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Original Article

Analysis on Imported Malaria Cases in Central Taiwan during, 2006-2010

Min- Tsung Lin[1], Sung-Hsi Wei[1], Ming-Chu Kuo[2], Du-Ling Lin[1], Chih-Tsung Tu[1], Horng-Ying Chiou[3], Tsuey-Feng Lee[4]

1. Third Branch, Centers for Disease Control, Taiwan
2. Research and Diagnostic Center, Centers for Disease Control, Taiwan
3. Fourth Branch, Centers for Disease Control, Taiwan
4. Maternal, Infant and Reproductive Health Division, Bureau of Health Promotion, Taiwan

Abstract

Certification of recognizing Taiwan as a malaria eradication area was issued by the World Health Organization in 1965. From then on, Taiwan entered the stage of prevention of reintroduction in malaria control, and, since then, most of the cases identified in Taiwan were imported, with an average of 30 reported cases per year. This study reviews the records of the epidemiological investigation conducted by the Third Branch of Taiwan Centers for Disease Control (Taiwan CDC) on confirmed malaria cases reported from central Taiwan areas during 2006-2010, and analyzes the characteristics of these cases. A total of 90 malaria cases were confirmed nationwide in Taiwan during the five-year period, 20 (22%) of them from the central Taiwan. Of the 20 cases, all were imported, among them, 12 were citizens and 8 were foreigners. The laboratory examination showed 14 cases were infected with *P. falciparum*, mainly from Africa, and 6 cases were infected with *P. vivax*, all from Asia. Fifteen of the twenty cases had a travel history to malaria infected areas, and 80% of these cases were either working or doing business there. Although 33.3% of the fifteen cases have used malaria chemoprophylactic drugs, they were still infected because interruption of taking drugs due to side effect, did not follow doctor's advice to take the drugs completely, and had used drugs not recommended by physician. Thirty percent of the 20 imported cases had visited private clinic for medical attention

INSIDE

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when they became ill after returned but were not correctly diagnosed by physician at the first time. This indicates that the physicians' vigilance and ability in malaria diagnosis still needs to be improved. All the drugs for treatment of malaria cases were provided by the Taiwan CDC within 24 hours after being laboratory confirmed. The treatment regimen for *P. falciparum* cases was artemisinin-based combination therapies (ACTs). For *P. vivax* cases, primaquine was added to the regimen for preventing relapse, and, for cases in severe condition, an intravenous therapy with artemisinine was used. Based on the results of the characteristic analysis on these cases, we suggest that citizens planning for a trip to malaria endemic areas should consult physician in travel medicine clinic before leaving the country and should take appropriate prevention methods to reduce the risk of malaria infection. In addition, clinical physicians should inquire patients with fever symptoms about their travel history and conduct microscopic examination of the blood smear for patients with travel history to malaria endemic areas since the examination is very helpful in the early diagnosis of malaria.

Keywords: malaria, imported case, *Plasmodium falciparum*, and *Plasmodium vivax*

Introduction

Malaria is caused by infection of red blood cells with Plasmodium parasite, which is usually spread through the bite of infected female anopheles mosquito, although an inductive infection (such as blood transfusion, organ transplantation, and syringe or needle sharing) or vertical transmission from mother to child also occurred occasionally. Four major Plasmodium species have been identified in human malaria cases. These are *P. falciparum,P. vivax, P. malariae, and P. ovalae*. Of these species, *P. falciparum* and *P. vivax* were most commonly seen in human cases while *P. falciparum* recorded the highest fatality rate. In recent years, several reports have documented human malaria cases that were infected with *P. knowlesi,* one of species causing monkey malaria, in forest areas of Southeast Asia [1-3].

Malaria is one of the most important issues in global public health. It is estimated that 50% of the world's population are living in poor countries and are staying in areas at risk of malaria infection. Based on reports of the World Health Organization (WHO), 225 million malaria cases were estimated to have occurred worldwide in 2009, including 780,000 fatal cases, and most of them (80%

and 91% of illness case and fatal cases, respectively) came from Africa [4]. International trips are continuously growing rapidly in number and frequency in recent years. The United Nations World Tourism Organization estimated that annual international travelers will reach 1.6 billion by 2020, and the number of tourists to tropical and subtropical destinations would greatly increase. Currently, more than 1.25 million international tourists had a trip to malaria epidemic areas each year and over 30 thousand malaria cases were identified among travelers from Europe and North America [5].

There were about 1.2 million malaria cases in Taiwan in 1945, but after the malaria control campaign was initiated in 1946, the number of cases decreased gradually, and, consequently, a malaria eradication certificate was awarded to Taiwan by WHO in 1965 [6-7]. From then on, the malaria control program in Taiwan entered the stage of prevention of reintroduction. No newly infected native malaria cases were identified up to now except 86 cases of *P. vivax* malaria locally infected in northern Taiwan during July 1966-August 1973, an inductive infection in the Taipei Veterans General Hospital in 1995, and an introduced infection detected in Taitung County in eastern Taiwan in 2003. A total of 1,601 malaria cases were reported nationwide from 1965 to 2010. Most of them were imported cases, with an average of about 30 cases per year. These cases mainly originated from areas in Southeast Asia, Africa, and Oceania. *P. vivax* was the species with the largest number of imported cases, followed by P. *falciparum*, but *P. malariae* and *P. ovalae* were found only in rare cases [8-9]. One of the imported cases was confirmed to be infected with simian malaria in 2005 [3].

Anopheles minimus used to be the major malaria vector in Taiwan areas, but, currently, it can be found only in mountain areas in Kaohsiung City (in Kaohsiung County before the reorganization of the administrative regions), Taitung County, Hualian County, Tainan City (in Tainan County before the reorganization of the administrative regions), and Pingtung County after a large scale of DDT spraying [10]. Therefore, an imported case is likely to result in a secondary infection or epidemic when the case is not detected in time and is bitten by the mosquito *Anopheles minimus*. Malaria is one of the category 2 communicable diseases in Taiwan, which requires physician to report any suspected case within 24 hours after diagnosis [11]. Moreover, public health authorities should initiate an epidemiologic investigation within 24 hours after receiving notification to understand travel history, to analyze infection source, and to avoid the spread of infection. This study analyzes data collected in the National Notifiable Disease Surveillance System (NNDSS) operated by the Centers for Disease Control (Taiwan CDC), reviews the records of epidemiological investigation on confirmed malaria cases conducted by the Third Branch Office of Taiwan CDC (Third Branch Office), and describes the characteristics of confirmed malaria cases reported from central Taiwan areas (central Taiwan) during 2006-2010.

Materials and Methods

Data source and analysis

The raw data of confirmed malaria cases who either resided in or were notified from Taichung City (including former Taichung City and Taichung County), Zhanghua County, or Nantou County, were retrieved from database of the NNDSS in Taiwan CDC during 2006-2010. By combining the raw data with the epidemiological investigation records made by the Third Branch Office, excel 2003 software was applied to analyze data on sex, age, nationality, occupation, place of infection, travel history, purpose of going abroad, use of prophylactic malaria medicines, clinical symptoms, experience of seeking medical service, date of diagnosis, and date of notification.

Specimen sampling and examination

On receiving the notification of malaria case from a hospital, Taiwan CDC will require the reporter to provide blood smear slide and whole blood specimens, and, at the same time, ask the hospital first to make staining and preliminary examination of the blood smear and, then, upload the microscopic images of plasmodium to the Taiwan CDC through the NNDSS. The Parasitic Disease Section of the Research and Diagnostic Center of Taiwan CDC then conducts distant examination on the images and sends the preliminary result about the type of the plasmodium to the reporter as soon as possible. On receiving the blood smear slide and whole blood specimen, the laboratory will perform microscopic examination and polymerase chain reaction (PCR) test to confirm the species of plasmodium [11-12].

Case definition

Confirmed case: A confirmed case is the reported case that has either a visualization of malaria parasite in blood smear by microscopic examination or a positive PCR reaction from blood specimen by molecular biological analysis [11].

Imported case: Malaria case who has been infected outside the country and no evidence of domestic infection has been found in epidemiological investigation is considered as an imported case [13].

