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Original Article

Effectiveness Analysis on Pilot Program for HIV Screening Test and Consultation Service for Patients Visiting the Emergency Rooms in Taiwan

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Abstract

In order to understand whether it is appropriate to extend HIV screening program to the sites of emergency rooms, the Taiwan CDC conducted a Pilot Program for HIV Screening Test and Consultation Service for Patients Visiting the Emergency Rooms at five hospitals, including the National Cheng Kung University Hospital, Chi Mei Hospital, Mackay Memorial Hospital in HsinChu, Kaohsiung Veterans General Hospital, and TaoYuan General Hospital of the Department of Health, during September 1, 2010 - December 31, 2010. A total of 8,646 patients visiting the emergency rooms agreed to participate in the screening program, and three new HIV-positive cases were identified, with a positive rate of 0.035%, which is equivalent to that from screening test for general population. The analysis shows that the effectiveness of screening test conducted at emergency rooms in Taiwan was lower than those documented in literatures. Therefore, a routine HIV screening test at hospital emergency rooms in Taiwan is not cost-effective.

Keywords: emergency room, HIV screening

Introduction

Since the first case infected with human immunodeficiency virus (HIV) was identified in Taiwan in 1984, the cumulative number of reported HIV infection cases with the nationality of the Republic of China has reached to 20,057 on December 31, 2010. Normally, the number of the reported cases represents only the tip of the iceberg of the number of actually infected cases. It is estimated that the real number of the cases infected with AIDS virus was about 1.5 times the number of the reported cases, which means that around ten thousand people may still not know that they have infected with HIV.

Under the current healthcare system in the United States, a very large proportion of patients visiting the hospital emergency room are those who live in a lower socioeconomic status and do not routinely have access to the healthcare resources. Therefore, the emergency rooms have become the only channel that they can obtain healthcare services and the only opportunity that the health care team can contact them [1-2]. The study conducted in the USA also indicated that the average HIV-positive rate among patients receiving screening test at the busy emergency rooms is around 1.0% [3] that is relatively higher than that among other populations. As long as the patients could be actually aware of the results of the screening test and received subsequent medical therapy, the screening test would be a cost-effective working mode [4-5]. Since the HIV screening tests were not routinely implemented in hospital emergency room in Taiwan, we conducted a pilot program by calling for five hospitals from the designated HIV/AIDS hospitals to participate in the study on a voluntary basis, for the objective of understanding whether it is appropriate to extend the HIV screening program to sites of emergency rooms. In this study, a simple, rapid HIV screening test was employed so that the time period waiting for the test results could be shortened and the patients visiting the emergency room could obtain the test results quickly on site at the emergency room. Following the test, the patients with positive results will be provided with consultation and referred for further therapy. Through the pilot program, we evaluate the feasibility of routinely implementing the HIV screening test at the emergency room in Taiwan.

Materials and Methods

The study projects were procured through open tender process, which the designated HIV/AIDS hospitals that the monthly average number of patients visiting the emergency rooms reached 3,500 or more in 2010 were invited to submit projects for panel evaluation. As a result, five hospitals were chosen as the members for implementation of the study, including the National Cheng Kung University Hospital, Chi Mei Hospital, Mackay Memorial Hospital in HsinChu, Kaohsiung Veterans General Hospital, and TaoYuan General Hospital of the Department of Health.

A. Study period: September 1, 2010 to December 31, 2010.

B. Target population of the study: Patients ages 20 to 55 years old visiting the emergency room of the designated hospitals participating in the study.

C. Qualification of staff implementing the study: having experience in providing HIV screening consultation or having completed at least five hours of training courses on how to provide patients with consultations before and after they receiving HIV screening tests.

D. Implementation methods

1. Before screening test: For patients meeting the criteria for screening test, study staff will provide them with explanations about the details of the study project and asked them to sign a consent form to participate in the study. Then, those who agreed to join the study

will have blood samples taken from finger tips for rapid HIV screening, and those who did not agree will be asked about the reasons of rejection.

2. After screening test: For patients with a positive result from the rapid screening test, the study staff will give them an illustration about the meaning of the results and collect venous blood samples for further screening test by enzyme-linked immunoassay or particle-agglutination method (simply referred to as ELISA/PA test hereafter). Patients with positive ELISA/PA test results will undergo Western Blot test (WB) for confirmation of HIV infection. At the same time, the study staff will assist the patients to make an appointment for medical services at infectious disease department. For patients with a negative rapid test result, if they have high-risk behaviors for HIV transmission, they will be educated about the methods for AIDS prevention, and be advised to receive additional screening test after an interval of a half year and avoid donating blood or occurring risky sexual behaviors during this period. The working procedures for screening test are shown in Figure.

