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Reflection on the New Delhi Metallo-β-lactamase 1 (NDM-1) Enterobacteriaceae Infection

Jih-Ching Huang[1], Li-Jung Chien[1], Jer-Jea Yen[1], Feng-Yee Chang [2]

1. Fifth Division, Centers for Disease Control, Taiwan
2. Director Office, Centers for Disease Control, Taiwan

Abstract

The recent discovery of Enterobacteriaceae which carry genes capable of producing New Delhi metallo-β-lactamase 1 (NDM-1) has brought attention and discussion among medical and public health researchers. In Taiwan, in order to prevent infection caused by NDM-1 Enterobacteriaceae, on September 9, 2010, NDM-1 Enterobacteriaceae infection was listed as category 4 communicable disease. Clinicians are required to report to public health authorities all patients with suspected NDM-1 Enterobacteriaceae infection. In addition, medical personnel should follow the "Standard Operating Procedure in the Management of Reported Cases of NDM-1 Enterobacteriaceae Infection" developed by the Taiwan Centers for Disease Control in the management of confirmed cases and their close contacts within the same room. In response to the identification of Taiwan's first asymptomatic carrier of NDM-1 Enterobacteriaceae, Taiwan has adopted the prevention and control strategies recommended by the World Health Organization in the relevant infection control measures and coping strategies against the emergence of multi-drug resistant bacteria. Policies that strengthen the control of nosocomial infection and promote good hand-washing habits among medical care providers and the general public have been consistently enacted. In addition, enhanced surveillance to identify multi-drug resistant bacteria has been discussed and planned in order to reduce the chance of transmission of drug-resistant bacteria.

Keywords: NDM-1 Enterobacteriaceae infection, multi-drug resistant bacteria, asymptomatic carrier of NDM-1 Enterobacteriaceae

Introduction

Since the beginning of the study in the pathogenesis of microbes, scientists have been

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trying to identify weapons to combat microorganisms that can cause diseases. In 1928, Scottish researcher Sir Alexander Fleming (1881-1955) unexpectedly discovered antibiotics [1]. Subsequently, animal studies were conducted 1940, and in 1941, Fleming first used Penicillin to treat infectious diseases in humans. Since then, antibiotics have completely changed the notion of high mortality of infectious diseases in human medical history, and rewritten the history of the fatal outbreaks of infectious diseases caused by bacteria in humans. Antibiotics have saved the lives of many individuals, and can be regarded as one of the great achievements of the 20th century in human medical science.

Two members of the Penicillin research team, Howard Florey (1898-1968) and Ernst B. Chain (1906-1979), published the results of the optimal doses of Penicillin in study animals in Lancet in 1940, and have since established an important milestone in the study of the use of antibiotics in the history of human medicine. Seventy years later, Kumarasamy et al. published the identification of infection caused by Carbapenem-resistant Enterobacteriaceae which carry genes capable of producing New Delhi metallo-β-lactamase 1 (NDM-1) [2-4], which is becoming an important issue that can no longer be ignored by medical and public health professionals in the 21st century.

The use of antibiotics has inevitably resulted in the emergence of antibiotic-resistant bacteria. As a result, the choice of antibiotics that are reserved for the last line of defense has become more and more limited and caused significant problems in the care of patients in clinical practice [5-7]. Gram negative bacteria have been generally regarded as important pathogens in recent years [8], and many Enterobacteriaceae that are resistant to Carbapenem, which is primarily used as the last line of defense against severe bacterial infection caused by Gram negative bacteria, have been identified [2, 9-10]. With the persistent spread of such antibiotic-resistant bacteria, it is crucial for both public health and clinical medicine to strengthen the prevention and control effort toward multi-drug resistant bacteria in Taiwan.

The recent report of the first imported asymptomatic infection of NDM-1 Enterobacteriaceae by the Taiwan Centers for Disease Control (Taiwan CDC) has led to broad discussion on this subject. This event also creates a good opportunity to seriously reflect on issues such as the proper use of antibiotics, control of nosocomial infection, and concerns about antibiotic-resistant bacteria in general, including policies, measures, and action in public health and

clinical medicine. The purpose of this article is to summarize the prevention and control measures adopted by Taiwan CDC toward NDM-1 Enterobacteriaceae infection and asymptomatic carriers, and to discuss the overall strategies against multi-drug resistant bacteria.

