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台灣地區腦膜炎雙球菌感染之流行病學和抗藥機轉之研究

研 究 報 告

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摘要:

台灣在 2001 年至今，腦膜炎雙球菌感染之病例有明顯增加。由現已完成之 43 株菌分析顯示，青黴菌之抗藥性有 3 株 (7%)，其最低抑菌濃度為 0.12 μg/mL。分子分型研究顯示血清型 W135，B 和 Y 型之擴散和 2001 年之群突發有關。和國外菌株分子分型之結果比較，台灣本土之菌株和國外之菌株是不同的。

中文關鍵詞(至少三個)：腦膜炎雙球菌，抗藥性，分子分型

Abstract

A remarkable increase in the number of cases of meningococcal disease (43 cases, 0.19/100,000) was noted in 2001 in Taiwan. Among the 43 preserved isolates of *Neisseria meningitidis* from 43 patients (41 in 2001 and one each in 1998 and 2000, respectively), three (7.0%) were resistant to penicillin (MICs ≥ 0.12 μg/mL). Wide dissemination of a limited number of domestic clones of *N. meningitides*, particularly serogroups W135, B, and Y contributed to the 2001 outbreak. The two clones of serogroup W135 involved in this outbreak were genetically different from the 2000 or 2001 Hajj-related W135 clone.

Key words: Meningococcal disease, reemergence, serogroup W135, outbreak, Taiwan

The incidence of meningococcal disease varies worldwide (1-3). In the United States, since the 1960s, the annual incidence rates of meningococcal disease has ranged from 0.9 to 1.5 cases per 100,000 population (1). In 1997, approximately 500,000 cases of meningococcal disease were reported worldwide[1]. A large outbreak that occurred in the African "Meningitis Belt" in 1996, involved more than 150,000 cases and about 10% of these victims died (4). Although there are at least 13 known serogroups, most reported sporadic cases or outbreaks of meningococcal disease are caused by serogroup A, B, or C (1). However, in recent years, some less commonly encountered serogroups, such as serogroups Y and W135, have emerged and were associated with several outbreaks in the United States and other countries (1, 5-8). Furthermore, international spread of serogroups A or W135 meningococcal disease associated with the Hajj have been reported (9, 10).

In Taiwan, meningococcal disease has been reported since the 1950s. From 1996 to 2000, 10 to 20 cases of meningococcal disease reported yearly to the Center for Disease Control (CDC, formerly the National Institute of Preventive Medicine) of Taiwan. Previous studies from different parts of Taiwan have shown a low prevalence of *Neisseria meningitidis* causing septicemia and meningitis in pediatric or adult patients (11, 12). Nasopharyngeal carriage rate of meningococcus among military recruits in 1974-1975 was reported to be 13.7% (13). However, an unusual increase in

the number of cases of meningococcal diseases was reported to the CDC of Taiwan during the period from January 2001 to December 2001.

This Study

From 1950 to December 2001, a total of 659 cases of meningococcal disease (meningitis and bacteremia) were reported to the Taiwan CDC. The annual incidence of the disease peaked in 1953 (78 patients, 0.94 per 100,000 population) and reached another very high level in 1959 (51 patients, 0.52 per 100,000 population) and then decreased dramatically in the following 30 years (Figure 1A). The disease nearly disappeared from 1975 to 1987 (only seven cases reported in 1975-1979 and no cases reported in 1980-87), reemerged beginning in 1988 and increased dramatically in 1997 (19 patients, 0.09 per 100,000 population). From January 2001 to December 2001, a dramatic increase in the number of cases of meningococcal disease (43 cases, 0.19/100,000) was noted. These 43 cases were dispersed in different parts of Taiwan (Figure 1B).

Relevant clinical information on the 128 patients with meningococcal disease (meningitis and bacteremia) found from January 1995 to December 2001 was evaluated. Serogroups data among the 128 isolates from these patients were obtained from CDC of Taiwan. Serogrouping of these isolates was performed with standard grouping sera for capsular types A, B, C, X, Y, Z, and W135 by the agglutination test

(Murex Biotech Ltd. Dartford, UK). The mean age of the 128 patients was 19.4 years: 43 (33.6%) aged from 11 to 30 years, 40 (31.3%) were aged below one year, and 67 (52.3%) were male. The disease occurred year round (except in August) and cases were concentrated from December to June. Among the 43 patients treated in 2001, the mean age was 19.4 years, 21 (48.9%) were male, nine (20.9%) were military recruits, and most (72.1%) developed disease during the period from February to June. The mortality was highest in 1999 (30.6%) and while none of the patients died in 1997 and 1998.

The majority of the 128 isolates belonged to serogroups B (48.4%) and W135 (35.9%). In 1999, serogroup B isolates accounted for 84.6% of all isolates (11 out of 13 isolates) but in 2001 this prevalence decreased remarkably (32.6%) while the prevalence of serogroup W135 (41.9%) increased and serogroup Y emerged (18.6%).