Provision of therapeutic medicine and follow-up of confirmed cases

WHO recommended that the treatment of malaria cases should be prescribed based on parasitological confirmation. The symptom-based treatment can be administered only in situations where parasitological diagnosis is unavailable [1]. The Taiwan CDC maintains stockpiles for malaria treatment medicine in each of the six Branch Offices, which physicians can apply for clinical use on the basis of the patient's clinical presentation, type of Plasmodium, and occurrence of antimalarial drug resistance in area where the patient got infection. The detailed treatment guidelines are provided on the Taiwan CDC's website (http://www.cdc.gov.tw/public/Attachment/11317174271.pdf) [10]. In addition, for monitoring the effectiveness of drug treatment, a blood specimen has to be taken daily prior to the administration of anti-malarial drugs during the treatment period. To assure the achievement of malaria control obtained in the prevention of reintroduction stage, a confirmed malaria case should be traced for

one year after the completion of medications [12].

Results

A total of 90 malaria cases were confirmed nationwide in Taiwan during 2006-2010, 20 (22%) of them were from the central Taiwan. The epidemiological investigation on incubation period, travel history, and possible infection source indicates that these cases were unlikely infected in Taiwan. Therefore, they are all categorized as imported cases. As shown in Table 1, of the 20 cases, 12 (60%) were citizens and 8 (40%) were foreigners. The nationality of the foreign cases includes India, Thailand, Indonesia, China, Mozambique, and France. Nineteen (12 citizens and 7 foreigners) of the twenty cases (95%) are male and one is female, a foreigner. The median age of them is 35.5 years (range 6-56 years), 80% in the age group of 20-49 years, and only one citizen at childhood age. For 12 citizen cases, 3 (25%) were mechanical engineers, 3 (25%) construction workers, 2 (16.7%) timber merchants, and 1 in each of the student, military personnel, businessman, and physician category. For 8 foreigner cases, 2 (25%) were sailors, 2 (25%) mechanical operators, and 1 in each of the care worker, acrobat, postdoctoral researcher, and oilfield worker category.

Based on the analysis of infection source, the imported cases can be classified into two patterns, one is cases having travel history to malaria infected areas, and another is those always residing in malaria infected areas. Fifteen (75%) of them belong to the former pattern, and 12 (80%) of the 15 cases have previously traveled to Africa, especially western Africa, and 3 (20%) to Southeast Asia (Table 2). Five (25%) of the imported cases belong to the latter pattern, and 3 (60%) of the five cases lived in Southeast Asia, and 2 (40%) in South Asia.

Of the fifteen cases with a travel history to malaria infected areas, 12 (80%) were traveling for the purpose of working or doing business (10 of the 12 were citizens), 2 (13.3%) for visiting relatives and friends (1 citizen and 1 foreigner), and 1 (6.7%) for tourism (citizen) (Table 2). The five cases residing in infected areas were all foreigners, 4 (80%) of them were all foreign workers

Table 1. Characteristics of imported cases in central Taiwan during 2006-2010

Characteristics	Total (N=20) No. (%)	Cases with R.O.C. nationality (N=12) No. (%)	Cases with foreign nationality (N=8) No. (%)
Sex			
Male	19 (95)	12 (100)	7 (87.5)
Female	1 (5)	0	1 (12.5)
Age			
5-9	1 (5)	1 (8.3)	0
20-49	16 (80)	8 (66.7)	8 (100)
>50	3 (15)	3 (25)	0
Nationality			
India	2 (10)	-	2 (25)
Thailand	2 (10)	-	2 (25)
Indonesia	1 (5)	-	1 (12.5)
China	1 (5)	-	1 (12.5)
Mozambique	1 (5)	-	1 (12.5)
France	1 (5)	-	1 (12.5)

coming to Taiwan for work, and 1 (20%) postdoctoral researcher staying here for study. Only five (33.3%) of the fifteen cases traveling to infected areas have used malaria chemoprophylactic drugs, including mefloquine (3 cases), doxycycline (1 case), and hydroxychloroquine (1 case). Before going abroad, all five travelers have consulted travel medicine clinic for health advice and had been taking chemoprophylactic drugs but were infected with *P. falciparum*since they visited the countries in Africa where chloroquine resistance has occurred. Follow doctor's advice, the case who had used doxycycline before departure, later he no longer took it after visiting clinic because of fever developed several days when arriving destination. All three cases who have received mefloquine did not follow doctor's advice to take the drug before departure although they took it after arriving destination but they did not continue because of vomiting side effect and did not take another chemoprophylactic drugs. Instead of following doctor's advice to take mefloquine because of concerning about the neurological side effect, the fifth case accepted tour guide's recommendation to take hydroxychloroquine, a drug to which *P. falciparum* in the travel destination has developed resistance.

Table 2. Analysis of travel destinations and purposes of the imported cases from central Taiwan during 2006-2010

	Total (N=15) **n (%)**	**Cases with R.O.C. nationality** (N=12) n **(%)**	**Cases with foreign nationality** (N=3) n **(%)**
Destinations			
Western Africa	9 (60)	7 (58.4)	2 (66.7)
Eastern Africa	2 (13.3)	2 (16.6)	0
Central Africa	1 (6.7)	0	1 (33.3)
Southeast Asia	3 (20)	3 (25)	0
South Asia	0	0	0
Purposes			
Working	9 (60)	7 (58.4)	2 (66.7)
Doing business	3 (20)	3 (25)	0
Visiting relatives & friend	2 (13.3)	1 (8.3)	1 (33.3)
Tourism	1 (6.7)	1 (8.3)	0

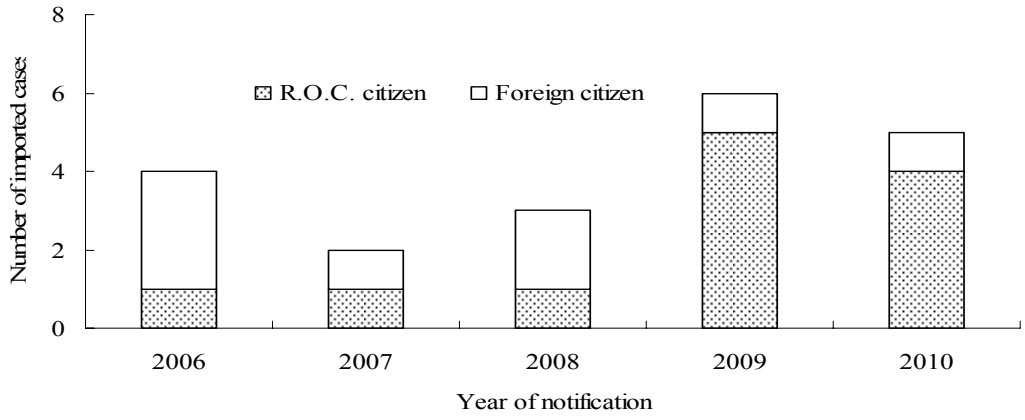

Figure Number of imported malaria cases in central Taiwan during 2006-2010, by year

Two to six imported malaria cases were reported in central Taiwan areas during the five-year period as shown in the Figure. From 2006 to 2008, the majority of the imported cases (6/9, 66.7%) were foreigners, and one citizen case was reported per year, for purpose of doing business (2 cases) or visiting relatives and friends (1 case). However, the nationality of the cases imported between 2009 and 2010 is different from other years, which almost all cases (9/11, 81.8%) are citizens. Of the nine cases, eight went abroad for the purpose of either working (7 cases) or doing business (1 case), and only 1 for tourism.

All the imported malaria cases in central Taiwan were reported by hospitals, and no cases were detected through fever screening station at international airport. The analysis of the timeliness of notification shows that all the cases have been reported within 24 hours after diagnosis, complying with the requirements of regulation. Fifty percent of the cases were reported by regional hospital, followed by medical center (35%), and local hospital (15%), but no cases were reported by private clinic. However, the study found that 30% of the cases would first visit private clinic for medical service when they got sick after arrival. The average time interval from the date of onset to the date of diagnosis was 3.4 days. Most of the cases (12/20, 60%) were diagnosed with malaria within three days from the date of onset, but 8 cases (40%) longer than three days. The longest time interval from the date of onset to the date of diagnosis was 8 days (in 2 cases). More than half of the cases (11/20, 55%) were diagnosed with the disease at their first visit to the hospital for medical service after returning country, 6 cases (30%) at their second visit, and 3 cases (15%) at their third visit. The clinical symptoms presented in these cases were consistent with those commonly seen in general malaria cases, which fever (100%) and chill (75%) were the most common symptoms. Other symptoms include sore muscle, headache, tiredness, vomiting, profuse sweating, jaundice, diarrhea, and stomach pain. No fatal case was found because of timely offering of therapeutic drugs by public health authority and appropriate treatment by physicians.