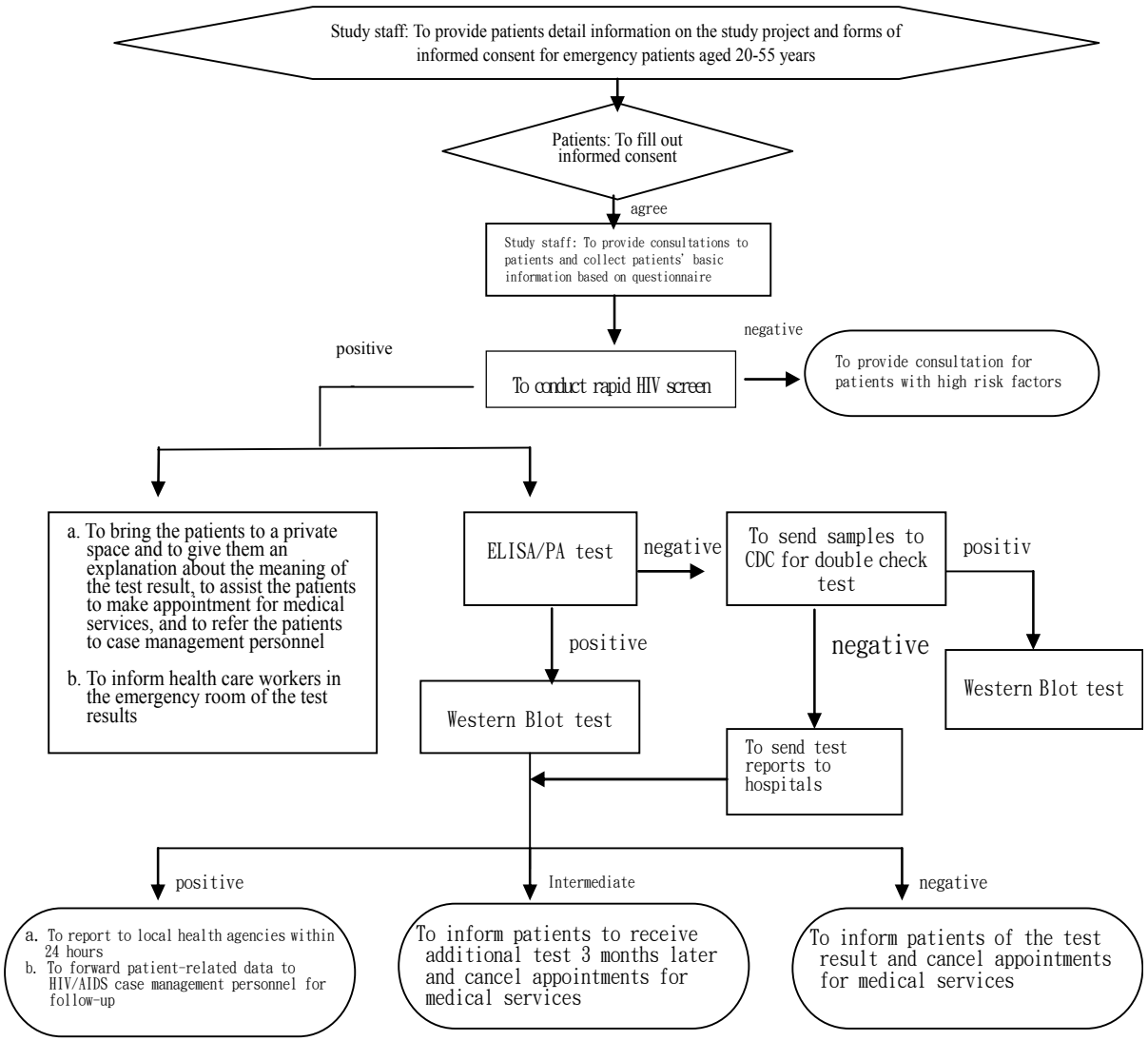

Figure 1. Flowchart for providing consultations and HIV screening tests to patients visiting the emergency rooms.

Results

During the study period, a total of 27,609 person-times ages 20-55 years old have visited the emergency rooms, and 13,319 person-times, accounting for 47.6%, have been asked to answer the questions about whether they agree to participate in the study project. As a result, 8,697 person-times, accounting for 66.2%, agreed to receive rapid HIV screening test. After eliminating duplicate records for the same patient, the actual number of patients receiving screening test was 8,646. Table 1 shows the analysis on basic information of patients receiving screening test. The analysis shows that the patients participating in the study were evenly distributed among the ation of patients participating in rapid HIV screening testage groups and the number of males was slightly higher than that of females, the group of married patients had the highest percentage (around 39.4%) among marital status groups, and patients visiting for internal medicine services recorded the highest percentage, 52.2%.

The distribution of diagnoses made at the emergency rooms for patients participating in the screening test program is shown in Table 2. The diagnosis of "other symptoms involving abdomen and pelvis" has the highest number of patients, 1,391 (18.1%) persons, followed by the diagnosis of "general symptoms," 1,269 (16.5%) persons.

Table 1. Analysis on basic inform of patients participating in rapid HIV screening test

Items	No. of patients (n=8,646)	Percentage (%)	Items	No. of patients (n=8,646)	Percentage (%)
Age (years)			Marital status		
20-24	1213	14	Married	3411	39.4
25-29	1317	15.2	Unmarried	2250	26.0
30-34	1360	15.7	Widowed	26	0.3
35-39	1061	12.3	Divorced	190	2.2
40-44	1108	12.8	Unknown	2769	32.1
45-49	1168	13.5			
50-55	1419	16.4	Clinics for emergency medicine services		
Sex			Internal	4514	52.2
Female	4094	47.4	Surgery	1074	12.4
Male	4552	52.6	Others	320	3.7
Having health insurance			Unanswered*	2738	31.7
No	63	0.7	*The emergency units visited by the patients were not split into different clinics.		
Yes	8583	99.3			

Table 2. Distribution of patients participating in the screen test program, by diseases of being diagnosed

Diagnosis	No. of patients	Percentage (%)
Other symptoms involving abdomen and pelvis	1391	18.1
General symptoms	1269	16.5
Symptoms involving respiratory system and other chest symptoms	402	5.2
Other noninfectious gastroenteritis and colitis	312	4.1
Other cellulitis and abscess	224	2.9
Symptoms involving head and neck	217	2.8
Other disorders of urethra and urinary tract	190	2.5
Gastrointestinal hemorrhage	169	2.2
Acute upper respiratory infections of multiple or unspecified site	144	1.9
Injury, other and unspecified	135	1.8