Identification of Taiwan's first imported asymptomatic carrier of NDM-1 Enterobacteriaceae

NDM-1 Enterobacteriaceae infection refers to infection caused by Enterobacteriaceae which carry genes capable of producing New Delhi metallo-β-lactamase 1 (NDM-1). The most common Enterobacteriaceae include *Escherichia coli* and *Klebsiella pneumoniae*. The first NDM-1 Enterobacteriaceae was isolated in 2008 from an Indian-Swedish national with a history of hospitalization in India [2, 11].

Taiwan has been very vigilant in the surveillance of NDM-1 Enterobacteriaceae infection. On September 9, 2010, NDM-1 Enterobacteriaceae infection was listed as category 4 communicable disease, which requires all medical care facilities to report cases compatible with the case-reporting criteria within 24 hours. In addition, specimen of the Carbapenem-resistant Enterobacteriaceae isolated from the patient should be sent to the Research and Diagnostic Center of Taiwan CDC to further examine whether the isolate carries NDM-1 gene.

On September 19, 2010, a gun shot incident occurred in India, which involved media workers from a Taiwanese TV station. Since India is one of the major countries with confirmed cases of NDM-1 Enterobacteriaceae infection mentioned in the study published in the Lancet Infections Diseases, Taiwan CDC strengthened its quarantine measures when the media workers returned to Taiwan from India.

When the three media workers returned to Taiwan, Taiwan CDC collected samples from all three workers for examination. One of the workers was tested positive for NDM-1 Enterobacteriaceae, and two other workers were both negative. Epidemiologic investigation indicated that the worker who was tested positive was hospitalized for emergency operation due to gunshot wound in India, while two other workers sustained only minor injury and was not hospitalized.

The worker who was tested positive did not present symptoms and signs of infection when he returned to Taiwan. The wound and drainage site did not have signs of inflammation and was negative for bacterial infection. As a result, he was considered an asymptomatic carrier. Taiwan CDC closely followed up on the health status of the worker after he returned to Taiwan for humanitarian reason, and the health care professionals attended to the worker during his hospitalization in Taiwan all followed procedures outlined in the "Guide to the prevention and control of the transmission of multi-drug resistant microorganisms" [12-13].

In order to prudently manage the follow-up of Taiwan's first asymptomatic carrier of NDM-1 Enterobacteriaceae, some experts proposed complete isolation and treatment in the hospital. As a result, Taiwan CDC held a consensus meeting chaired by Deputy Director Shen-Chwen Chang of the Department of Health on October 9, 2010. Members from the infection control group of

the Advisory Committee on Communicable Disease Control of the Department and the commanders and vice commanders of the Communicable Disease Control Medical Network all participated in the meeting.

Practical approach to the management of cases of multi-drug resistant microorganisms infection and asymptomatic carriers

A. Asymptomatic NDM-1 nterobacteriaceae carriers

The emergence of multi-drug resistant bacteria complicates the clinical treatment of patients with infection. Antibiotics alone may not adequately control infection. In order to prevent the spread of these multi-drug resistant bacteria within the hospitals, the fundamental approaches include proper isolation of patients and enhanced infection control measures within the hospital throughout the course of treatment [13].

As to the question of whether asymptomatic NDM-1 Enterobacteriaceae carriers should be isolated in the hospital until they are bacteria-free, many factors need to be taken into account. There may be an increased risk of infection to other hospitalized patients due to their decreased immunity. According to our current understanding of NDM-1 Enterobacteriaceae, asymptomatic carriers do not transmit infection to others through air droplets. There is also no scientific evidence supporting the continued use of antimicrobial treatment in order to completely eliminate the bacteria within the body. As a result, the current management of asymptomatic carriers in Taiwan does not require isolation within the hospital. Persons who are asymptomatic carriers are asked to implement self health management in the community and maintain adequate personal hygiene practices.