The ways for confirmation of these cases include having personnel from the Parasitic Disease Section to conduct examination at the reporting hospital in 4 cases (20%), and conducting distant examination on images to provide preliminary results to hospitals in 16 cases (80%). The preliminary results from image observation were all consistent with those obtained from real specimens. The type of the Plasmodium parasite and places of acquiring infection for

Table 3. Type of Plasmodium parasites and places of acquiring infection of the imported cases in central Taiwan during 2006-2010

Places of acquiring infection	Type of Plasmodium parasites		Total (N=20) n (%)
	P. falciparum (N=14) n (%)	P. viavx (N=6) n (%)	
Western Africa	9 (64.3)	0	9 (45)
Eastern Africa	2 (14.3)	0	2 (10)
Central Africa	1 (7.1)	0	1 (5)
Southeast Asia	2 (14.3)	4 (66.7)	6 (30)
South Asia	0	2 (33.3)	2 (10)

all of these cases is presented in Table 3. Of the 20 cases, 14 cases (70%) were caused by *P. falciparum*, and 6 cases (30%) by *P. vivax*, but no cases by *P. malariae*, *P. ovalae*, or co-infection of multiple Plasmodium parasites (simultaneously infected with more than two Plasmodium parasites). The majority of the *P. falciparum* cases (12/14, 85.7%) acquired infection in Africa, two cases (14.3%) in Asia, but six *P. vivax* cases were all infected in Asia.

Distant examination that allows the test results about the type of Plasmodium parasites to be instantly given to the notification hospitals makes it possible to administer medication treatment of malaria cases within 24 hours after notification. The treatment regimen for the 14 cases of *P. falciparum* was artemisinin-based combination therapies (ACTs), which Artesunate and Mefloquine were combined for use. The six *P. vivax* cases were treated with Artesunate, Artesunate plus Mefloquine, Mefloquine, or Chloroquine, followed by Primaquine for preventing relapse. Of the 20 imported cases, 6 were diagnosed as severe cases, which intravenous artemisinin were administered. A follow-up was conducted for all of the 20 cases by public health authorities in central Taiwan after they were cured. However, six of them (30%) were not traced because they departed for other country after they were cured and discharged from hospitals.

Discussion

A large proportion of the 20 imported cases in central Taiwan during 2006-2010 were infected with *P. falciparum*, followed by *P. vivax*, but no *P. ovalae*, *P. malariae*, or co-infection of multiple Plasmodium parasites which was not consistent with the national data (the majority of the cases were *P. vivax*, followed by *P. falciparum*) [9]. This difference was probably associated with the destination choice made by most of the malaria cases in central Taiwan. Previous study indicated that *P. falciparum* mainly occurred in sub-Saharan Africa; *P. vivax* was commonly seen in India sub-continent, Mexico, Central America, and China; while *P. falciparum* and *P. vivax* often co-existed in Southeast Asia and South America [14]. Since 80% of malaria cases in central Taiwan had chosen countries in sub-Saharan Africa as their travel destinations, more cases were infected with *P. falciparum*. The rest of the cases have been to countries in South Asia and Southeast Asia, so more cases were caused by *P. vivax* although few cases were infected with *P. falciparum* in these areas. This finding is similar to the surveillance data obtained in the United States and United Kingdom [2, 15].

The analysis shows that the reason that the number of imported malaria cases in central Taiwan increased apparently during 2009-2010 mainly resulted from the increase of cases that went abroad for working or business. However, when look at the national data, the number of imported cases that went abroad for working or business during 2009-2010 was not obviously increased. Working or doing business is always the major purpose for the majority of outgoing travelers in this country, followed by visiting friends and relatives, or tourism [9]. This finding was very different from the surveillance data in the United States and United Kingdom, which 50% of the imported malaria cases were going abroad for the

purpose of visiting friends and relatives (VFR)[2, 15]. Although the number of cases going abroad for VFR is low (only 2 cases) currently, the future impact of VFR on the number of imported malaria cases is worthy of attention and observation from public health authorities. We make this remark because, based on the annual report released by the Ministry of Interior, the number of foreign spouse applying for citizenship presented in an increasing trend. About ten thousand foreign spouses per year have applied for R.O.C. citizenship since 2005. The majority (99%) of them came from Southeast Asia countries (Vietnam, Indonesia, Cambodia, Philippines, and Myanmar) [16], where are still malaria endemic areas. When families in Taiwan go along with the foreign spouse returning their home countries for VFR, it is very likely that they may be infected with malaria if they do not take suitable protection measures. Therefore, health dissemination should also be strengthened for foreign spouses and the accompanying Taiwanese families who are planning to make a trip to their home countries for VFR, to educate them about the knowledge of disease prevention, except for those of citizens who go to malaria endemic areas for working or doing business. Especially, previous study reported that some foreign immigrants might wrongly believe that they still have immunity to the diseases in their home countries, and others might be reluctant to seek health consultation before going abroad due to economic reason [5].

The preventive measures for malaria are generally grouped into two categories: physical and chemical. Physical measures mean to take actions to avoid mosquito bites, such as the use of liquid mosquito repellents, the use of mosquito net when sleeping, and avoidance of outdoor activities at dawn and dusk. Chemical measures are to take chemoprophylactic drugs. Although chemoprophylactic antimalarial drugs are not 100% effective in preventing malaria infection, to use them accurately can certainly avoid death or decrease the severity of disease in case of acquiring infection. [5]. In this study, 66.7% of the imported cases did not use chemoprophylactic antimalarial drugs, which is similar to those found in the United Kingdom and United States (75% and 71.7%, respectively). These finding indicates that failing to use chemoprophylactic antimalarial drugs could be the major cause of acquiring malaria infection for people destined to endemic areas. For cases who have used chemoprophylactic drugs but were still infected with malaria, we may need to further examine whether they have effectively complied with doctor's advice and whether they have chosen correct chemoprophylactic drugs. Previous study indicated that the key point of successfully preventing malaria infection is the patient's compliance with doctor's advice on chemoprophylactic drugs, and, therefore, suggested that travelers should be reminded of the importance of taking chemoprophylactic antimalarial drugs correctly and completely [14]. Except patient's knowledge, attitude, and behavior on the disease, another important factor affecting patient's compliance is the side effect of the chemoprophylactic drugs and their severity. In this study, we have noticed that the side effect of the drugs have indeed affected the

travelers' willingness of taking the drugs for malaria prophylaxis.

To choose effective chemoprophylactic antimalarial drug for travelers to endemic areas, we need to consider travelers' personal circumstance (such as health conditions, accessibility of medical resources at travel destination, duration of activity in a specific areas, and experience in use of chemoprophylactic antimalarial drugs), geographical locations of destination (including the major type of Plasmodium parasite and their sensitivity to antimalarial drugs in the locations), and personal economic situation [14]. In order to provide citizens with professional consultation, Taiwan CDC has contracted with 11 hospitals to establish travel medicine clinics since November 2006, which provide integrated services over travel-related medicine, health consultation, international travel vaccination (for yellow fever and meningococcal meningitis), and provision of chemoprophylactic antimalarial drugs for people having an international travel, to assure their safety while traveling abroad. In addition, Taiwan CDC has created an international travel section on its website to provide information for people in preparing their international travel. Therefore, the important issues for public health authorities are to enhance the utilization of travel medicine clinics, to provide correct chemoprophylactic drugs through physician, and to improve travelers' awareness of disease prevention through health education.