Among the patients agreeing to receive rapid screening test, 18 (0.2%) were positive. Two of the eighteen patients did not receive further testing, including one patient who expressed at the site of emergency room that he has previously been diagnosed as HIV positive and another patient (a foreign spouse) who refused to undergo further testing. Six (37.5%) of the sixteen patients receiving ELISA/PA tests were positive. Of the six patients, one refused to receive further screening test and the rest five were confirmed to be HIV infection by using WB test. The five HIV positive patients were reported by the Chi Mei Hospital, Mackay Memorial Hospital in HsinChu, Kaohsiung Veterans General Hospital, and TaoYuan General Hospital of the Department of Health. Table 3 shows the analysis on basic information of the five HIV positive patients. Four of the five patients were male and one was female, and the reasons for visiting the emergency room were abdominal pain in four patients and herpes zoster in one patient. Cross-checking of data obtained from the five HIV positive patients with those maintained in the databank of HIV notification system found that two of them were the previously reported cases. The newly identified HIV positive cases were referred to infectious disease departments at designated HIV/AIDS hospitals. A further investigation about why the two previously reported HIV positive cases have again received the HIV screening test found that because they were accompanied by their families when visiting the emergency rooms and they were worried that their rejection to the screening test may be stirring skepticism from families and others, they continuously complete the ELISA/PA and WB test following the positive results of the rapid HIV screening test

Over all, a total of ten full-time study staff and additional part-time staff working for 917.5 hours have been hired by the five hospitals participating in the study projects and an amount of 3,498,684 New Taiwan Dollars (TWD) has been spent for payments of the study staff, general and administrative expenses, and reagents for rapid screening test. In this study, five of the 8,646 patients agreeing to receive screening test were confirmed to be HIV positive, with a positive rate of 0.057%. The positive rate was 0.035% while it was calculated by using the three newly identified HIV positive cases as the numerator. An average of 699,729 TWD has been spent for screening out a HIV-infected case.

Table 3. Analysis on data of confirmed cases of HIV infection

No.	Age (years)	Sex	Marital status	Symptoms	Diagnosis	Risk factors	Remark
1	55	F	Divorced	Skin blisters	Herpes zoster	Heterosexual behaviors	New case
2	45	M	Unmarried	Abdominal pain	Other symptoms involving abdomen and pelvis	Unspecified	New case
3	31	M	Unmarried	Abdominal pain	Other and unspecified noninfectious gastroenteritis and colitis	MSM behaviors	New case
4	33	M	Married	Abdominal pain	Other symptoms involving abdomen and pelvis	Injecting drug user	Old case reported in June 2010
5	53	M	Married	Upper abdominal pain	Peritoneal adhesions	Heterosexual behaviors	Old case reported in August 2008

In this study, although the patients who did not agree to receive rapid screening test were not asked for providing their basic information, they were requested to answer questions about the reasons for refusal. The analysis on reasons for refusing rapid screening tests was shown in Table 4. There were 4,623 person-times (33.0%) refusing to receive rapid screening test. The reason of "considering themselves without risky sexual behavior, impossible to get infected" recorded the highest number of person-times, 2,008 (43.4%), followed by the reason of "having received screening test within the past three months," 495 (10.7%).

Discussions and limitations

In this study, the number of patients agreeing to receive HIV screening test accounted for 66.2 percent of the patients, ages 20-55 years old, visiting the emergency rooms of five designated HIV/AIDS hospitals during a three-month period. This outcome shows that people in Taiwan have currently had a higher level of reception in the participation of HIV screening test than before. However, owing to the health care manpower and the environmental factors in the emergency rooms, about only half of the patients visiting the emergency rooms have been given the message for the screening test. Therefore, we were unable to know whether the patients who were not asked for providing basic information had different distributions of basic information. Similarly, since the majority of the patients agreeing to receive screening test were those of patients ages 50-55 years old and married male, and the study only collected basic information from these patients, we were unable to compare the difference in distribution of basic information between the patients agreeing and disagreeing to participate in the screening test. Nevertheless, the facts that most of the patients refusing for screening test considered themselves to be at low risk of infection because they did not have risky sexual behaviors and most of them were married and had only a single sexual partner reveals that people in this

Table 4. Analysis on reasons for refusing to receive rapid screening tests

	No. of person-times (N=4,623)	Percentage (%)
Considering themselves without risky sexual behavior so impossible to get infected	2008	43.4
Having received screening test within the past three months	495	10.7
Waiting too long for undergoing screening test	352	7.6
Not being in a good body condition and just wanting to take a rest	257	5. 6
Rejection and objection from families, uncommunicated with families, fear of the result of AIDS screening test, etc.	179	3.9
Having ever been tested for HIV infection	116	2.5
For fear of finger prick, refusing for finger prick	74	1.6
Not to understand procedures of screening test	61	1.3
Space for screening test not private enough	46	1
For fear of knowing the results of screening test	36	0.8
Having been a HIV infection case	36	0.8
For being hurry up their time, discharged of moved to ward, waiting for surgery not wanting to undergo screening test	32	0.7
Unmarried, without having sexual behaviors, or having only a single sexual partner	14	0.3
For unfriendly manner of the health care workers	1	<0.1
Not to give the reason for refusal	916	19.8

country probably considered that HIV infections only occurred in some specific groups and there is nothing to do with them. The study found that patients with heterosexual behaviors had a higher percentage of delayed diagnosis. This is probably because most of the patients in this population did not consider themselves at high risk of HIV infection and, therefore, neglected to undergo the screening test. Thus, the HIV/AIDS health education for general population should be improved.

