Investigation report of a bacillary dysentery infection event from a university laboratory in central Taiwan in 2006

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Abstract

The bacillary dysentery event from a university laboratory in Taichung city in August 2006 involving a post-graduate student was the first confirmed laboratory-related infection after the use of "Regulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases" since March 26, 2006. According to the investigations done by the Centers for Disease Control (CDC) at the laboratory, this event was thought to be caused by indirect contact transmission from contaminated gloves while handling bacteria culture to the laboratory equipments or facilities due to inappropriate laboratory design and lighting. Although the university has established "Committee for Safety of Biological Experiments" in 2001, there is still room for improvement for its surveillance regards to the management of biosafety in laboratories. Biological research institutes handling infectious materials throughout the Nation should learn from this incidence to reinforce the function of their biosafety committees, regularly examine laboratories to ensure the safety of all facilities and equipments, and fulfill safety training and education for the personnel in order to prevent laboratory-related infections.

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Background

The Fifth Branch Office of the Centers for Disease Control (CDC) was informed on August 17, 2006 by a hospital in Kaohsiung City about a suspected case of bacillary dysentery. This event was thought to be a laboratory-related infection because the case was a post-graduate student who has been performing laboratory experiments related to Shigella bacteria prior having this incidence.

Disease surveillance

This case was a PhD student from a university in Taichung. On August 9, the case performed experiments on extracting genomic DNA from *Shigella flexneri*. On August 10, the case had shrimp fried rice at a night market, and returned his home in Kaohsiung city the next day. In the middle of the day, the case had symptoms of fever (up to 38.5℃), and diarrhea with three times of watery stools, and was seen by a hospital in Kaohsiung that night where gastroenteritis was suspected. The case thought it could be related to his laboratory work involving handling Shigella bacteria and hence gave a stool specimen for bacteria culture. The culture turned out to be negative for Shigella but the case's diarrhea persists. A repeated stool culture was done at the request of the case and the result showed Shigella bacterial on August 17. The hospital informed Kaohsiung City Government and the CDC immediately, and on the same day, the Fifth Branch of the CDC asked the Health Bureau of the Kaohsiung City Government to perform pertinent disinfection procedures. After being informed with Shigella bacterial infection, the case notified his laboratory for lab disinfection. Disease surveillance from the Fifth Branch of the CDC revealed that the case's activity was mainly in Taichung City before onset of the disease, and hence the Third Branch was notified to perform pertinent disease control procedures. This incident was a

single case event since the case's parents who live in Kaohsiung City, together with other eight post-graduate students in the lab had no suspected symptoms.

Laboratory confirmation

Research and Diagnostic Center (RDC) of the CDC contacted the director of the lab immediately after getting the news from the Third Branch on August 17. The director of the lab was attending a meeting overseas on that day and was not contacted till the next day. He was asked to provide the three Shigella strains that the case had worked with in the laboratory to clarify whether this event was a laboratory-related infection or not. The Central regional laboratory of the RDC performed a pulsed-field gel electrophoresis (PFGE) for genotyping and drug sensitivity tests for the three strains from the laboratory together with the isolated gathered from the case on August 21. According to the DNA fingerprint maps of the PFGE (Fig. 1), the map of the isolate from the case (SH010579) matched well with two strains in the lab (SH2308 and SH3160). The sensitivity tests of the 17 drugs (Table 1) revealed that the drug sensitivity maps of the four strains were all quite similar, but the map from strain SH2308 was identical to the map from the strain in question. Despite the case felt the shrimp fried rice he had at the night market might be unclean, the fact is that Taiwan is not an epidemic area for bacillary dysentery and the strain type, *S. flexneri* 2a isolated from the case mainly circulating in aboriginal tribes among mountainous villages of central Taiwan (1) and has rarely been isolated in recent years. It is quite unlikely that the infection was caused by food contamination, so it is more likely that the case was infected by strain SH2308 in the laboratory due to inappropriate handling. Supporting fact is that the case worked on strain SH2308 in his lab two days prior the onset of his disease.