B. Cases of NDM-1 Enterobacteriaceae infection and their contacts

When patients with suspected NDM-1 Enterobacteriaceae infection are identified and reported, or when patients become confirmed cases of NDM-1 Enterobacteriaceae infection, the management of these patients, along with their close contacts within the same room, should follow the guidelines listed in the "Standard Operating Procedure in the Management of Reported Cases of NDM-1 Enterobacteriaceae Infection" [14].

Generally speaking, enhanced community isolation is aimed at patients infected with pathogens of high infectivity, pathogenicity, and mortality. In addition, recommendations on the control strategies against multi-drug resistant bacteria from the US Centers for Disease Control and Prevention and the World Health Organization both emphasize adequate infection control measures only for those patients requiring hospitalization. The main considerations are that due to the characteristics of hospitalized patients and the frequent medical procedures performed, drug-resistant bacteria are more likely to spread within the hospital and cause severe diseases. As a result, there is no need in Taiwan to implement heightened infection control measures (such as enhanced community isolation) that are employed in

unknown emerging infectious diseases (such as viral hemorrhagic fever and SARS) to asymptomatic NDM-1 Enterobacteriaceae carriers who do not require hospitalization.

Surveillance of multi-drug resistant bacteria

Surveillance of multi-drug resistant bacteria can provide information on the type and trend of drug-resistant bacteria and their respective drug sensitivity, prevalence and incidence of infections caused by multi-drug resistant microorganisms, the assessment of infection control measures in medical care facilities, and the potential genetic mutation of drug-resistant bacteria. The quality of the surveillance system may affect studies of the trend of changes in antibiotic resistance and the timely implementation of prevention and control strategies in response to the changes in antimicrobial resistance.

Currently, surveillance of drug-resistant microorganisms in Taiwan includes the following:

A. Taiwan Surveillance of Antimicrobial Resistance (TSAR)

The National Health Research Institute started the "Taiwan Surveillance of Antimicrobial Resistance" program since 1998, which aims at the antimicrobial resistance of microorganisms of outpatients and inpatients in the hospital. This is an ongoing program with participation from 26 medical centers and regional hospitals in Taiwan. This program collects and analyzes long term data on changes in antimicrobial resistance of drug-resistant bacteria and their genetic variations in order to monitor changes in the trend of antimicrobial resistance and the emergence of new strains of drug-resistant microorganisms [15].

B. Taiwan Nosocomial Infectious Surveillance System (TNIS)

Taiwan CDC started the "Taiwan Nosocomial Infectious Surveillance System" on January 1, 2007, which provides the platforms for hospitals to report information on patients with nosocomial infection. Taiwan CDC analyzes and presents the data on a regular basis. This system not only helps hospitals better manage the current status of nosocomial infection, but also improves the quality of hospital infection control through inter-hospital comparison. Taiwan CDC also utilizes the data to estimate the incidence of nosocomial infection of all hospitals in Taiwan.

The current status of antimicrobial resistance in Taiwan can be analyzed using data from both National Health Research Institute's TSAR system and Taiwan CDC's TNIS system. However, information is still not available for hospitals that do not participate in TSAR, and for patients with infections that were not considered hospital-acquired. Further discussion and planning to include data of antibiotic resistance in this area are needed in order to better evaluate and ensure adequate drug safety for all patients receiving antimicrobial treatment [16].

Conclusion

The issue of multi-drug resistance has been receiving increased attention, and experts around the world have gained significant knowledge on multi-drug resistant bacteria,

including NDM-1 Enterobacteriaceae. WHO has urged countries to take measures to combat antimicrobial resistance, including increased surveillance for antimicrobial resistance, rational antibiotic use, introducing or enforcing legislation to stop the selling of antibiotics without prescription, and strict adherence to infection prevention and control measures [17]. US Centers for Disease Control and Prevention also have similar recommendations.