In terms of the interval days from the date of onset to the date of diagnosis, the result (3.4 days) in central Taiwan was largely shortened, as compared with that (14.2 days) from previous nationwide surveillance [9]. This improvement was probably contributed by the enhancement of travelers' vigilance to illness occurred in traveler themselves and the requirement that physician should ask for patients' travel history during disease diagnosis. What was unsatisfactory was that 30% of the 20 imported cases were not diagnosed as malaria cases by physician at the first time when they became illness and visited private clinic for medical attention. The symptoms of malaria at its early stage are not specific and similar to those caused by some viruses [1], so it is uneasy for physicians in the non-endemic Taiwan areas to diagnose it. However, to inquire patients with fever symptoms about the international travel history before making a diagnosis would allow physicians to elevate their own vigilance on the diagnosis of travel-related diseases. For patients with travel history in malaria endemic areas, the first priority would be to rule out malaria. Moreover, to perform microscopic examination of blood smear for these patients would be helpful for physician to diagnose the disease in its early stages. For primary health care clinics without microscopic examination technique or equipment, the pertinent procedures for them are to immediately refer the patient to hospitals with the examination ability. In addition, several cases were not diagnosed until they visited hospital for medical attention at the third time. This indicates that the physicians' vigilance and ability in malaria diagnosis still needs to be improved.

Since only 20 imported cases in central Taiwan during 2006-2010 have been

identified, the results presented in this study may be unable to represent the whole picture of the imported cases. However, based on the results of the characteristic analysis on these cases, we would like to make suggestions on malaria control strategy, and, therefore, to elevate citizen vigilance on malaria infection. For citizens planning a trip to malaria endemic areas, we suggest that they should visit travel medicine clinic before leaving the country to consult with physician about health information and should take chemical and physical methods to reduce the risk of malaria infection. For clinical physicians, we suggest that they should inquire patients with fever symptoms about their travel history in malaria endemic areas and conduct microscopic examination of the blood smear for patients with travel history or living in malaria endemic areas since the examination is very helpful in the diagnosis of malaria in the early stage. To continually maintain the achievement of malaria control in the prevention of reintroduction stage needs not only the efforts of public health authorities but also the cooperation of medical care sector and all the people in this country.

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Report on Surveillance of Gonococci-National Isolate Collection for Epidemiology (G-NICE), 2009

Kun-Yen Lin, Chun-Chen Chen, Shu-Ying Li

Research and Diagnostic Center, Centers for Disease Control, Taiwan

Abstract

The surveillance program of Gonococci-National Isolate Collection for Epidemiology (G-NICE) was implemented during January to December, 2009, enrolling 31 hospitals and clinics. The program collected 519 gonococcal isolates nationwide, including 427 isolates from northern Taiwan, 63 from southern Taiwan, and 29 from central Taiwan. None was obtained from eastern Taiwan or offshore islands. Using disk diffusion test, the proportions of isolates resistant to penicillin, ciprofloxacin, cefixime, cefpodoxime, and ceftriaxone were 66.7%, 93.4%, 3.3%, 5.2%, and 1.3%, respectively. The Minimum Inhibition Concentration (MIC) of ceftriaxone and cefixime were 0.125mg/L and 0.38mg/L, respectively.

The 519 isolates were delineated into 193 sequence types (STs) by *Neisseria gonorrhoeae*- multi-antigen sequence type (NG- MAST) molecular subtyping method. Among the 193 STs, 13 had more than 5 isolates collected, including ST421 (n=60), ST419 (n=31), ST225 (n=20), ST2175 (n=19), ST2194 (n=18), ST2178 (n=14), ST3684 (n=12), ST2179 (n=9), ST 3382 (n=8), ST3694 (n=8), ST359 (n=6), ST2253 (n=6), and ST1971 (n=5). Each ST exhibited distinct antibiotic susceptibility pattern. For example, ST2253 was highly resistant to penicillin, ciprofloxacin, cefixime, and cefpodoxim and was only sensitive to ceftriaxone while ST539 was sensitive to all antibiotics tested.

Based on the surveillance results of G-NICE for drug resistance, quinolones is no longer recommended for treating gonorrhea infection, and the 3rd generation of

cephalosporin should be used instead. Combined molecular subtypes and drug susceptibility patterns of their isolates, patients could be clustered into different sexual networks. Patients from high-risk sexual network should receive medical consultation, diagnosis and treatment of other concurrent sexually-transmitted infections, and encourage their sexual partners to seek proper medical care.

Keywords: sexually-transmitted infections, gonococcus, drug susceptibility test, molecular epidemiology

Introduction

Gonorrhea, caused by *Neisseria gonorrhoeae* (gonococci), is the second most prevalent bacterial transmitted infections worldwide. Gonococci attack preferably columnar epithelium, such as urethra, cervix, and rectal mucosa. Male patients usually present with symptoms, including purulent urethral discharge, dysuria and a burning sensation during urination. Patients of men who have sex with men (MSM) often have rectal involvement. Female patients might have urethritis and cervicitis; complications including endometritis, salpingo-oophritis, and long-term sequelae such as pelvic inflammatory disease, ectopic pregnancy and infertility.

According to the survey by World Health Organization (WHO), 62 million people worldwide become infected with gonorrhea each year [1]. Recently, in many countries the relative increases of gonorrhea were greatest among men who have sex with men (MSM) and young people [2]. In Taiwan, the number of reported cases was 2,137 in 2009, indicating an annual incidence of 9.2 per 100,000 persons [3].

Currently, no vaccine is available for preventing gonorrhea and antibiotics remain the mainstay for treating gonococcal infections. However, over the last decades, *N. gonorrhoeae* strains developed a high level of resistance against several antimicrobial agents such as penicillin and tetracycline. Resistance to quinolone emerging in early 2000 in Asia and subsequently worldwide has further limited treatment choices [4-7]. In Taiwan, 9% of the gonococci were resistant to cefixime, an oral cephalosporin, in 2003 using disk diffusion test. The proportion of gonococci resistant to cefixime and another third-generation cephalosporin, cefpodoxime, increased to 16.4% and 21.2%, respectively during 2006-2007 [8-9]. Since 2006, we have started a surveillance program of Gonococci-National Isolate Collection for Epidemiology (G-NICE) to collect gonococcal isolates from hospitals and clinics to trace the resistance trend and molecular epidemiology of gonococci in Taiwan.

Molecular typing such as muti-antigen sequence typing (NG-MAST) is helpful in identifying the transmission networks among high-risk populations and the emergence and international spread of drug-resistant gonococci. In London, distinct sexual network as defined by sequence types (ST) was found to be associated with ethnicity, gender, and HIV infection status; and rare sequence types were more frequently isolated from elder people with contact history overseas [10]. Similarly, in Netherland,

specific epidemiologic patterns of gonorrhea infections were identified among high-risk populations, including MSM, heterosexual people, and those seeking partner via the Internet [11]. NG-MAST methods were also used to identify high-risk populations carrying drug-resistant strains [9, 12] and to trace quinolone-resistant gonococci in the United Kingdom and Greece [13-14].

Herein, we report the resistance pattern and NG-MAST sequence type distribution of the 519 gonococcal isolates collected by G-NICE 2009 surveillance program in Taiwan.

Materials and Methods

Collection of gonococcal isolates

In this G-NICE study, 519 gonococcal isolates were collected from 31 hospitals and clinics between January and December 2009, which represents 24.3% of the reported cases in 2009. All hospitals and clinics participating in G-NICE submitted the isolates together with a questionnaire to Research and Diagnostic Center, Taiwan Centers for Disease Control. All bacterial isolates were subcultured and stored under -80℃.

Tests for drug susceptibility

Gonococcal isolates were inoculated on chocolate agar and incubated at 37℃ for 16 to 18 hours. Fresh colonies were added to Mueller-Hinton solutions and the turbidity was adjusted to 0.5-0.6 McFarland standard using a Nephelometer (BD Diagnostic Systems, Franklin Lakes, NJ, USA). Antimicrobial susceptibility of *N. gonorrhoeae* isolates to penicillin, ciprofloxacin, cefixime, cefpodoxime, and ceftriaxone were analyzed using disk diffusion method. Isolates exhibited resistance to cefixime and ceftriaxone by disc assay were further evaluated for their Minimum Inhibition Concentration (MIC) using E-test. Drug susceptibility was determined by the size of inhibition zone to each antibiotic using BIOMIC® V3 (Giles Scientific, Santa Barbara, CA, USA). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for *N. gonorrhoeae* [15]. Isolates with MIC of ceftriaxone and cefixime ≤ 0.25 mg/L were considered to be susceptible (S).