Moreover, only three new cases infected with HIV were identified by the screening test, with a positive rate of 0.035%, which was equivalent to that obtained from HIV screening test for general population (for example, the positive rate of screening test conducted by the Taiwan CDC for general population in 2010 was 0.02%) but was far lower than 2% that from anonymous screening test of high risk population. This might be because that more than 99.3% of Taiwanese citizens were covered by the National Health Insurance system and can easily have access to over 400 hospitals nationwide for health care services; they usually visited private clinics or clinics at hospital instead of emergency rooms for diagnosis and treatments when getting sick. Therefore, the use of medical resources at the hospital emergency rooms in Taiwan was largely different from that in other countries (such as the United States) where citizens have to pay total amount of the medical charges by their own. This may influence the accessibility to health care services for people in weak economy in these countries. Very often they just visited the emergency rooms at hospitals once they were getting sick or suffering from serious symptoms. It was not easy for public health workers and health care workers to contact these people. Therefore, the emergency rooms probably were the only sites where people have the chance to be asked for HIV screening test, so that a higher HIV-positive rate was found among these people. Take the United States as an example, the positive rate of HIV screening test conducted for people visiting the emergency rooms in the three states during January 2005--March 2006 reached 1% [2]. From the cost-effectiveness point of view, the average expenses for screening out one HIV-positive case were 699,729 TWD. In contrast, the average cost for identifying one HIV-positive case was 35,964 TWD in the anonymous screening program conducted in 2011, which 531 of the 24,711 people receiving screening test were positive, with a positive rate of 2.2%, and the total amount of the screening test was 19,097,182 TWD. Apparently, routine HIV screening test at hospital emergency rooms in Taiwan was not cost-effective. In addition, since patients visiting emergency rooms were urgently getting relief from the uncomfortable condition, it was more difficult for health care workers to provide complete consultations to participants before and after screening tests, as compared with other screening program. This was another limitation that should also be considered when HIV screening test was conducted in the emergency rooms.

In addition, the study found that the majority of the patients visiting the emergency rooms for reasons of “other symptoms involving abdomen and pelvis” and “general symptoms” but no patients for reason of sex-related diseases. These findings were obviously

different from those in the U.S. and these were probably the reason why the HIV positive rate of screening test at emergency rooms in Taiwan was lower than that in the U.S.

The major limitations of the study were that the study population, patients ages 20-55 years old, mostly visited the emergency rooms during the night time period. However, the study project did not allocate extra budgets to hire extra workers for the time period. The subject enrollments and screening tests were completed by health care workers at the emergency rooms by taking a break from their works, leading that the number of subjects enrolled was relatively low. Moreover, since most patients visiting the emergency rooms just wanted to quickly relieve uncomfortable symptoms, they would tend to refuse the screening tests if they were not asked for participation in the study project at the appropriate time.

Conclusions

A higher positive rate of HIV screening tests was found among people visiting the emergency rooms in other counties than in Taiwan. The reason might be that more than 99.3% National Health Insurance (NHI) coverage to Taiwanese citizens and over 400 hospitals having a contract with NHI have provided people with high convenience and easy access to health care services, but citizens in other countries have to pay total amount of the medical charges by their own, which may influence the people's access to health care services, so that they usually did not go to clinics and just visited the emergency rooms at hospitals once they were getting sick or suffering from serious symptoms. Moreover, the study found that no patients were visiting the emergency rooms for reasons of sex-related diseases. These findings were obviously different from those in the U.S. and these were probably the reason why the HIV positive rate of screening test at emergency rooms in Taiwan was lower than that in the U.S. In addition, a higher average cost was needed to identify a positive HIV-infected case from screening test of patients visiting emergency rooms, as compared with that from other screening program (such as anonymous screening test) for population at high risk of HIV infections. Therefore, routine HIV screening test at hospital emergency rooms in Taiwan was less cost-effective.

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Surveillance of Gonococci-National Isolate Collection for Epidemiology (G-NICE), 2010

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Abstract

The surveillance program of Gonococci-National Isolate Collection for Epidemiology 2010 (G-NICE 2010) was implemented during January to December, 2010, enrolling 21 hospitals and clinics. The program collected 644 gonococcal isolates nationwide. Using disk diffusion test, the proportions of isolates resistant to penicillin, ciprofloxacin, cefpodoxime, cefixime, and ceftriaxone were 70.34%, 89.60%, 2.95%, 3.73%, and 1.24%, respectively. 4.35% and 0.78% of isolates showed reduced susceptibility to cefixime (≥0.125 mg/L) and ceftriaxone (≥0.125 mg/L). The highest Minimum Inhibitory Concentration (MIC) of cefixime and ceftriaxone were 0.38 mg/L and 0.125 mg/L, respectively. The 644 isolates were delineated into 208 sequence types (STs) by *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) method. Among the 208 STs, 13 had more than 6 isolates collected, including ST421 (n = 82), ST359 (n = 35), ST419(n = 32), ST3684 (n = 28), ST225 (n = 23), ST2175 (n = 17), ST1971 (n = 13), ST3680 (n = 13), ST4378 (n = 13), ST2178 (n = 11), ST1614 (n = 9), ST4352 (n = 8), and ST4376 (n = 6). Among them, ST359, ST3684, ST3680, ST4378, and ST1614 are new emerging STs. ST4378 was resistant to ciprofloxacin, penicillin and cefpodoxime and was only susceptible to cefixime and ceftriaxone. The ST4378 is phylogenetically closely related to ST1407, a successful clone causing cephalosporin treatment failure in many countries.Based on the G-NICE 2010 surveillance, the gonococcal isolates in Taiwan showed high resistance rate to penicillin and

ciprofloxacin. Regarding the 3rd generation of cephalosporin, the isolates with MIC ≥ 0.125 mg/L to oral cefixime remained under 5%. Isolates with reduced susceptibility to cephalosporin were mostly multidrug-resistant and from high-risk sexual networks. People should be alerted that contact with high-risk sexual networks will not only increase the risk of infecting with HIV and other sexually-transmitted diseases but also bear higher possibility of contracting "super drug" gonorrhea.

Keywords: antimicrobial susceptibility testing, cefixime, ceftriaxone, *Neisseria gonorrhoeae*, *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST)

Introduction

Gonorrhea caused by *Neisseria gonorrhoeae* is the second most prevalent sexually transmitted infections worldwide. Recently, in many countries the relative increases of gonorrhea were greatest among men who have sex with men (MSM) and young people [1]. The World Health Organization (WHO) has estimated that 62 million people worldwide become infected with gonorrhea each year [2]. In Taiwan, the number of reported cases was 2,265 in 2010, indicating an annual incidence of 9.79 per 100,000 persons which is higher than that in 2009 (9.26) and is ranked the highest incidence since 2006 [3].