Taiwan CDC follows the recommendations of WHO in the management of multi-drug resistant bacteria, including enhanced surveillance and reporting system, increased infection control effort in healthcare facilities (including the use of hand-washing measures), and rational use of antibiotics. WHO considers hand-washing one of the most important public health issues [18]. Taiwan CDC also promote hand-washing in healthcare settings and in the communities by advocating the five indications for hand-washing and good hand-washing habits in order to reduce the chance of infection and spread of various Enterobacteriaceae [19].

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Laboratory Biosafety Certification of BSL-2 Negative Pressure Laboratories of *M. tuberculosis* in Taiwan, 2009

Wen-Chao Wu, Shu-Hui Tseng, Jer-Jea Yan

Fifth Division, Centers for Disease Control, Taiwan

Abstract

In Taiwan, culture and identification, as well as drug sensitivity test, of *Mycobacterium tuberculosis (M. tuberculosis)* are mandatory to be performed in biosafety level 2 (BSL-2) laboratory under negative pressure system. In 2009, Taiwan Centers for Diseases Control (Taiwan CDC) conducted an onsite laboratory biosafety certification

and inspection on 34 BSL-2 laboratories manipulating *M. tuberculosis*, including 15 medical centers, 13 regional hospitals, 3 district hospitals, 1 outpatient clinic, and 2 private laboratories. Based on the laboratory safety manual published by World Health Organization (WHO) in 2004, we established our national standards for laboratory biosafety certification, including 15 major categories and 104 items. Each laboratory was inspected by 3 to 4 biosafety commission members and the examination lasted for about 3 hours for each laboratory. The overall failure rate among the 5 different levels of health care facilities was 7.2%. The average failure rate in medical center was the lowest (6.5%) and in outpatient clinic was the highest (11.5%). Individually, one of the medical centers had the lowest failure rate (2.9%) while one district hospital had the highest rate (16.3%). The standard deviation (SD) of failure rate was highest among regional hospitals (3.5%). "General engineering control", "Laboratory control" and "Facility" were the most common major categories that laboratories failed to meet the standards; the number of failed items in each category were 64, 40, and 30 respectively. As for individual items, laboratories often failed in the following three: "All penetrations in laboratory should be sealed or sealable for decontamination" (16 labs), "Autoclaves should have exhaust filter" (10 labs), and "Access to negative pressure laboratory should be limited to authorized personnel" (9 labs). Through this onsite examination, we urge these health care facilities to take laboratory biosafety issues seriously and to assure that all laboratory personnel are aware of these regulations.

Keywords: *Mycobacterium tuberculosis*, negative pressure laboratory, biosafety, laboratory certification

Introduction

M. tuberculosis is essentially an airborne pathogen included in Risk Group 3 according to the international classification. The incidence of *M. tuberculosis* infection among laboratory personnel involved in tuberculosis diagnosis is known to be three to five times higher as compared to personnel manipulating other microbial specimens [1]. Health care facilities should adopt strict biosafety rules in all diagnostic and research laboratories where tubercle bacilli are manipulated, identified, and tested for drug sensitivity to protect the personnel. According to the official documents of the Department of Health on January 20, 2009, biosafety level 2 Plus (BSL-2+) laboratories under negative pressure system can perform amplification of *M. tuberculosis* isolates and drug sensitivity test if they have education and training programs about biosafety issues and personal protection for laboratory personnel, and annual check-up for laboratory facilities as biosafety level 3 (BSL-3) laboratories.

To prevent laboratory-acquired infections, biosafety is an important issue. Worldwide, there were more than 5,000 laboratory-acquired infections and at least 200 associated deaths among laboratory workers since 1890 [2]. Reviewing the literature, there were recorded 1,342 laboratory-acquired infections and 39 associated deaths, involving 69 different

pathogens, in the earlier article published by Sulkin and Pike in American Journal of Public Health in 1951 [3]. The establishment and implementation of biosafety regulations and the knowledge and attitude of laboratory personnel is the key to prevent laboratory-acquired infections. A laboratory–acquired Severe Acute Respiratory Syndrome (SARS) occurred in 2003 was the best counterexample [4]. To know more about the performance status of biosafety regulations in all BSL-3 laboratories, Taiwan CDC launched an annual evaluation since 2004 [5-6]. By examination on site, health care facilities and laboratories were urged to set-up control measures and improve the knowledge on biosafety among laboratory personnel [7].

