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NOTES

Multicenter Surveillance of Antimicrobial Resistance of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in Taiwan during the 1998–1999 Respiratory Season

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A susceptibility surveillance study of 276 isolates of *Streptococcus pneumoniae*, 301 of *Haemophilus influenzae*, and 110 of *Moraxella catarrhalis* was carried out from November 1998 to May 1999 in Taiwan. High rates of nonsusceptibility to penicillin (76%), extended-spectrum cephalosporins (56%), azithromycin (94%), clarithromycin (95%), and trimethoprim-sulfamethoxazole (TMP-SMX) (65%) for *S. pneumoniae* isolates and high rates of nonsusceptibility to amoxicillin (58%) and TMP-SMX (52%) for *H. influenzae* isolates were found. Higher percentages of *S. pneumoniae* isolates nonsusceptible to aminopenicillins, extended-spectrum cephalosporins, macrolides, and TMP-SMX were observed among penicillin-intermediate and -resistant isolates. All quinolones tested were active in vitro against these three organisms.

Streptococcus pneumoniae, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the three major bacterial pathogens causing a variety of community-acquired infections, predominantly respiratory tract infections (16). Increasing resistance to β-lactam antibiotics, macrolides, and/or trimethoprim-sulfamethoxazole (TMP-SMX) among clinical isolates of the three pathogens has been widely documented in many countries, particularly in Taiwan (2, 3, 6–8, 10, 11). The high rate of antimicrobial resistance among these isolates compromises the choice of antibiotics available for empiric treatment of infections caused by these organisms. The aim of the present study was to describe the susceptibilities of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in a nationwide, prospective, antimicrobial resistance surveillance study. All consecutive clinical isolates (from November 1998 to May 1999, winter to early spring) were collected from patients with community-acquired infections at five hospitals in three different regions of Taiwan: the northern region (National Taiwan University Hospital [NTUH], Taipei, and Chang Gung Memorial Hospital [CGMH]), the central region (Veterans General Hospital, Taichung [VGH-Taichung]), and the southern region (National Cheng-Kung University Hospital, Tainan, and Veterans General Hospital, Kaohsiung [VGH-Kaohsiung]). These institutions are all teaching hospitals and vary in size from 1,200 beds to more than 2,000 beds.

A total of 678 isolates of *S. pneumoniae* (267 isolates), *H. influenzae* (301), and *M. catarrhalis* (110) were collected during a 7-month period. The majority (86.4%) of these isolates was recovered from respiratory tract secretions (sputum, bronchial washing fluid, and ear and sinus secretions), and 8.8% of the isolates were recovered from blood, cerebrospinal fluid, pleural effusion, and ascites fluid. The identity of these isolates was further confirmed at the Microbiology Laboratory of NTUH.

MICs for these isolates were determined at the Microbiology Laboratory of NTUH by using the Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. For susceptibility testing of *S. pneumoniae*, Mueller-Hinton agar supplemented with 5% sheep blood was used. For *H. influenzae*, *Haemophilus* test medium agar was used, and for *M. catarrhalis*, Mueller-Hinton agar was used. All cultures were incubated 24 h at 35°C under an ambient air atmosphere. The following organisms were included as control strains: *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, *Escherichia coli* ATCC 35218, and *Staphylococcus aureus* ATCC 29213. The organisms were categorized into susceptible, intermediate, and resistant based on the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (12). When the MIC read between the traditional $\log_2$ concentrations, the result was rounded up to the next value and then interpreted. Production of β-lactamase was assayed by using the Cefinase disk test (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

MICs of antimicrobial agents for the five control strains were within the MIC ranges provided by the NCCLS (12). In vitro susceptibilities of this collection of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* isolates to 15 selected antimicrobial agents are shown in Table 1. Seventy-six percent of *S. pneumoniae* isolates were penicillin nonsusceptible, 51% were penicillin intermediate, and 25% showed full resistance to penicillin. Fifty-six percent of the *H. influenzae* isolates produced

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TABLE 1. In vitro antimicrobial susceptibility testing results for 678 isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* recovered from five major teaching hospitals in Taiwan (November 1998 to May 1999)