Control measurements

After confirming the event as a laboratory-related infection on August 21, Taiwan CDC announced a news release titled "Infection accident in a university in central Taiwan, never overlook biosafety in the laboratory" on August 22 explaining to the public event background and the measurements taken by the CDC. Institutes that hold, preserve, or handle at least the second level or higher infective bio-materials in Taiwan should strengthen biosafety management in their laboratories to avoid recurrence of similar events. According to the twelfth term of the "Regulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases", the university was asked to stop the laboratory from doing experiments related to the Shigella bacteria, perform surveillance by its biosafety committee, and report the results. The university reported back to us on August 29 on their investigation results and the subsequent coping strategies, which include: 1. suspending experiments related to Shigella bacteria; 2. modify standard operation procedures in the laboratory: UV light exposure once a day, pairing up workers for doing experiments, wearing masks during experiments, using latex gloves, disinfecting disposals on the same day, and disinfecting laboratory coats using 70% ethanol; 3. renewing biosafety negative pressure cabinets; 4. modify laboratory design so that all experiments are performed in area B; 5. carrying out biosafety education to all personnel working in the laboratories.

Strains involved in this laboratory-related event were isolated from a case in Nanto County by National Institute of Preventive Medicine (NIPM, one of the former body of the CDC) in 2006. Experiments involving Shigella bacteria can be done at Biosafety Level 2 laboratories. Issues related to the preservation and changes of infectious bio-materials in the country have been managed by the "Regulations

Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases" since March 26, 2006. Prior to this event, the university has already submitted to the CDC the name list of their biosafety committee and also a list of basic information about strains kept in their laboratories.

Field visit

The RDC visited the chief member of the biosafety committee of the university on September 12 to understand how the committee operates, their attitude towards dealing with this infection event, and also in an attempt to identify possible causes for the infection through visiting the laboratory and meeting with related personnel. The laboratory had no experiments on that day. According to our understanding, the post-graduate student took strain SH2308 and SH2308-10A from the -20℃ refrigerator in area A (Fig. 2, floor plan of the laboratory) on the August 7, inoculated four cultures in biosafety cabinet (I), and cultured the plates in a 37℃ incubator in area B. On the August 8, the plates were taken from the 37℃ incubator in area B and put into to the biosafety cabinet (I) in area A for inoculation in some culture media. Cultures were then moved to the 37℃ incubator in area B. On the August 9, the culture media were removed from the 37℃ incubator in area B and put into the biosafety cabinet (I) in area A for genomic DNA extraction. Gloves were worn all time during each step and disposed in the waste barrel in the bisafety cabinet after use. Since the biosafety cabinet and 37℃ incubator were located in two different areas separated by a sliding wood door (Fig. 3). The case might contaminate the gloves while handling culture media, which then contaminated the plates, incubator, biosafety cabinet, or even the sliding wood door. Even though the hands were washed, the fact is the wash hand sink was located in area B, there was still a possibility for contact

transmission caused by contaminated equipments from area A or B prior to the time of leaving the lab.

Advices for the laboratory

This infection event was probably caused by contact transmission due to contaminated gloves and improper laboratory design. Hence, our advice would be as follows:

1. All procedures related to Shigella bacteria should be done in area B to minimize risk of contamination.
2. Change the sliding wood door to an automated or foot-operated one.
3. Move the wash hand sink in area B to the exit and use an automated sensor or foot-operated switch instead if limited by the present space.
4. Good hygiene and attitude of biosafety should be emphasized to all post-graduate students. While handling infective bio-materials, especially those transmitted via oral-fecal route, extra-attention should be paid to avoid gloves contamination by using laboratory equipments to prevent contact transmission.

Other suggestions

1. The surveillance report and coping strategies provided by the university to the CDC contained only policies but no further implementation plans. We suggested that the biosafety committee of the university should assign professionals with microbiology and laboratory safety management background to monitor microbiology laboratories and follow up implementation results, including periodic checkups for various pieces of biosafety equipments (cabinets and autoclaves) and a certain hours of biosafety training courses for all laboratory personnel in order to fulfill biosafety management of university laboratories.
2. Among the proposals, the idea of pairing up workers for experiments will need to be assessed for its appropriateness and feasibility. This incident was not

caused by insufficient people to perform the experiment, but inappropriate operating habits. For level two infectious microorganisms, it is not necessary for two people to perform the experiments. The university should set feasible and appropriate protocols according to the characteristics of infectious materials.

3. The two biosafety cabinets carry risks for air leak. Especially the one located close to the entrance, which can lead to safety problem due to air turbulence resulted from people moving in and out of the room. The pathogen in this case is highly infectious, only 10 to 100 bacteria are enough to cause infection (2). The infection can therefore be easily transmitted by contaminated handles, equipments, or via oral transmission but not by air. Therefore, the biosafety cabinets are not related to this event. Nevertheless, the university should still strictly examine all biosafety cabinets to prevent air-born infection in order to ensure safety of lab personnel.