Currently, no vaccine is available for preventing gonorrhea and antibiotic therapy remains the mainstay for treating gonococcal infections. However, over the last decades, *N. gonorrhoeae* strains developed high levels of resistance to several antimicrobial agents such as penicillin, tetracycline, and quinolone [4]. Now the 3rd cephalosporins [e.g., cefixime (oral) and ceftriaxone (injectable)] are the first-line drugs recommended for treatment of gonorrhea infections in many countries. Unfortunately, *N. gonorrhoeae* isolates with reduced susceptibility to cephalosporins, even treatment failure cases, have been increasingly reported [5]. The GISP (gonococcal isolate surveillance project) of United States CDC in 2010 estimated that 1.4% of isolates had cefixime MIC ≥0.25 mg/L [6]. The results of GRASP (Gonococcal Resistance to Antimicrobials Surveillance Program) of Health Protection Agency (HPA) in the United Kingdom in 2010 showed that 17.4% of isolates had cefixime MIC ≥0. 125 mg/L, and 6.3% of that had cefixime MIC ≥0.25 mg/L. Because the percentage of *N. gonorrhoeae* isolates with cefixime MIC ≥0.25 mg/L has exceeded 5%, the HPA has announced that cefixime is no longer the recommended first-line drug for treating gonorrhea infection [7]. In Taiwan, G-NICE program indicated that 3.47% and 0.96% of isolates had cefixime MICs ≥0.125 mg/L and 0.25 mg/L respectively in 2009 [8].

G-NICE 2010 surveillance program collected 644 gonococcal isolates and determined their resistance pattern to understand the resistance trend in Taiwan. The gender, age group and geographic distribution of the isolates were analyzed to better understand the epidemiological characteristics of gonorrhea in Taiwan. The isolates were subtyped by *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST). Sexual networks were delineated

by integrating resistance patterns with sequence types and epidemiological information. These results are valuable to understand the introduction/emergence and dynamic transmission of gonococci among high-risk sexual networks domestically as well as internationally.

Materials and methods

A. Collection of isolates

In this G-NICE study, 644 gonococcal isolates were collected from 21 hospitals and clinics between January and December 2010. All hospitals and clinics participating in G-NICE submitted the isolates together with a questionnaire to Research and Diagnostic Center, Taiwan CDC. All bacterial isolates were subcultured and stored under -80℃ for further experiments. .

B. 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 System). Antimicrobial susceptibility of *N. gonorrhoeae* isolates to penicillin, ciprofloxacin, cefpodoxime, cefixime, and ceftriaxone were analyzed using disk diffusion method. Isolates exhibited resistance to cefixime and ceftriaxone by disc assay were further evaluated for their minimum inhibitory concentration (MIC) using the E-test method. Drug susceptibility was determined by the size of inhibition zone to each antibiotic using BIOMIC® V3 (Giles Scientific, Santa Barbara, California, USA). The interpretation followed the Clinical and Laboratory Standards Institute (CLSI) guidelines for *N. gonorrhoeae* in 2010 (M100-S20, 2010) [9]. Isolates with MIC ≤0.25 mg/L to ceftriaxone and cefixime were considered to be susceptible (S).

C. Extraction of gonococcus DNA

DNA was extracted by using MasterPure™ Yeast DNA Purification Kit (EPICENTRE Biotechnologies, Madison, Wisconsin, 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 fully suspend and dissolve the cells. After additional 150µl Protein Precipitation Solution was added, the mixture was vortexed for 10 seconds and centrifugation under 12000xg 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. A spectrophotometer was used to examine the quantity of DNA under –80℃, and all extracted DNA was stored under –20℃ for further studies.

D.*Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST)

The NG-MAST molecular typing of gonococcal isolates was conducted by sequencing of internal fragments of 2 highly polymorphic loci, *porB* and *tbpB* [10]. The *porB* 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 *tbpB* 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'.

E. Comparing the DNA sequences of isolated gonococci and establishing a database

DNA sequences of *porB* gene and *tbpB* gene were analyzed using the BioNumerics 6.5 software. The sequence data of each *porB* and *tbpB* gene was uploaded onto the database of the NG-MAST website (http://www.ng-mast.net) to obtain the allele numbers and the sequence types (ST).

Results

A. Distribution and epidemiological characteristics of gonococcal isolates

In the 2010 G-NICE study, 644 isolates were collected from 21 hospitals and clinics. More isolates were from northern Taiwan (n = 563). Fewer were from southern Taiwan (n = 68), central Taiwan (n = 12) and eastern Taiwan (n = 1), while none was from off-shore islands (Figure 1). Among the 644 isolates, 560 isolates were from male patients while 72 isolates were from female patients and gender for 12 isolates was unknown. The male to female ratio was 7.8. The range of patient age was between 16 and 84. Among them, 23.6% of the male patients were 25-29 years old; 31.9% of the female patients were 20-24 years old, constituting the most prevalent age groups. The second and third most prevalent age groups were somewhat different by genders. In male, 21.6% were in 30-34 years old and 19.6% were in 20-24 years old. While in female, 19.4% were in 25-29 years old and 15.3% were in 15-19 years old (Table 1).