Extraction of gonococcus DNA

DNA was extracted by using MasterPure™ Yeast DNA Purification Kit (EPICENTRE Biotechnologies, Madison, WI, USA). Gonococcal isolates were cultured on chocolate agar at 37℃ for 16-18 hours. Sufficient bacteria was inoculated into 100µl PBS solution and mixed with 250µl Cell Lysis Solution to dilute and dissolve the cells. After addition of 150µl Protein Precipitation Solution, the mixture was vortexed for 10 seconds and centrifuged under 12,000xg for 10 minutes. To facilitate the precipitation of DNA, 500µl of 100% isopropanol was added to the supernatant. The precipitated DNA was rinsed with 70% alcohol, and 100µl Hydration Solution was then added to dissolve the extracted DNA. The concentration of the extracted DNA was read by a spectrophotometer. The DNA was stored under –20℃ for further studies.

***Neisseria gonorrhoeae*-Multiantigen sequence typing (NG-MAST)**

The NG-MAST molecular typing of gonococcal isolates was conducted by sequencing of internal fragments of 2 highly polymorphic loci, *por* and *tbpB* [16]. The *por* gene (750 bp) was amplified by PCR using forward primer 5'-CAA GAA GAC GAC CTC GGC AA-3' and reverse primer 5'-CCG ACA ACC ACT TGG T-3'. The *tbp*B gene (600 bp) was amplified by PCR with the forward primer: 5'-CGT TGT CGG CAG CGC GAA AAC-3' and reverse primer: 5'-TTC ATC GGT GCG CTC GCC TTG-3'. The PCR condition was as previously described [16].

Comparing the DNA sequences of isolated gonococci and establishing a database

DNA sequences of *por* gene and *tbpB* gene were analyzed using the BioNumerics 5.0 software. The sequence data of each *por* and *tbpB* gene was uploaded onto the database of the NG-MAST website (www.ng-mast.net) to obtain the allele number and the sequence type (ST).

Results

The number of gonococcus isolates and epidemiologic studies

In the 2009 G-NICE study, 519 isolates were collected from 31 hospitals and clinics. More isolates were from northern Taiwan (n=427). Fewer were from southern Taiwan (n=63) and central Taiwan (n=29) while none was from eastern Taiwan and off-shore islands (Figure 1). Among the 519 isolates, 449 isolates were from male patients while 66 isolates were from female patients, and gender for 4 isolates was unknown. The male to female ratio was 6.8:1. The age range of patients was between 13 and 84. Among them, 25.8% of the male patients and 24.2% of the female patients were 25 to 29-years

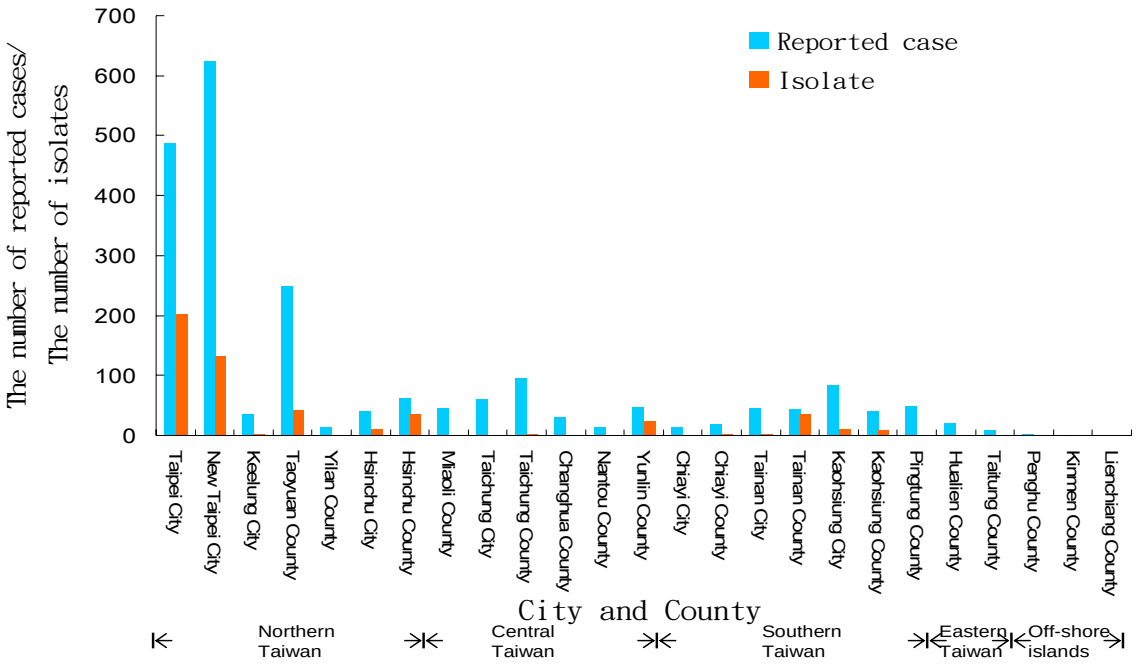

Figure 1. Geographic distribution of the number of reported cases and bacterial isolates, 2009, Taiwan

old, constituting the most prevalent age group. The second and third most prevalent age groups were somewhat different by genders. In male, 19.6% were in 30-34 years-old and 17.4% were in 20-24 years-old. In female, 22.7% were in 20-24 years-old while 13.6% were in 15-19 years-old (Table 1).

Results of drug susceptibility tests

The drug susceptibility using disc diffusion assay for penicillin, ciprofloxacin, cefixime, cefpodoxime, and ceftriaxone of the 519 bacterial isolates were 66.7%, 93.4%, 3.3%, 5.2%, and 1.3%, respectively. The results of E-test showed that the MIC to ceftriaxone in all tested isolates was less than 0.25mg/L (range, 0.002-0.125 mg/L) and the MIC to cefixime was > 0.25 mg/L in 5 isolates with 4 had MICs equal to 0.25mg/L and one had MIC = 0.38mg/L. The other 18 isolates showed decreased susceptibility with MICs ≥ 0.125 mg/L (range, 0.125-0.19 mg/L). The MIC_{50} of ceftriaxone and cefixime were 0.012mg/L and 016 mg/L, respectively. The MIC_{90} of ceftriaxone and cefixime were both 0.064 mg/L.

NG-MAST sequence types of isolates

Among the 519 isolates collected in 2009, 193 STs were identified; 120 of the 193 STs had only one isolate, while the other 73 STs had 2 to 60 isolates. Thirteen STs including ST421, ST419, ST225, ST2194, ST2178, ST3684, ST2179, ST3382, ST3694, ST359, ST2253, and ST1971 had more than 5 isolates. The three most prevalent STs, i.e., ST421, ST419, and ST225, had more than 20 isolates. All isolates of ST2179, ST3694, ST359, ST2253, and ST1971 were collected from male patients. The male to female ratio of ST4198 and ST2178 were 14.5:1 and 13:1, respectively, which were higher than the ratio of ST421 (3.5) and ST225 (2.1) (Figure 2).

Figure 3 showed the proportion of isolates resistant to five different antibiotics: penicillin, ceftriaxone, cefixime, cefpodoxime, and ciprofloxacin, by disc diffusion assay in different STs. For most STs, 87.5%-100% of isolates were resistant to

Table 1. Age distribution of patients

Age group	Number of isolates from male patients	%	Number of isolates from female patients	%
<15	1	0.2%	2	3.0%
15-19	23	5.1%	9	13.6%
20-24	78	17.4%	15	22.7%
25-29	116	25.8%	16	24.2%
30-34	88	19.6%	7	10.6%
35-39	56	12.5%	3	4.5%
40-44	29	6.5%	7	10.6%
45-49	17	3.8%	1	1.5%
50-54	10	2.2%	1	1.5%
55-59	8	1.8%	0	0.0%
60-64	5	1.1%	0	0.0%
>65	11	2.4%	1	1.5%
Unknown	7	1.6%	4	6.1%
Summation	449		66	
Total number of isolates	519			

ciprofloxacin. For penicillin, the proportion of resistant strain in most STs was 84.2%-100%, while that in ST421, ST2194, and ST3684 were lower (range, 50%-58.3%). Isolates in most STs were found to be susceptible to cefixime and ceftriaxone except 2 (33.3%) and 3 (50%) separate isolates in ST2253 were resistant to cefixime and ceftriaxone, respectively. For cefpodoxime, one isolate of ST225 (5.0%), one isolate of ST2194 (5.6%), and 5 isolates of ST2253 (83.3%) were resistant. Among isolates of other 15 minor