In 2003, species identification of mycobacterium was performed at 34 laboratories in 14 medical centers, 8 regional hospitals, 10 district hospitals, and 2 private laboratories; drug sensitivity test was performed at 32 laboratories in 13 medical centers, 8 regional hospitals, 8 district hospitals, and 3 laboratories [8]. In 2009, 36 laboratories (2 BSL-3 and 34 BSL-2+) in different health care facilities participated in the laboratory certification program held by Taiwan CDC. The on-site biosafety evaluation and certification described in this article only targeted on the 34 BSL-2+ laboratories that manipulate *M. tuberculosis* (abbreviated as *M. tuberculosis* laboratory).

Materials and Methods

A. Subjects

We targeted on the 34 *M. tuberculosis* laboratories in 15 medical centers, 13 regional hospitals, 3 district hospitals, 1 outpatient clinic, and 2 private laboratories that perform species identification and drug sensitivity test of *M. tuberculosis*.

B. Making the checklist

We used the 8th chapter, which describes the guidelines for laboratory and facility certification, in the Laboratory Biosafety Manual published by WHO as a reference [9]. Experts were invited to make a checklist which can meet the standard requirements and consistent for every institute. The checklists included 15 major categories and 104 items. The major criteria are “Laboratory control”, “Operating procedures”, “General practice and procedures”, “Personal protection”, “Gas cylinders”, “Chemicals”, “Refrigerators/ freezers /cold rooms”, “Electrical equipment”, “Heating blocks” , “Waste management”, “Decontamination”, “Biosafety cabinet, BSC”, “Laboratory design”, “General engineering control”, “Facility”, “ Fire protection”, and “Records”. The document is retrievable from: http:// www. cdc. gov. tw/ public/ Data/ 9651427971. doc. Two major categories, “Chemicals”, and “Heating blocks” were excluded from this checklist after discussion with the commission members.

In addition, the function and operation of the institutional biosafety committee (IBC) of each facility was also evaluated on site by reviewing the

following documents: 1. the meeting records in recent two years, 2. records of internal quality audit in recent two years, 3. control measures, and lists of the preserving infectious material in Risk Group 2 and higher, including the person in charge, 4. transaction records of the infectious material in Risk Group 2 and higher, 5. emergency response and reporting procedure, and 6. biohazard warning signs, signs of infectious material equal or higher than Risk Group 2, and laboratory signs for biosafety level.

C. Arrangement of the schedule

Biosafety certification of the 34 aforementioned laboratories was arranged between June and December 2009; each on-site evaluation lasted for 3 hours.

D. Organizing a biosafety certification team

The biosafety certification team was composed by 3 to 4 specialists with expertise in laboratory biosafety issues, microbiology, and ventilation system.

E. Agenda for onsite evaluation

1. Conference before evaluation (20 minutes): opening, introduction of laboratory personnel and team members, laboratory briefing.
2. On-site evaluation (90 minutes).
3. Team members panel discussion (30 minutes).
4. Closing summary (30 minutes).

F. Calculation of the failure rates

1. For each laboratory, the failure rate for a designated major category was dividing the number of failed items in this category by the total item number in this category; and the rate for failed item was dividing total number of failed items by 104.
2. For each level of health care facility (e.g., medical center or regional hospital), the failure rate for a designated major category was the sum of the failure rates from every laboratory in the same level dividing by the total number of the laboratories in this level; the rate for failed item was the sum of the failure rates for failed item from each laboratory dividing by the total number of the laboratories in this level.
3. Overall failure rate for a major category was the sum of failure rates of the 34 laboratories dividing by 34; the rate for failed items was the sum of failure rates of the 34 laboratories dividing by 34.

