Laboratory*. To ensure the safety of BSL-3 and -4 laboratories, new laboratory shall undertake deliberation before the opening and receive regular inspection afterwards. From 2004 to 2010, a total of 19 BSL-3 and 1 BSL-4 laboratories have been in operation. Besides, 34 laboratories conducting identification and drug sensitivity test of *Mycobacterium tuberculosis* have been under reviewed since 2009. Laboratory shall improve any deficiency found within a limited time and have the second check through written or onsite review.

In light of the SARS incident, a series of events such as trainings, seminars, and forums, have been held annually for personnel working in BSL-2 and above laboratories since 2005 to strengthen laboratory safety and personnel protection. Up to 2,578 persons have participated in the education and training until 2010. In addition, digital learning materials have been developed to enrich the training system.

After all, Taiwan CDC is responsible for supervising laboratory biosafety affairs. However, it is essential for biosafety committees to make efforts on laboratory biosafety. Furthermore, laboratory biorisk management and emergency response are also critical to laboratory biosafety.

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