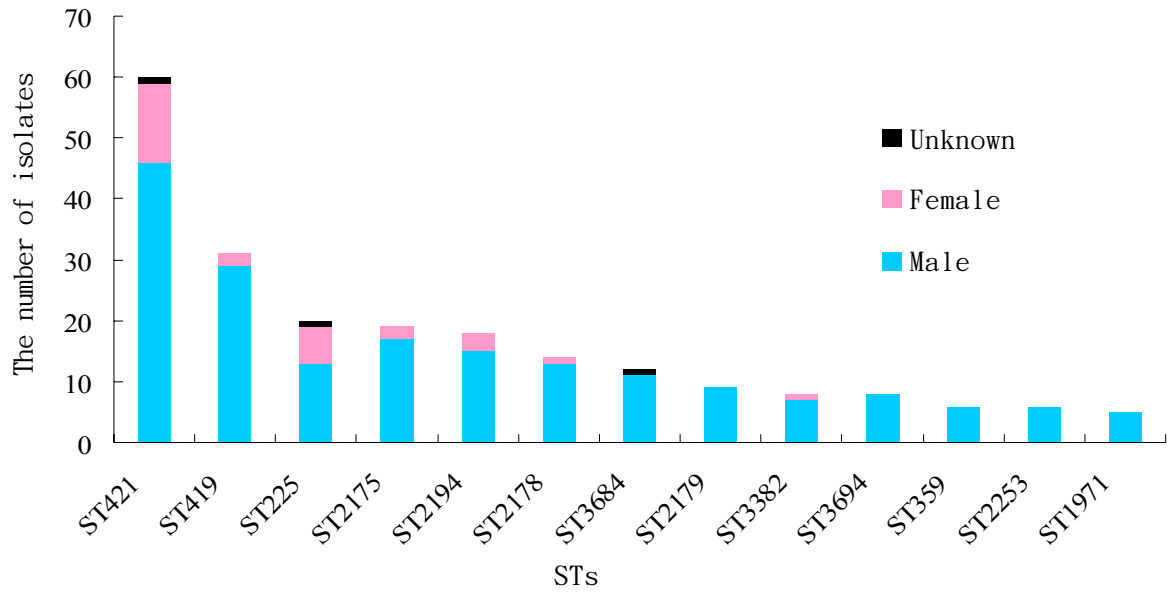

Figure 2. Gender distribution of different sequence types (only STs with at least 5 isolates were included)

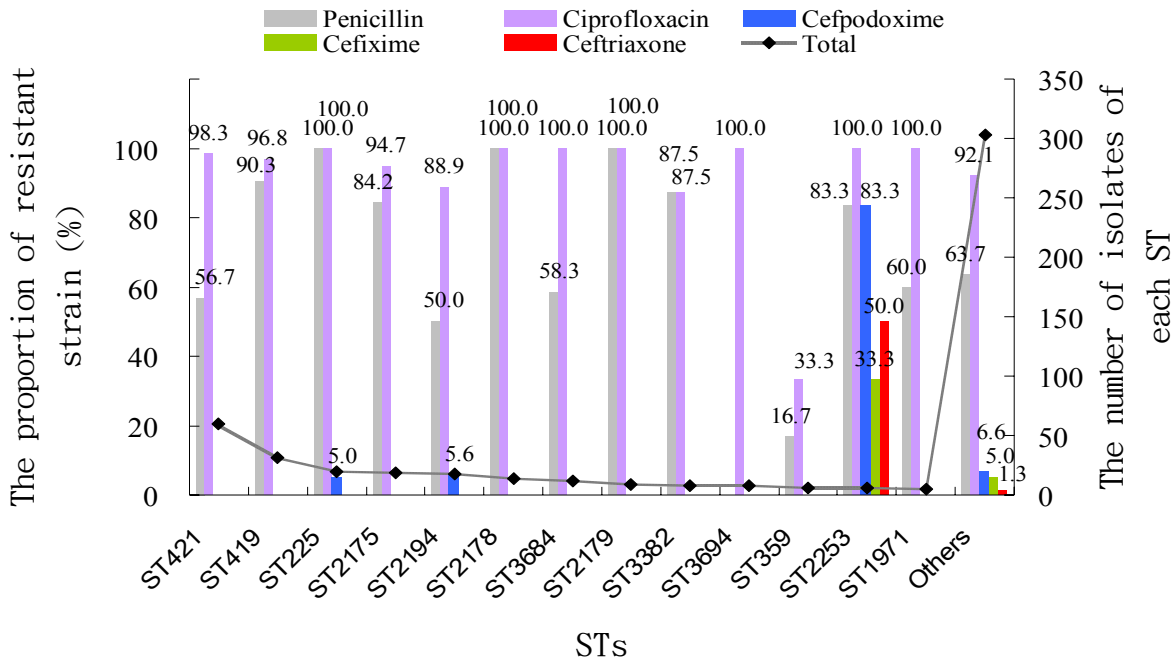

Figure 3. The proportion of resistant strain in different sequence types. STs in others have ≤4 isolates

STs, 20, 15, and 4 isolates were resistant to cefpodoxime, cefixime, and ceftriaxone, respectively.

Analysis of the antibiotic susceptibility patterns of drug-resistant gonococci

The 519 isolates were classified into 6 resistant patterns (Type 1 to Type 6) according to their susceptibility by disc diffusion assay to penicillin, cefixime, cefpodoxime, ceftriaxone, and ciprofloxacin (Table 2). As shown in Figure 4, Type 1 carried the highest drug resistance and Type 6 was susceptible to most antibiotics. Most of the isolates belonged to Type 3 or Type 4 showed intermediate or resistant to penicillin. All isolates of ST225, ST2178, and ST2179 were Type 3. All of the isolate of ST3382 were also Type 3, except one being Type 6. All isolates of ST3694 were Type 4. As for isolates of ST2194 and ST3684, both Type 3 and Type 4 patterns could be found.

According to our previous surveillance, isolates of ST359, ST2253, and ST1971 were mainly from homosexual male patients, while the isolates of ST421, ST419, ST225, ST2175, ST2194, ST2178, ST3684, and ST2179 were from heterosexual patients. Distinct

Table 2. Antibiotic susceptibility patterns

	Penicillin	Cefixime	Cefpodoxime	Ceftriaxone	Ciprofloxacin
Type 1	R[a]	R	R	S	R
Type 2	R	S	R	S	R
Type 3	R	S	S	S	R
Type 4	I	S	S	S	R
Type 5	I	S	S	S	I
Type 6	I	S	S	S	S

[a] "R": resistant; "I": intermediate; "S": susceptible.

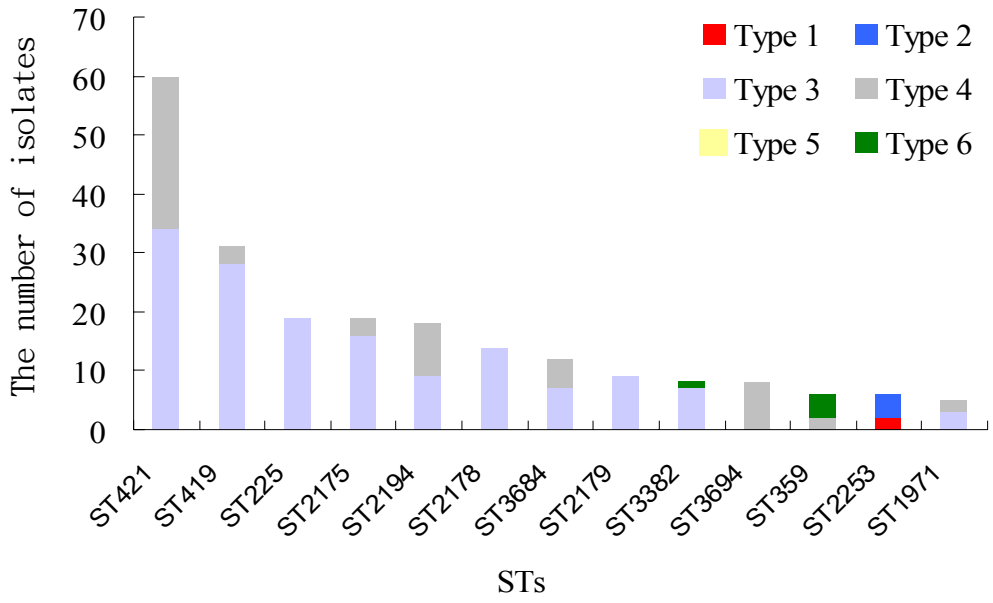

Figure 4. Patterns of antibiotics resistance in different sequence types (only STs with at least 5 isolates were included)

susceptibility patterns could be found in these two groups. Heterosexual patients usually had gonococci with medium resistance (Type 3 or Type 4). By comparison, homosexual patients could have isolates with either very strong (Type 1 or Type 2) or very low resistance (Type 6).