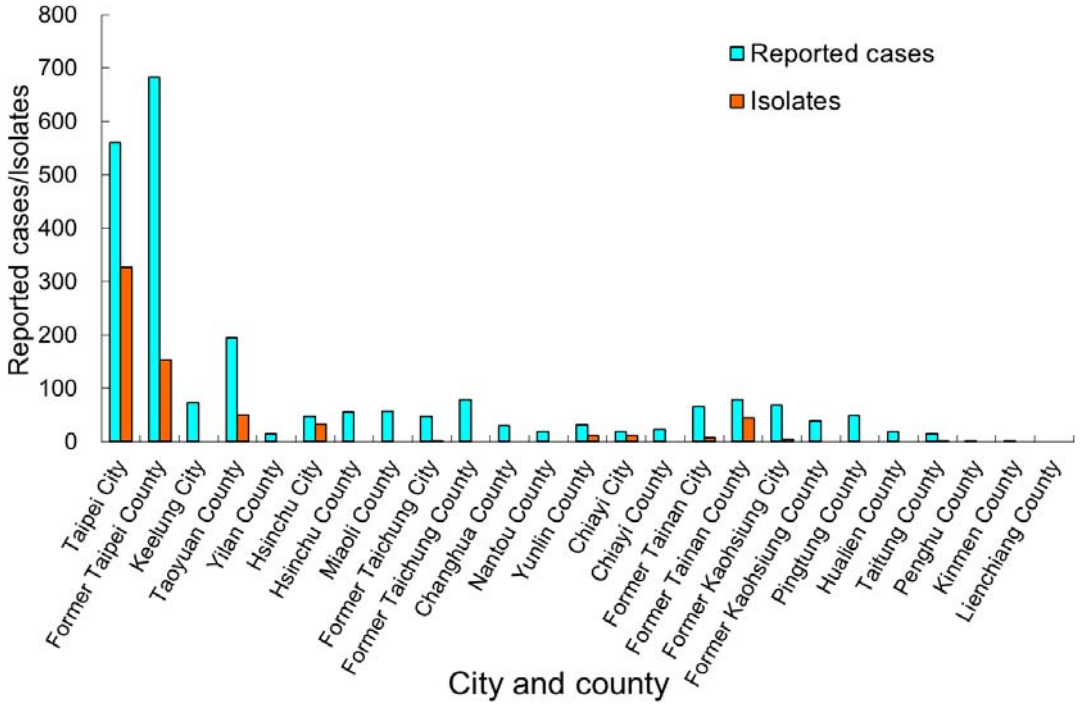


Figure 1. Geographic distribution of the numbers of reported cases and bacterial isolates, 2010, Taiwan

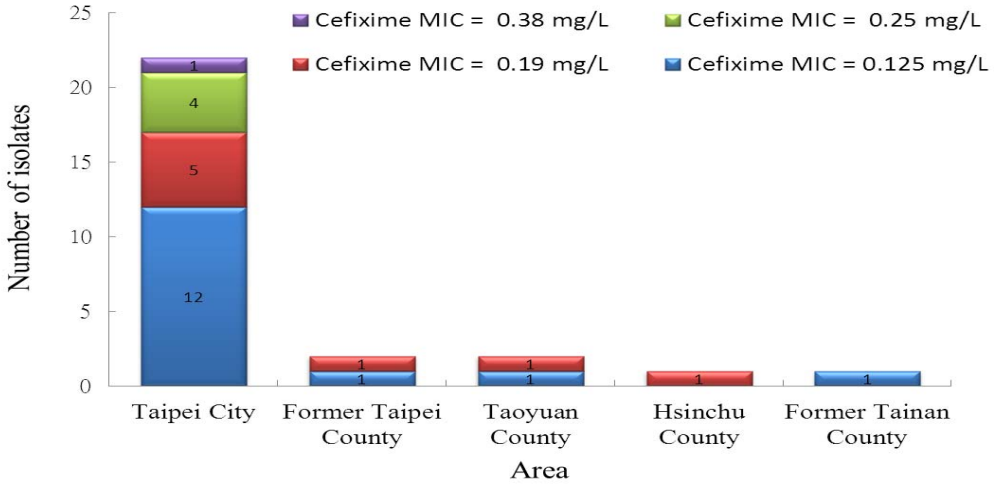
B. Results of drug susceptibility tests and geographical distribution of resistant isolates

Using disc diffusion assay, the resistance rate for penicillin, ciprofloxacin, cefpodoxime, cefixime, and ceftriaxone of the 644 bacterial isolates was 70.34%, 89.60%, 2.95%, 3.73%, and 1.24%, respectively. The results of E-test of ceftriaxone range was 0.012-0.125 mg/L, with only one isolate exhibited MIC = 0.125 mg/L, the rest of all tested isolates was less than 0.125mg/L. For cefixime (range 0.125-0.38 mg/L), 28 isolates (4.35%) showed decreased susceptibility with MICs ≥0.125 mg/L, with 4 had MICs 0.25 mg/L and 1 had MIC 0.38 mg/L. The MIC_{50} of cefixime and ceftriaxone were 0.125 mg/L and 0.032 mg/L, respectively. The MIC_{90} of cefixime was 0.19 mg/L, and that of ceftriaxone was 0.064 mg/L.

Only 1 strain with reduced susceptibility to ceftriaxone (MIC ≥0.125 mg/L) was isolated and it is from the former Taipei County (data not shown). Relatively, the strains with reduced susceptibility to cefixime (MIC ≥0.125 mg/L) spread in several areas, including Taipei City (22 isolates, 10 of them had cefixime MIC 0.19 to 0.38 mg/L), former Taipei County (2 isolates), Taoyuan County (2 isolates), Hsinchu County (1 isolate), and Tainan County (1 isolate) (Figure 2).

Table 1. Age distribution of patients

Age group	Male	%	Female	%
< 15	0	0.0%	0	0.0%
15-19	12	2.1%	11	15.3%
20-24	110	19.6%	23	31.9%
25-29	132	23.6%	14	19.4%
30-34	121	21.6%	10	13.9%
35-39	69	12.3%	3	4.2%
40-44	37	6.6%	4	5.6%
45-49	21	3.8%	0	0.0%
50-54	18	3.2%	4	5.6%
55-59	15	2.7%	2	2.8%
60-64	4	0.7%	0	0.0%
≥ 65	8	1.4%	0	0.0%
Unknown	13	2.3%	1	1.4%
Summation	560		72	
Unknown	12			
Total	644			



Figur 2. Geographical distribution of *N. gonorrhoeae* isolates with reduced susceptibility to cefixime