Results

The overall average rate of failed items was 7.2%, ranged from 2.9% to 16.3%. Among the 5 different levels of health care facilities, the average rate of failed items was lowest in medical center (6.5%) and highest in outpatient clinics (11.5%). The first three commonly failed items included “All penetrations in laboratory should be sealed or sealable for decontamination”, “Autoclaves should have exhaust filter, as known as high efficiency particulate air filter (HEPA)”, and “Access to negative-pressure ventilated laboratory should be limited to authorized personnel”, which revealed the fact that M. tuberculosis laboratories were not knowledgeable about these aforementioned issues（Table 1）.

The first three commonly failed major categories among the 34 laboratories, in number

of items, were 64 in "General engineering control", 40 in "Laboratory control" and 30 in "Facility". Among the 5 different levels of health care facilities, medical centers and regional hospitals commonly failed in " General engineering control", "Laboratory control", and" Facility";district hospitals often failed in "General engineering control", "Biosafety cabinet", and "Laboratory control"; outpatient clinic failed in "Biosafety cabinet", and private laboratories failed in "General engineering control", "Facility", and "Decontamination". The standard deviation (SD) of failure rate was highest among regional hospitals (3.68) （Table 2）.

Table 1. The failure statistics of the 34 *M. tuberculosis* laboratories in biosafety certification and inspection, 2009

Class of health care facilities	Medical center														
Serial number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Number of failures	8	4	4	6	7	9	7	6	3	6	8	4	10	10	9
Failure rate(%)	7.7	3.8	3.8	5.8	6.7	8.7	6.7	5.8	2.9	5.8	7.7	3.8	9.6	9.6	8.7
Average failure rate(%)	6.5														

Class of health care facilities	Regional hospital													District hospital			Outpatient clinic	Private laboratory	
Serial number	01	02	03	04	05	06	07	08	09	10	11	12	13	01	02	03	01	01	02
Number of failures	7	6	6	4	17	8	4	8	11	7	6	13	4	8	10	8	12	6	8
Failure rate(%)	6.7	5.8	5.8	3.8	16.3	1.7	3.8	7.7	10.6	6.7	5.8	12.5	3.8	7.7	9.6	7.7	11.5	5.8	7.7
Average failure rate(%)	7.5													8.3			11.5	6.7	

Note：Total average failure rate 7.2%

Table 2. The number of failed items of the 34 M. tuberculosis laboratories among the 5 different health care providers in biosafety certification and inspection, 2009

Major category（number of items）			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Class of health care providers	Number of hospital		（8）	（16）	（3）	（8）	（4）	（2）	（6）	（11）	（4）	（9）	（6）	（9）	（7）	（6）	（5）	（104）
Medical center	15	Number of failed item	20	1	2	4	3	0	0	8	8	9	1	24	11	1	9	101
		Standard deviation	1.07	0.25	0.34	0.57	0.54	0.00	0.00	0.62	0.50	0.61	0.25	1.08	0.57	0.25	0.61	2.21
Regional hospital	13	Number of failed item	14	0	1	3	3	0	3	9	8	7	3	27	13	0	10	101
		Standard deviation	1.21	0.00	0.27	0.58	0.58	0.00	0.42	0.72	0.62	0.84	0.42	1.54	0.88	0.00	1.19	3.68
District hospital	3	Number of failed item	3	0	0	1	0	0	0	2	1	6	2	7	2	0	1	26
		Standard deviation	0.82	0.00	0.00	0.47	0.00	0.00	0.00	0.94	0.47	0.84	0.47	0.47	0.82	0.00	0.47	0.94
Outpatient clinic	1	Number of failed item	2	0	0	0	0	0	0	1	1	3	0	2	0	0	2	12
		Standard deviation																
Private laboratory	2	Number of failed item	1	0	0	0	0	0	0	2	3	1	0	4	4	0	0	14
		Standard deviation	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	1.00	0.00	0.00	1.00
Total	34	Number of failed item	40	1	3	8	6	0	3	22	21	26	6	64	30	1	22	254
		Standard deviation	1.10	0.17	0.28	0.55	0.51	0.00	0.28	0.68	0.59	0.91	0.38	1.25	0.82	0.17	0.90	2.88