Conclusion

After a laboratory-infected SARS event in late 2003, the Nation started to emphasize on laboratory safety. The CDC invited professionals around the world to set up "tRegulations Governing Management of Infectious Biological Materials and Collection of Specimens from Patients of Communicable Diseases", and implement it since March 26, 2006. According to the principles of leveled management, the CDC periodically examines laboratories of level 3 and above in the country. Laboratories of level 2 and below are supervised by the biosafety committees or professionals from the institutes they belong to, and examined at least once a year. Biosafety levels of laboratories should be set according to the characteristics of infectious bio-materials, the safety condition of equipment and facility, the degree of personnel safety protection in the laboratories, along with a comfortable operating environment, to undergo reasonable risk assessment in

order to determine the appropriate safety management regulations to ensure biosafety in the laboratories.

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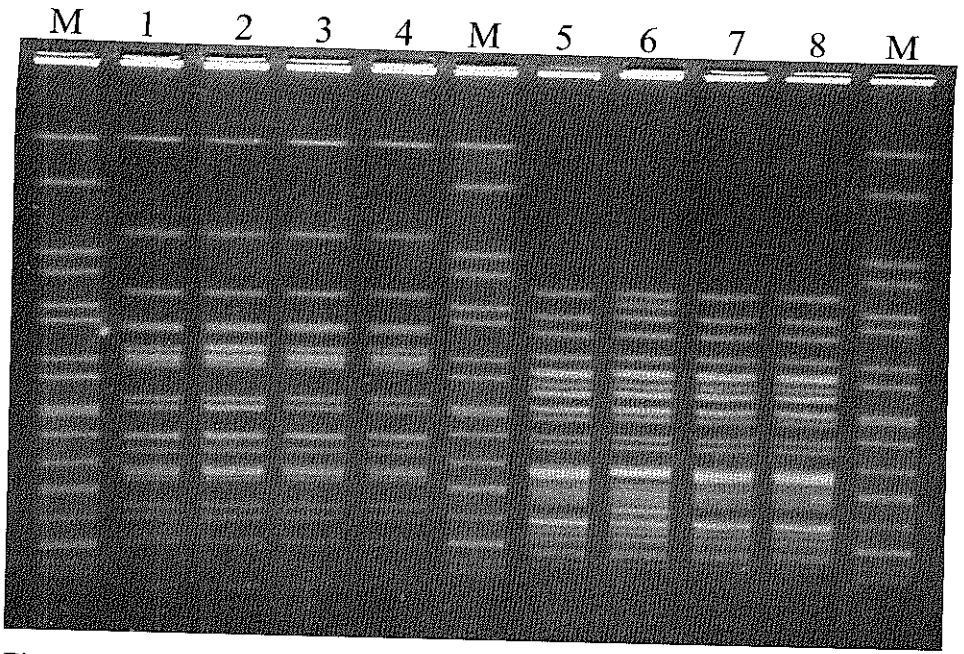

Fig. 1 **PFGE DNA fingerprint map of strains. Strains were processed according to standardized PFGE procedures (3) for embedment, washing and electrophoresis. Before electrophoresis, genomic DNA was cut by restriction enzyme NotI (lanes 1-4) and XbaI (lanes 5-8).Lanes 1 and 5: laboratory strain SH2308; lanes 2 and 6: strain SH2308-10A; lanes 3 and 7: SH3160; lanes 4 and 8: strain SH010579 isolated from the case. M: XbaI-digested genomic DNA of *Salmonella enterica* subsp. *enterica* serotype Braenderup H9812 as the marker.**