C. NG-MAST sequence types of isolates

Among the 644 isolates collected in 2010, 208 STs were identified. 133 of the 208 STs had only one isolate, while the other 75 STs had 2 to 82 isolates. Thirteen STs (ST421, ST359, ST419, ST3684, ST225, ST2175, ST1971, ST3680, ST4378, ST2178, ST1614, ST4352, and ST4376) had more than 6 isolates. The three most prevalent STs, i.e., ST421, ST359, and ST225, had more than 32 isolates. ST359 (from 6 isolates in 2009 to 35 in 2010), ST3684 (from 12 to 28), ST3680 (from 3 to 13), ST4378 (from 0 to 13) and ST1614 (from 2 to 9) increased significantly in 2010 compared to 2009 and became major STs. All isolates of ST359, ST3684, ST3680, ST4378, ST1614, ST4352, and ST4376 were collected from male patients. The male to female ratio of ST2175 was 7:1, which were higher than the ratio of ST1971, ST419, ST225, and ST421 (ranged from 3.3:1 to 5.2:1) and ST2178 (0.7:1) (Figure 3).

D. Analysis of the antibiotic susceptibility patterns of drug-resistant gonococci

In this study, gonococcal isolates were classified into 6 resistant patterns (Type 1 to Type 6) according to their susceptibility by disc diffusion assay to penicillin, ciprofloxacin, cefpodoxime, cefixime, and ceftriaxone (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 resistant to ciprofloxacin and intermediate or resistant to penicillin. All isolates of ST2178, ST4352, and ST4376 were type 3. All of the isolates of ST2175 were type 3, except one being type 2. In ST419, ST225, and ST1971, all isolates were type 3 with only 1 to 3 isolates being type 4. As for isolates of ST421, ST359, and ST3984, both type 3 and type 4 patterns could be found. Notably, isolates of ST3680 (Type 1 and Type 2) and ST4378 (Type 2) had higher resistance than that of the other STs.

According to our previous surveillance, isolates of ST421, ST419, ST3684, ST225, ST2175, and ST2178 were mainly from heterosexual male patients, while the isolates of ST359, ST1971, ST3680, ST4378, ST1614, ST4352, and ST4376 were from homosexual male patients [11]. Distinct 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 (e.g., Type 1 or Type 2 in ST3680; Type 2 and Type 3 in ST4378) or low resistance (e.g., 89% of ST359 isolates were type 4).

Discussion

G-NICE program collected 644 isolates in 2010 (19% higher than the 519 isolates in 2009). The proportion of isolates to reported cases was 28.4% (644 isolates/2265 cases), and was 4.1% higher than 24.3% (519/2137) in 2009. These results may reflect increased transmission of gonorrhea in Taiwan. On the other hand, this may reflect that more patients are willing to take medical treatment and more clinician to report cases to TCDC.