The overall mortality of the 43 patients seen in 2001 was 25.6%: 42.8% of patients infected with serogroup B isolates and 37.5% of patients with serogroup W135 isolates died. None of these 43 patients were associated with international travel or contact with travelers to Saudi Arabia during 2001 or attended the 2000 or 2001 Hajj or had any contact with the pilgrims returning from the 2000 or 2001 Hajj.

A total of 43 isolates recovered from 43 patients with meningococcal diseases were preserved for further study. Among these isolates, 41 were isolated from 41

patients seen during the period from January 2001 to December 2001, and one each was isolated from a patient seen in March 1998 and March 2000, respectively

Minimum inhibitory concentrations (MICs) of 14 antimicrobial agents were determined using the agar dilution method according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (14). *Streptococcus pneumoniae* ATCC 49619 was included as the control strain. The MIC breakpoints for susceptibility and resistance used for *S. pneumoniae* or *N. gonorrhoeae* were applied to *N. meningitides* (31). Production of β-lactamase was assayed by the Cefinase disk test (BBL Microbiology Systems). The polymorphism of *penA* gene was analyzed by investigating restriction endonuclease patterns for amplified *penA* following digestion with three enzymes (*Taq*I, *Hpa*II, and *Hae*III) (15).

Genotypes of the 43 isolates of *N. meningitidis* were identified by random amplified polymorphic DNA (RAPD) patterns generated by arbitrarily primed PCR (APPCR) and pulsed-field gel electrophoresis (PFGE) as previous description (33-35). The four random primers used in APPCR analysis were M13 (5'-GAGGGTGGCGGTTCT-3' (Gibco BRL products, Gaithersburg, MD), ERIC1 (5'-GTGAATCCCCAGGAGCTTACAT-3' (Gibco BRL Products), OPH-03 (5'-AGACGTCCAC-3'), and OPH-09 (5'-CTGACCAGCC-3') (OPERON

Technologies, Inc., Alameda, CA). The restriction enzymes used for PFGE analysis were *Bgl*II, *Spe*I, and *Nhe*I (16, 17).

To interpret RAPD patterns, both faint and intensive bands were included. RAPD patterns were considered identical only if they differed by no more than one band. Interpretation of PFGE profiles (pulsotypes) was in accordance with the criteria previously described (18). Isolates were defined as being of the same clone (highly related isolates) if they had identical serogroups, RAPD patterns, and pulsotypes.

All isolates were susceptible to ceftriaxone, cefepime, imipenem, meropenem, faropenem, moxifloxacin, gatifloxacin, tigecycline, and rifampicin (MICs, ≤ 0.25 μg/mL), and chloramphenicol, erythromycin and clarithromycin (MICs, ≤1 μg/mL). Sixteen isolates (37.2%) were resistant to trimethoprim-slfamethoxazole (MICs, ≥4/76 μg/mL). Three isolates (3.2%) were resistant to penicillin (all had MICs of 0.5 μg/mL) and these isolates were all β-lactamase negative.

Among the 43 isolates of *N. meningitidis*, nine clones were identified based on the PFGE profiles (pulsotypes) and RAPD patterns (Tables 1). Four major clones, i.e. clones 1 (serogroup W135- 17 isolates), 3 (serogroup Y- 8 isolates), 4 (serogroup B- 9 isolates), and 5 (serogroup B- 4 isolates) were found in different time and different regions of Taiwan in 2001. All three penicillin-resistant isolates belonged to clone 1. Two isolates recovered in 1998 and 2000 were genetically different from isolates

found in 2001 and belonged to clones 6 and 7, respectively. None of the pulsotypes of the nine clones were identical or closely related to those reported from other countries, including that of the epidemic Hajj-related W135 clone (MenW135) or (W) ET-37 clone (16, 17, 19)

The three penicillin-resistant isolates (clone 1) had identical restriction profiles of *pen*A, which were different from those of the three penicillin susceptible isolates. The DNA sequences of the *penA* amplicon from the three penicillin-resistant strains and three randomly selected susceptible isolates with penicillin MICs of ≤0.03 μg/mL (clone 1), ≤0.03 μg/mL (clone 2), and 0.06 μg/mL (clone 4), were subsequently determined. All three *penA* sequences from the susceptible strains showed more than 98% similarity. However, the *penA* from the resistant strains showed much lower similarity (about 75%) to those of other strains.

Conclusions

The reasons for the reemergence of meningococcal disease ~~d~~in recent years and the upsurge in 2001 are difficult to clarify. Spreading of some clones of the organism islandwide seems to be the most likely explanation for a substantial increase in the number of cases during a short time. However, increased alertness and better handling of clinical samples (particularly cerebrospinal fluids) and better recognition of *N. meningitidis* by microbiology laboratories due to programs of intensive training for

clinical microbiology staffs and frequent proficiencyt tests monitored by the Department of Health of Taiwan during the period may also have contributed to increased recognition). More efficient and timely notification of this disease to the CDC by clinicians and microbiology staffs may also have contributed to increased incidence.