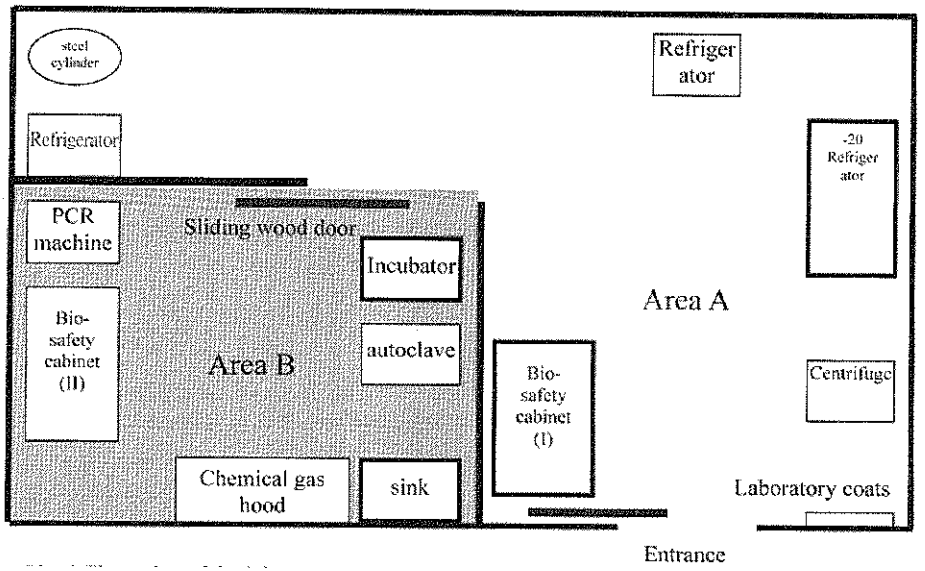

Fig. 2 Floor plan of the laboratory

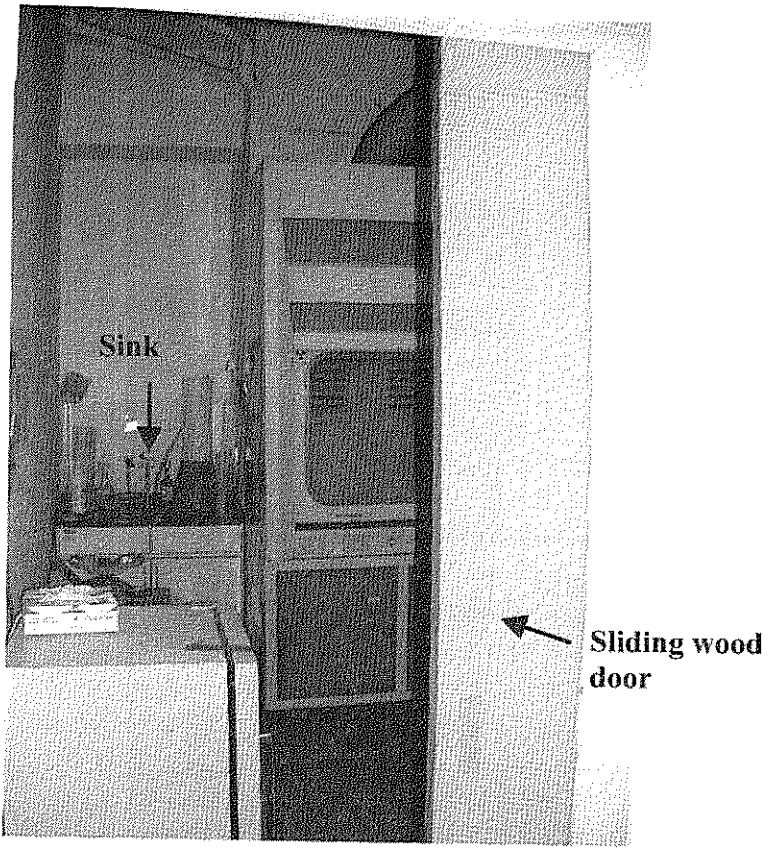

Fig 3. Area A and B in the laboratory were separated by a sliding wood door. The sink was located at the back of area B

Table 1. Results of drug sensitivity tests of the *Shigella flexneri* 2a strain isolated from the case and the three laboratory strains

Drugs[b]	Strains[a]			
	SH010579	SH3160	SH2308-10A	SH2308
Amikacin	S	S	S	S
Ampicillin	R	R	R	R
Cefazolin	S	S	S	S
Cefixime	S	S	S	S
Cefotaxime	S	S	S	S
Cephalothin	S	S	S	S
Chloramphenicol	I	R	R	I
Ciprofloxacin	S	S	S	S
Enrofloxacin	S	S	S	S
Gentamicin	S	S	S	S
Kanamycin	S	S	S	S
Nalidixic acid	S	S	S	S
Norfloxacin	S	S	S	S
Ofloxacin	S	S	S	S
Streptomycin	R	R	R	R
Tetracycline	R	R	R	R
Tobramycin	S	S	S	S

[a]Strain SH010579 was isolated from the case, the rest three are laboratory strains

[b] Drug sensitivity tests were done according to the NCCLS paper disc diffusion method (4)