Major category			
1:Laboratory control	4:Personal protection	8:Waste management	12:General engineering control
2:Operating procedures	5:Gas cylinders	9: Decontamination	13: Facility
3:General practice and procedures	6:Refrigerators/ freezers/ cold rooms	10:BSC	14:Fire protection
	7:Electrical equipment	11:Laboratory design	15:Records

The problems found in the operation and function of the IBC included: 1. unable to follow up and correct the mistakes found in previous internal quality audit; 2. unable to keep the updated lists of pathogens preserving in the laboratory; 3. unable to report the bacterial isolates in Risk Group 2 or above to the central government; and 4. unable to report the transaction records of the infectious material in Risk Group 3 to the central government.

Discussion

A. Issues of why keep all penetrations in a negative pressure laboratory sealed, why autoclaves should have exhaust filter, and why access to the laboratory should be limited.

The negative pressure ventilation can keep the unexpected or unintentional infectious material or pathogens inside the laboratory, which are removed by HEPA filter after adequate interception and diffusion. Normally, the air pressure is negative inside the laboratory and infectious material is contained. But once the negative pressure is lost, leakage from un-sealed penetrations could become a problem. For example, the ventilation system could be suspended because of an annual check-up. Fumigation and decontamination should be done to protect the technicians and the maintenance staff from getting infections. If the penetrations are un-sealed, not only the efficacy of fumigation and decontamination can be compromised owing to sub-optimal concentration of the disinfectants, but the leaked aerosols can also be harmful for staff in surrounding area. In this evaluation and certification program, we have found that some laboratories never performed fumigation and decontamination, and some other laboratories had records of leakage of disinfectant aerosols which raised a protest in their neighborhoods.

In a laboratory, autoclaves are often used to disinfect the contaminated waste. Most of the laboratory workers know that regular check-up for the sterilizing effects is necessary, but they are not aware if the autoclaves are gravity displacement or vacuum assisted. In vacuum assisted autoclaves, steam enters the clave chamber, displaces the heavier air downwards, and expels it through a valve before the temperature and pressure meet the settings. If the expelled air is not adequately filtered, the surrounding areas could be contaminated. Currently, some manufacturers have made autoclaves equipped with HEPA filters which could solve the problem.

Generally speaking, amplification and manipulation of highly infectious pathogens should be performed in negative-pressure laboratories; access to these laboratories should be limited, standardized, and monitored. For unauthorized visitors and those who never been adequately trained, they should be kept outside the laboratories to prevent unwanted accidents. In this evaluation and certification program, we found that some laboratory entrance were automatic doors, people were free to enter the laboratories. We also noticed that although being against the access control regulations, some

laboratory workers shared one password used to admit to the laboratory.

B. The laboratory personnel's knowledge about biosafety issues and laboratory designs should be reinforced.

The most common failed major category was "General engineering control", especially the item "Laboratory should be equipped with exhausts and ventilation system". The associated problems included: 1. the air supply and exhausts of the laboratory ventilation system were too close, resulting in short and close circulation; 2. lack of back-up ventilation system; 3. BSC and ventilation system shared the same exhaust duct; 4. exhaust tubes of Class II A2 BSC incorrectly connected, not using canopy method; 5. exhaust tubes inadequately assembled, resulting in positive pressure around the exhaust hood; 6. abnormality associated with the damper of the exhaust; 7. leakage from unsealed testing side holes of HEPA filter; 8. lack of space berneath the door separating the anteroom and the laboratory, leading to ineffective air flow; 9. use of closed ventilation system, without induction of air from outside the laboratory; 10. ventilation stack without drainage openings; and 11. leakage from flexible hose of the exhaust.

In this evaluation and certification program, we found most laboratory workers knew little about the correct settings of negative pressure and the importance of sufficient frequency of gas exchange. Considering the value of the air pressure, some thought the more negative the better, mistaking that the more negative pressure could be more efficient in containing the infectious material. However, excessive negative pressure could be due to damaged filter of the HEPA. Although air expelled without filter could do no harm to the laboratory workers, the pathogens suspended in the expelled air could contaminate the surrounding areas. If the exhaust of HEPA is very close to the air supply port of other ventilation system, the other ventilation system could be contaminated as well. Air expelled without HEPA filtration could spread out and harm the neighborhoods. Therefore, the settings of negative pressure ventilation should be treated carefully.