Discussion

The development of resistance of *Neisseria gonorrhoeae* to antibiotics such as penicillins, tetracycline, fluoroquinolones has diminished therapeutic options. To monitor the trends of drug resistance of gonococci in Taiwan, we launched G-NICE program in 2006 to collect gonococci clinical isolates. Meanwhile, NG-MAST molecular typing technique was also used to analyze the emergence, epidemiology of drug-resistant strains, and international transmission patterns. In 2009, 519 gonococcal isolates were collected. The male to female ratio was 6.8:1. The reason for high male to female ratio may be due to either increased transmissions among MSM or more asymptomatic infections and less motivation to seek medical care of female patients. Therefore, to reduce potential reservoir, sexual partners of confirmed patients should be encouraged to receive proper medical management. The age range of the patients was between 13 and 84, with the highest proportion among those aged between 25 and 29 in both genders (25.8% in male and 24.2% in female). Analyzing the drug resistance of isolates, the proportion of strains resistant to penicillin, ciprofloxacin, cefixime, cefpodoxime, and ceftriaxone were 66.7%, 93.4%, 3.3%, 5.2%, and 1.3%, respectively. Therefore, quinolones was no longer recommended in treating gonorrhea and the third-generation cephalosporins such as the oral drug cefixime and the parenteral drug ceftriaxone should be used instead. However, gonococcal resistant or reduced susceptibility to third-generation cephalosporins has been increasingly reported, Recently, the first gonococci highly resistant to cefixime (8 mg/L) and ceftriaxone (2-4 mg/L) has been isolated and characterized in Japan [17]. The threat has become imminent that gonococci may become superbug and usher in era of untreatable gonorrhea. The G-NICE 2009 showed that the MIC to ceftriaxone and cefixime has reached 0.125 mg/L and 0.38 mg/L, respectively. This indicated that several strains with decreased susceptibility have emerged and should be monitored carefully in Taiwan.

Gonococcal strains were subtyped and designated to different STs by NG-MAST. Patients carrying the same ST may belong to the same transmission network. In addition, each ST was found to display specific antibiotic susceptibility pattern. This finding is helpful to trace the spread of antibiotic-resistant strains. A previous study in London showed that there were 6 main drug-resistant clones identified in high-risk populations [18]. In Taiwan, ST359 and ST2253 were the dominant STs found in MSM. These 2 STs also exhibited quite distinct drug-resistant patterns (Type 6 and Type 1, respectively). It is postulated that isolates of these distinct STs may have been introduced via MSM patients through foreign contact and then spread to their sexual

partners in Taiwan. For those ST strains harboring Type 3 or Type 4 resistance patterns, they are susceptible to most cephalosporins, commonly seen in heterosexual patients and might be domestic strains. Regarding the gender differences, the number of female patients was far less than that of male. The 66 isolates collected from female patients scattered in diverse STs, suggesting that there was no major transmission network in female. In the future, more isolates should be collected to clarify the high-risk groups, such as MSM, heterosexual partners, and sex workers, and to identify the drug susceptibility patterns and transmission chains.

To help clinicians to decide antibiotic for treatment, and to help identifying high-risk populations, these results of molecular typing and drug susceptibility analysis have been fed back immediately to hospitals and clinics participated in G-NICE program. Further education, examination, and treatment to patients and their sexual partners should be performed and tailored according to respective at-risk groups. Hospitals and clinics are encouraged to participate in future G-NICE studies.

Acknowledgement

We deeply appreciate the hospitals and clinics participating in G-NICE program between 2006 and 2009. These hospitals and clinics are (sorted from A to Z): Buddhist Tzu Chi General Hospital Dalin Branch, Buddhist Tzu Chi General Hospital Taipei Branch, Cathay General Hospital Hsinchu Branch, Cathay General Hospital Sijhih Branch, Catholic Mercy Hospital, Changhua Christian Hospital, Cheng Hsin General Hospital, Chi Mei Medical Center LiouYing Branch, Chi Mei Medical Center YongKang Branch, China Medical University Hospital, Chung Shan Medical University Hospital, Chutung Hospital, Department of Health, Cyuanmin Hospital, Ditmanson Medical Foundation Chia-Yi Christian Hospital, Far Eastern Memorial Hospital, Hsin Chu Armed Force Hospital, Hsinchu Hospital, Department of Health, Kaohsiung Armed Forces General Hospital, Kaohsiung Chang Gung Memorial Hospital of the C.G.M.F., Kaohsiung Municipal United Hospital, Keelung Chang Gung Memorial Hospital of the C.G.M.F., Landseed Hospital, Mackay Memorial Hospital Taipei Branch, Mackay Memorial Hospital Tamshui Branch, Min-Sheng General Hospital, National Taiwan University Hospital, National Taiwan University Hospital Yun-Lin Branch, New Taipei City Hospital Sanchong Branch, Saint Paul's Hospital, Sin-Lau Medical Foundation, the Presbyterian Church in Taiwan, Taichung Hospital, Department of Health, Taichung Veterans General Hospital, Tainan Municipal Hospital, Taipei City Hospital KunMing Branch, Taipei City Hospital RinAi Branch, Taipei Hospital, Department of Health, Taipei Medical University Hospital, Taoyuan Hospital, Department of Health, Taoyuan Veterans Hospital, Ton Yen General Hospital, and Tungs' Taichung MetroHarbor Hospital.

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Biosafety and Biosecurity

The Role of Institute in the Enforcement of the Biosafety Self-management System

Yu-Yen Shih, Wen-Chao Wu, Jer-Jea Yan

Fifth Division, Centers for Disease Control, Taiwan

A wide range of infectious biomaterials are being manipulated in a biological laboratory and each of them possesses different risks to involved personnel and requires protection equipments for different biosafety level. Therefore, it is essential that the institute owning biological laboratories (hereafter refers as “the install units”) should establish a biosafety self-management system. Health authority usually regulates the principles of protection and safety for personnel involving in the manipulation of infectious biomaterials. However, manipulation of infectious materials is a daily routine work in a biological laboratory, and even a little negligence in safety and protection procedures could lead to the infection of personnel working in the laboratory. Therefore, the only way for an institute to effectively prevent and terminate the occurrence of laboratory-related infection is to set up its own self-management system on laboratory biosafety practices. Based on this system, an install unit will be able to actively conduct internal evaluation on possible risk itself, find potential problems on biosafety management, clarify and discuss the problems, and finally confirm the improvement and solution of the problems.

An install unit should develop a set of self-management system appropriate for all laboratories installed within the institute on the basis of the regulations and policies set by the competent health authority [1]. The implementation of the biosafety self-management system largely depends on the effective operation of the established Institutional Biosafety Committees (IBCs) (or specifically authorized personnel) over the issues that include setting up the goals of the laboratory biosafety policy and clearly defining the focus and items of the laboratory biosafety management and implementation. Moreover, the IBCs should demonstrate their strong ambitions in achieving the policy goals through the support of high level supervisor, post notice to inform workers in the laboratory of the implementation details of the policy, and then fully implement the laboratory biosafety management activities through empowering procedures [2-3].

The enforcement of the laboratory biosafety self-management system primarily relies on the awareness of the personnel at all levels in the institute about the system. Personnel should keep alert and conduct risk assessment at all times and places on issues of all operation procedures related to the

manipulation of infectious biomaterial, the efficiency and effectiveness of the personal protective equipments and biosafety equipments, the functionality and appropriateness of the facilities and environment where biomaterials are manipulated, and the potential risks and hazards of the infectious biomaterials to personnel involving during manipulation.