In addition to the reemerging nature of meningococcal disease in Taiwan, four important points were clearly demonstrated in this study. First, in addition to the predominance of serogroup B, the emergence of serogroups Y isolates and the remarkable increase of serogroup W135 isolates in 2001 are impressive. Second, epidemiological information and typing results indicate that wide dissemination of a limited number of domestic (Taiwanese) clones of *N. meningitides*, particularly serogroups W135 and Y contributed to the Taiwan 2001 outbreak (16, 17, 19). The two clones of serogroup W135 involved in this outbreak were genetically unrelated to the W135 clone, which was associated with the epidemic in the 2000 or 2001 Hajj pilgrimage (16, 17, 19).

Third, the low prevalence of penicillin resistance (3.2%) and high prevalence of trimethoprim-sulfamethoxazole resistance (37.2%) among our recent isolates is interesting, particularly as it occurred in a region with a high prevalence of penicillin and/or trimethoprim-sulfamethoxazole resistance among *Streptococcus pneumoniae*

and other respiratory pathogens (20). In this study, the three penicillin-resistant isolates were β-lactamase negative and exhibited alteration of *penA* sequence, which is in accordance with the previous findings that extremely rare penicillin-resistant isolates of *N. Meningitidis* were β-lactamase producers (1, 21). In comparison with other isolates of clone 1, the penicillin-resistant isolates had identical pulsotypes and RAPD patterns and serogroups but had different penicillin MIC and less similarly, *penA* sequence. These findings suggested that the possibility of horizontal transfer of *penA* gene between these penicillin-resistant isolates and other bacteria (22). Finally, clonal spreading of *N. meningitidis*, particularly by the four major clones (serogroups W135, Y and B) may have partially contributed to the 2001 outbreak.

Resistance to sulfonamides, rifampicin, ciprofloxacin, and ceftriaxone is of particular concern in the management of patients with meningococcal disease (1, 21). Resistance to sulfonamide (sulfadiazine or trimethoprim-sulfamethoxazole) has been reported worldwide with rates of up to 30% in the United Kingdom and 54% in the United States (11, 21). A previous report from Taiwan on nasopharyngeal carriage isolates in military recruits also showed a high-level of resistance (54.1%) to sulfadiazine (13). Fortunately, our isolates were all susceptible to the latter three agents. Other agents, such as carbapenems, macrolides, newer fluoroquinolones, and chloramphenicol were also active against our isolates. These findings support

observations by previous studies (21, 23). The MIC_{90} of our isolates for tigecycline, a newer semisynthetic glycylcyline, was 0.12 μg/mL, which is consistent with the findings of Gales et al (24). Accordingly, penicillin is still recommended as the drug of choice for treating meningococcal disease in Taiwan.

Close contacts are at increased risk of contracting meningococcal disease and warrant chemoprophylaxis (25). Meningococcal vaccination is beneficial for individuals at high-risk or travelers to countries recognized as having epidemic disease caused by a vaccine-preventable serogroup (25). During the 2001 outbreak in Taiwan, three persons, who had close contact (frequently had slept or ate meals in the same dwelling) with one of the 41 patients, had nasopharyngeal colonizations with *N. meningitidis*. The three isolates from the three contacts and the patient's isolate all belonged to serogroup B indicating the possibility of close relatedness of the four isolates, although the three isolates were not preserved for further typing.

Moreover, the persistently high annual proportion of serogroup B isolates causing invasive meningococcal disease in the recent six years and the predominance of serogroup B (54.3%) among nasopharyngeal colonizers in a surveillance on military recruits in 2001 (data shown elsewhere) suggest that the quadrivalent vaccine (A, C, Y, and W135) has a limited role in controlling the present reemergence of meningococcal disease. Introduction of developing conjugated or outer membrane

based serogroup B meningococcal vaccine into Taiwan is warranted.

In conclusion, meningococcal disease, after a 7-year period of dormancy, has reemerged in Taiwan. Wide dissemination of a limited number of domestic clones of *N. meningitides*, particularly serogroups W135, B, and Y contributed to the Taiwan 2001 outbreak. The high proportion of serogroup B meningococcal disease involved in the recent outbreak contraindicates the need for vaccination of risk populations with quadrivalent vaccine to control this reemerging disease in Taiwan.

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Table 1. Microbiological characteristics of 43 isolates of *N. meningitides* recovered from 1998 (one isolate), 2000 (one isolates), and 2001 (41 isolates).

Serogroup	No. of isolates tested	Genotype (no. of isolates)		Year of isolation	Clone
		RAPD pattern (M13/ERIC1/ OPH-03/OPH-09)	Pulsotype (*Spe*I/*Bgl*II/*Nhe*I)		
W135	18	W1 (17)	w1 (17)	2001	1
		W2 (1)	w2 (1)	2001	2
Y	8	Y1 (8)	y1 (8)	2001	3
B	15	B1 (9)	b1 (9)	2001	4
		B2 (4)	b2 (6)	2001	5
		B3 (1)	b3 (1)	1998	6
		B4 (1)	b4 (1)	2000	7
A	1	A1 (1)	a1 (1)	2001	8
C	1	C1 (1)	c1 (1)	2001	9