According to the Tuberculosis Examination Manual published by Taiwan CDC in March 2004 [10], the air pressure should be kept at least 30 Pa below the atmosphere pressure, and the frequency of air exchange should be 6-12 air changes per hour (ACH). Generally speaking, the aforementioned air exchange frequency could expel at least 99% of infectious droplets in the laboratory within 30 to 40 minutes. More frequent air exchange could not lead to better effects but result in energy wastage.

In this certification program, we also investigated the values of the negative pressure and the frequency of air exchange of the 34 *M. tuberculosis* laboratories. Ten of them used sub-optimal negative pressure, including 5 medical centers, 2 regional hospitals, 2 district hospitals, and one private laboratory. Only one medical center and one regional hospital had adequate frequency of air change between

6 and 12 ACH. Twenty *M. tuberculosis* laboratories, including 8 medical centers, 8 regional hospitals, 2 district hospitals, one outpatient clinic, and one private laboratory, had higher air exchange frequency. Eleven laboratories in 6 medical centers, 4 regional hospitals, and 1 private laboratory did not check the air exchange frequency. The ventilation system in one regional hospital failed to function normally and the air exchange frequency was 0 ACH.

C. The IBC should be put into effect for biosafety and biosecurity.

According to the Regulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases, institutes with 5 or more laboratory workers that preserve or manipulate infectious biological material at Risk Group 2 or higher should establish biosafety committees. For those with less than 5 staff, one designated member should be in charge of all control and prevention measures regarding biosafety issues. The objective of this committee is to protect the laboratory personnel, prevent laboratory-acquired infections, and adequately preserve the infectious biological materials so that they would not be misused, wasted, or stolen. The IBC should annually check the microbiologic laboratories.

In this evaluation and certification program, members of the IBC were asked to participate. We could see how this institute weighed the importance of biosafety issues from the attendee's ranking and title. We also found some committees failed to perform regular check-up and some laboratories were hot and uncomfortable owing to abnormalities of the ventilation system. Our findings depicted that the institutional committees still have some space for improvement.

D. The failed items of laboratories have been assisted to improve by the certification team and subsequent re-check ensure the problems have been fixed or improved.

In order to make the problems clearly understood, one of our certification team members would summarize the evaluation and detailed on how to improve in the closing conference. Laboratory members could consult these team members and asked for help subsequently. To follow up the improvement status, laboratories were asked to straighten things up within two months unless some major modifications of the constructions were necessary. As a result, of the 34 *M. tuberculosis* laboratories, 24 fulfilled the committee's requirements on time, 7 were still under facilities renovation project, 1 transferred all tasks manipulating *M. tuberculosis* to a BSL-3 lab, and the remaining 2 stopped to perform species identification or drug sensitivity test. Taiwan CDC would keep following up the 7 laboratories under renovation.

Through this evaluation, Taiwan CDC now learns about the situations regarding the problems in software and hardware of the *M. tuberculosis* laboratories. But the effects could be limited if we only annually check-up each item. What could be more effective is to prioritize the problems and set up goals, so that the laboratory biosafety could further advance.

Conclusion

The overall failure rate among the 34 *M. tuberculosis* laboratories was better in medical centers (6.5%) and worse in outpatient clinics (11.5%). The commonly failed major categories, including "General engineering control", "Facility", and "Laboratory control", all pointed out the problems might be associated with initial laboratory designs. For example, some failures come from the storey where the laboratory located, the height, and the size of the building and were difficult to deal with.

As the threat from multi-drug resistant tuberculosis emerged, all laboratory workers who participate in manipulating *M. tuberculosis* have become more and more cautious. Taiwan CDC also arranged annual evaluation and certification for *M. tuberculosis* laboratories since 2009, hoping that the IBC could have better function under the supervision of our commission. Through onsite evaluation and communication, laboratory personnel could learn and practice more.

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