Whenever changes in the process, equipments, or environment of the manipulation occurred, the existing biosafety procedures should be appropriately reviewed, and the measures on biosafety improvement and prevention should be formed based on the level of risk probably resulted from the changes. For example, the old, malfunctioned equipments should be replaced with a new one; the standard operating procedures and relevant laboratory biosafety equipments should be updated in accordance with current necessity; biosafety operation standard should be established; personnel training should be strengthened; an emergency response plan for dealing with laboratory incident and disaster should be developed [2-3]. Since the goals of laboratory biosafety policy will be unable to be achieved when it is implemented totally through the external audits performed by competent health authority, all relevant units should maintain vigilance over possible risks at all times to early identify errors and correct it immediately so that the goal of "zero risk" could be accomplished.

Some issues that personnel should be aware of in the implementation of biosafety self-management activities include following the current laws and regulations, appropriately updating internal standard operating procedures, continually enriching and being sophisticated in the biosafety associated knowledge that covers the fields of risk assessment on infectious biomaterials, microbiological practice techniques, and inspection and maintenance of hardware equipments and software. What is more important is that the install units should have a well-established internal biosafety self-management system and should annually perform the internal biosafety audit program to assure that the laboratories have fully enforced the requirements about the functional testing or validation testing of the security facilities and equipments, the implementation of inventory management plan for infectious biomaterial, and training of staff in biosecurity issues. Through the audit activities, the deficiencies associated with biosafety management could be immediately found and corrected, the personnel performance evaluation could be conducted in referring to the audit findings, the effectiveness of the existing program or system could be verified, and the results could be used as a basis in revising the policy and program related to biosafety management. A well designed biosafety management system must include the concept of PDCA (Plan-Do-Check-Act) cycle. Only through the continuous operation of the PDCA cycle, the goal set for biosafety management system could be achieved and the spirits of self-management could be effectively enforced [2-3].

In any case, to fully enforce a well designed biosafety self-management system is the most effective and economic way to avoid the occurrence of laboratory-acquired

infection among the workers. Therefore, the institute should consider the implementation of the system as a must-do activity and continually enforce it to make the consciousness on biosafety internalize into the minds of each worker to let the activities become a habit and a natural behavior among the workers, and to ensure safety and health of the workers in laboratory and residents in the community.

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Organization, Role, and Mission of the Institutional Biosafety Committee

Yu-Yen Shih, Wen-Chao Wu, Jer-Jea Yan

Fifth Division, Centers for Disease Control, Taiwan

Three laboratory-acquired severe acute respiratory syndrome (SARS) infections occurred in different countries during 2003-2004 [1] have evoked a great concern on issues of laboratory biosafety to the countries in the world. As one of the countries with incidental infections, Centers for Disease Control, Taiwan (Taiwan CDC) has the inevitable responsibilities to work more actively over issues related to laboratory biosafety. Therefore, Taiwan CDC has formulated the Regulations on Management of Infectious Biomaterials and Specimen Sampling from Communicable Disease Cases in 2005 based on the Communicable Disease Control Act. In referring to the essence of self monitoring and voluntary notification remarked in the International Health Regulation (IHR), the Article 3 and Article 4 of the Regulations has defined the mission of an institute has the biological laboratory and its Institutional Biosafety Committee (IBC), to regulate the rights and duties of an institute in performing self-management activities in biosafety [2].

The establishment of the IBC should be designed in adopting recommendations made by the World Health Organization, which the members of the Committee should include personnel with various academic backgrounds from different units in the institute, such as biosafety officer, researchers, medical personnel, veterinarian (for institute where animal experimentation is performed), engineering personnel, and head of the laboratory [2]. In addition, the organization and operation strategies of the IBC should be adjusted in accordance with the characteristics and size of the institute. For example, the large-sized research institutes, or universities and their hospitals may have to

develop a set of well-designed and sufficiently decentralized management mechanism to avoid the occurrence of error or negligence in the performance of management, owing that these institutes may have more number and larger size of laboratories that may conduct experiments involving human, animal, or plant genetic modification. On the contrary, the operation and management of the IBC in a small-sized institute may be performed under the oversight of other organization. However, whether the institutes are operated independently or by affiliating to other organization, they have to establish their own IBC with a well-designed framework, constitution, and mission, and periodically hold IBC meetings to effectively implement the laboratory biosafety businesses [2].

To effectively update the status of the biosafety management, the institute is recommended to hold IBC meeting at least quarterly and could invite experts from different disciplines (such as radiation protection, industry safety, fire safety) to attend the meeting for providing expertise and professional opinions when necessary. The resolutions reached by the IBC meeting should be thoroughly transmitted to all personnel involving laboratory activities through various available channels or routes for them to know and follow [3]. In addition, the IBC chairperson would be preferable at the level of Deputy Director-General or above of the institute, as in the instance of the Labor Safety and Health Committee, so that the matters of biosafety management could be effectively implemented.

Except that the IBC must have a well-designed organizational framework and definite constitution and missions, what is more important for an effectively operated IBC is to completely fulfill the activities for each of the missions that have been stated in the Regulations on Management of Infectious Biomaterials and Specimen Sampling from Communicable Disease Cases, which include issues on infectious biomaterials, laboratory biosafety, and oversight, assessment, and management of biosafety incidents [2]. In foreign countries, the biosafety officer usually is the person who represents the head of the research institute or laboratory in pushing the implementation of the matters related to aforementioned biosafety related issues [3-4]. Although we currently do not have relevant regulations about the duties of the biosafety officer, the implementation model still can be performed by assigning a specific person as the biosafety executive personnel or secretary.

The major deficiencies found during a routine check conducted recently by the Taiwan CDC over the operation of the IBC include the following items. First of all, the IBC does not accurately review the documents regarding the adjustment of biosafety level of infectious biomaterials. For example, the infectious biomaterials related to avian influenza A (H5N1) virus that are supposed to be classified as biosafety level 3 was incorrectly categorized as biosafety level 2 in the documents submitted by the laboratory. However, the IBC was unable to correctly identify the error and directly approved the application as biosafety level 2. Since the assignment of biomaterials at biosafety level 2 was not required to report to the central competent authority to request for

recognition, the conclusions reached by the IBC were not forwarded to the Taiwan CDC. It would be very regretful if the laboratory personnel be infected due to the wrongly classification that make them conduct the experiments under an inappropriate environment and operation requirements. Secondly, the IBC did not fulfill their missions and perform their function effectively. Some medical colleges did not establish an IBC. As a result, any applications related to infectious biomaterials from the laboratory of the medical college were entrusted to the IBC operated by the hospital of the medical college for reviewing. Another problem was that the mission and function of the IBC operated by the hospital did not include the supervision and management of biosafety related matters from the laboratory of medical college. In other words, whether there are two or one IBC, the missions and functions of the IBC should cover matters of biosafety occurred in both medical college and hospital of the medical college. Thirdly, the requirements and regulations associated with biosafety management were not effectively implemented. The coordinators of study program in some institutes were served by their Director Generals that were staying in a position rank higher than members of the IBC. Therefore, it seems to be very difficult for the IBC to ask the coordinators to follow the requirements related to the infectious biomaterial management. To solve this problem, the members of the IBC should keep themselves updating the latest laws and regulations, have enough academic knowledge in biosafety, and be equipped with the abilities to communicate and coordinate with other units, so that they can provide academic consultation to laboratory personnel and fulfill their supervisory responsibilities. The IBC should not just be a rubber stamp. On the contrary, it should act in a manner of actively conducting follow-up and management on matters pertaining to biosafety, and should preside over the effective operation of biosafety management of the institute. Therefore, the IBC should not have “evasive mentality” that requires themselves to meet only the minimum requirements of the regulations.

In order to intensify academic ability of laboratory personnel on biosafety issues, the Taiwan CDC has successively developed digital courses on the topics of laboratory biosafety since 2010. Members of the IBC could get access to these online courses at all times during the off-hours period to learn more about biosafety knowledge so that they will be capable of shouldering their own responsibilities for safeguarding safety of laboratory workers, early identifying possible source of health hazards, preventing occurrence of laboratory infection incident, and thoroughly fulfilling the essence of self-management.

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