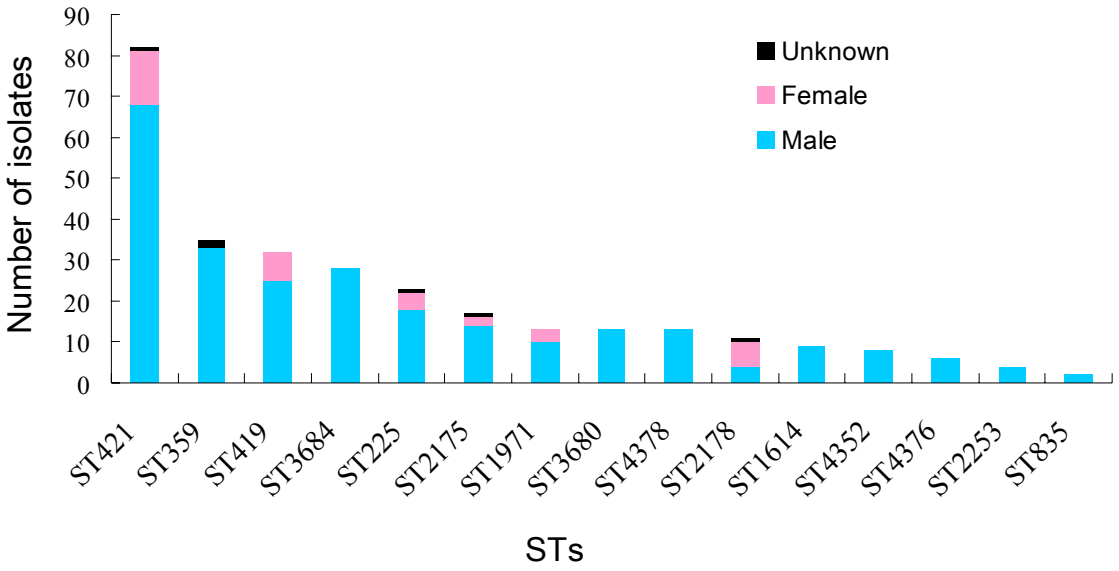


Figure 3. Gender distribution of different major sequence types

Table 2. Antibiotic susceptibility patterns

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

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

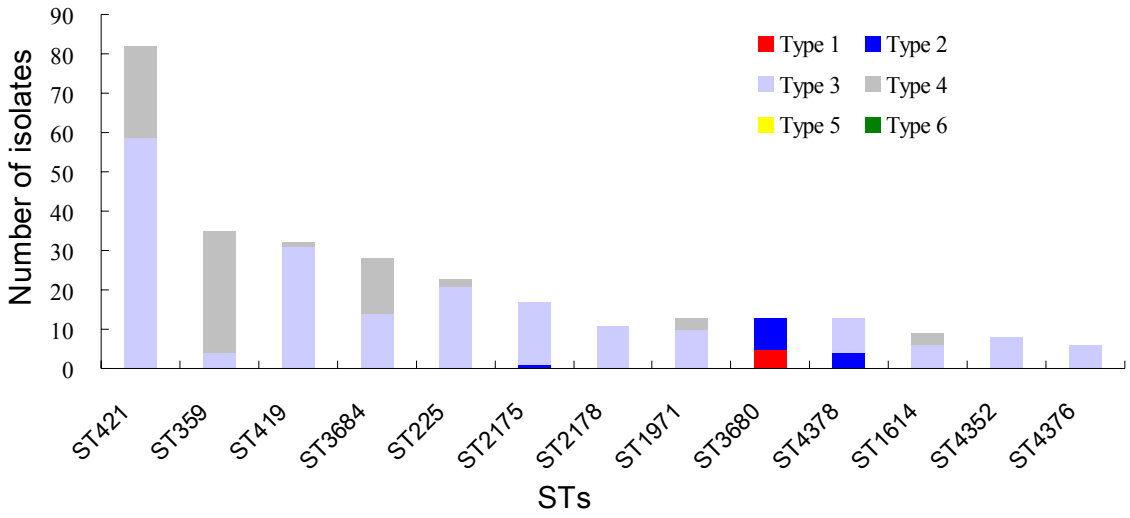


Figure 4. Patterns of antibiotics resistance in different major sequence types

The male to female ratio was 7.8:1, higher than in 2009 (6.8:1). The incidence of gonorrhea per 100,000 people was 9.8. The reason for high male to female ratio may be due to either increased transmissions among men who have sex with men (MSM) or more asymptomatic infections and less motivation to seek medical care of female patients.

According to the analysis of age and gender distribution, 64.8% of male patients were in 20-34 years old group, and 66.6% of female patients were in 15-29 years old group, with the 20-24 years old being the most prevalent group, constituting 31.9% (9.2% more than that in 2009). Also, female patients increased by 1.7% in 15-19 years old group compared to 2009; oppositely, 6.1% decrease was found in 25-29 years old group of female. The increasing of female cases in younger age groups warrants further attention and investigation.

The results of disk diffusion tests showed that high proportion of *N. gonorrhoeae* isolates had resistance to penicillin (3.6% higher than that in 2009) and ciprofloxacin in Taiwan. For 3rd generation cephalosporins, only the isolates with resistance to cefixime increased slightly by 0.6%, the isolates with resistance to both cefpodoxime (by 2.2%) and ceftriaxone decreased. According to the standards from CLSI 2010, isolates with cefixime and ceftriaxone MICs ≤0.25 mg/L were considered as susceptible. In G-NICE 2010 program, we identified only 1 isolate with MIC 0.125 mg/L to ceftriaxone, and no isolate with ceftriaxone MIC ≥0.25 mg/L. 28 isolates (4.35%) had cefixime MIC ≥0.125 mg/L, and 5 (0.78%) of them were ≥0.25 mg/L. The maximum MICs of cefixime and ceftriaxone remained the same as in 2009, 0.125 mg/L and 0.38 mg/L, respectively. But the MIC_{50} and MIC_{90} of cefixime and ceftriaxone were both higher than that in 2009. For clinical treatment, we still recommended cefixime (oral) and ceftriaxone (injectable) for treatment of gonococcal infections. However, gonococcal resistant or reduced susceptible to third-generation cephalosporins has been increasingly reported. Recently, after the first high-resistant strain H041 (cefixime MIC = 8 mg/L, ceftriaxone MIC = 2 mg/L) was reported in Japan, another *N. gonorrhoeae* isolate, F89, displayed highly resistant to cefixime (4 mg/L) and ceftriaxone (1-2 mg/L) was isolated in France [12]. The threat has become imminent that gonococci may become superbug and usher in era of untreatable gonorrhea. Briefly, susceptibility to 3rd generation cephalosporins has decreased in Taiwan, a public health response plan and continuous and enhanced surveillance program with national and global perspectives are essential.

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 a specific antibiotic susceptibility pattern. This finding is helpful to trace the spread of antibiotic-resistant strains. In Taiwan, ST359 and ST2253 are the dominant STs found in MSM. These two 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 STs 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 patient 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.

Compared with NG-MAST data of G-NICE 2009; ST359, ST3684, ST3680, ST4378 and ST1614 increased substantially. Isolates of ST3684 are mainly from heterosexual patients. ST3684 differs from ST421, the most predominant heterosexual ST in Taiwan, by only one nucleotide in *porB* gene (from glycine to threonine). Both ST3684 and ST421 share similar resistance pattern. It is very probably that ST3684 have evolved from ST421. The rest of the newly emerging clones such as ST359, ST3680, ST4378, and ST1614 are mostly from homo- or bi-sexual patients. ST359 showed marked increase especially during October to December and a total of 35 isolates were discovered in 2010. ST3680 intensively appeared during late February. ST3680 showed multiple resistant to penicillin and ciprofloxacin and 84% of the isolates were cefixime MIC ≥0.125 mg/L. Isolates of ST4378 also displayed multidrug resistance to penicillin and ciprofloxacin with some showed decreased susceptibility to cephalosporins. All of these newly emerged clones were with distinct resistance profile and showed sharp increased during short time period. It is postulated that these clones were introduced into Taiwan via MSM and circulate in high-risk sexual network thereafter. The striking increase of ST4378 deserves special attention. ST4378 is closely related to a global successful clone ST1407, recently increasingly reported to cause treatment failure. All of these reinforce the importance of global collaborative surveillance.

In conclusion, G-NICE 2010 surveillance showed the gonococcal isolates in Taiwan exhibited high resistance rates to penicillin and ciprofloxacin. For the third generation cephalosporins, resistance rates to cefpodoxime and ceftriaxone decreased and to cefixime leveled off or increased slightly. Rate of isolates with cefixime MIC ≥0.125 mg/L remained under 5%. Special note should be given regarding whether the newly emerging ST4378 will follow in the footstep of its close relative ST1407 to become strains that causing treatment failure. People should be warned against contacting with high-risk sexual network, which not only increase the possibility of infection with HIV and other sexually transmitted diseases but also increase the risk of contracting "Super bug" strains of gonorrhea.

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

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