Organism (no. of isolates) and drug	MIC (μg/ml)			% of isolates in category[a]		
	Range	50%	90%	S	I	R
S. pneumoniae (267)						
Penicillin	0.016–8	1	4	24	51	25
Amoxicillin	<0.016–4	0.5	1	67	32	1
Amoxicillin-clavulanate	<0.016–4	0.5	1	60	37	3
Cefaclor	0.25–>256	32	>256	NA[b]	NA	NA
Cefuroxime	<0.016–32	2	4	33	16	51
Ceftriaxone	<0.016–4	1	2	44	54	2
Cefpirome	<0.016–2	0.5	1	NA	NA	NA
Azithromycin	0.5–>256	>256	>256	6	4	90
Clarithromycin	<0.016–>256	>256	>256	5	6	89
TMP-SMX	0.047–>32	2	>32	35	33	32
Ofloxacin	0.5–>32	2	2	93	6	1
Grepafloxacin	0.064–>256	0.12	0.25	99	0	1
Sparfloxacin	0.032–>256	0.25	0.25	99	0	1
Trovafloxacin	0.032–>32	0.125	0.19	99	0	1
Levofloxacin	0.25–>32	1	1	99	0	1
H. influenzae (301)						
Penicillin	0.25–>32	>32	>32	NA	NA	NA
Amoxicillin	0.016–>256	8	>256	42	2	56
Amoxicillin-clavulanate	0.125–8	0.5	2	99	0	1
Cefaclor	0.25–>256	4	32	83	8	9
Cefuroxime	0.032–>256	1	2	97	2	1
Ceftriaxone	<0.016–>256	<0.016	0.016	99	NA	NA
Cefpirome	0.016–8	0.125	0.25	NA	NA	NA
Azithromycin	0.064–>256	4	8	69	NA	NA
Clarithromycin	0.032–>256	8	16	66	32	2
TMP-SMX	0.016–>32	12	>32	48	1	51
Ofloxacin	0.016–2	0.063	1	99	NA	NA
Grepafloxacin	0.004–1	0.063	0.25	100	0	0
Sparfloxacin	0.004–2	0.032	0.25	100	0	0
Trovafloxacin	0.004–1	0.032	0.25	100	0	0
Levofloxacin	0.004–1	0.016	0.032	100	0	0
M. catarrhalis (110)						
Penicillin	0.25–>32	>32	>32	NA	NA	NA
Amoxicillin	0.032–16	2	4	NA	NA	NA
Amoxicillin-clavulanate	0.016–2	0.125	0.25	NA	NA	NA
Cefaclor	0.5–64	1	8	NA	NA	NA
Cefuroxime	0.125–8	1	8	NA	NA	NA
Ceftriaxone	<0.016–2	0.5	1	NA	NA	NA
Cefpirome	0.5–4	1	2	NA	NA	NA
Azithromycin	0.032–8	0.063	0.125	NA	NA	NA
Clarithromycin	0.032–16	0.125	0.5	NA	NA	NA
TMP-SMX	0.063–>32	0.25	1	NA	NA	NA
Ofloxacin	0.032–2	0.125	0.125	NA	NA	NA
Grepafloxacin	0.032–2	0.063	0.25	NA	NA	NA
Sparfloxacin	0.016–1	0.032	0.25	NA	NA	NA
Trovafloxacin	0.016–1	0.032	0.25	NA	NA	NA
Levofloxacin	0.016–1	0.063	0.063	NA	NA	NA

[a] S, susceptible; I, intermediate; R, resistant.
[b] NA, not applicable; no NCCLS breakpoint criteria (12).

β-lactamase, as did nearly all *M. catarrhalis* isolates (95.7%). Five isolates (1.7%) of *H. influenzae* were β-lactamase negative and amoxicillin resistant (MICs, 8 to 16 μg/ml). Macrolides had remarkably poor activities against *S. pneumoniae* isolates (the MIC at which 90% of the isolates were inhibited [MIC_{90}] was >256 μg/ml) and *H. influenzae* isolates (MIC_{90}, 16 μg/ml for clarithromycin and 8 μg/ml for azithromycin) but had good potency against the *M. catarrhalis* isolates (MIC_{90}, 0.38 μg/ml for clarithromycin and 0.094 μg/ml for azithromycin). More than 50% of *S. pneumoniae* and *H. influenzae* were nonsusceptible to TMP-SMX. All quinolones tested were the most active agents against the three pathogens tested, including penicillin-nonsusceptible and extended-spectrum cephalosporin-nonsusceptible *S. pneumoniae*, amoxicillin-resistant *H. influenzae*, and *M. catarrhalis*. For one multidrug-resistant *S. pneumoniae* isolate (penicillin MIC of 4 μg/ml and azithromycin MIC of >256 μg/ml), MICs of ofloxacin (>32 μg/ml), grepafloxacin (>256 μg/ml), sparfloxacin (>256 μg/ml), trovafloxacin (>32 μg/ml), and levofloxacin (>32 μg/ml) were higher.

Higher percentages of *S. pneumoniae* nonsusceptible to aminopenicillins, extended-spectrum cephalosporins, macrolides, and TMP-SMX were observed among penicillin-interme-

TABLE 2. In vitro activities of antimicrobial agents against 267 *S. pneumoniae* isolates classified by penicillin susceptibility and proportions of intermediate and resistant isolates

Antimicrobial agent	*S. pneumoniae* isolates											
	Penicillin susceptible (n = 64)				Penicillin intermediate (n = 136)				Penicillin resistant (n = 67)			
	MIC (μg/ml)		% of isolates[a]		MIC (μg/ml)		% of isolates		MIC (μg/ml)		% of isolates	
	Range	90%	I	R	Range	90%	I	R	Range	90%	I	R
Amoxicillin	<0.016–0.063	0.016	0	0	0.016–2	1	21	1	0.5–4	2	86	3
Amoxicillin-clavulanate	<0.016–0.5	0.032	0	0	0.016–2	1	32	1	0.5–4	2	81	10
Cefaclor	0.25–16	0.75	NA[b]	NA	0.250–>256	>256	NA	NA	32–>256	>256	NA	NA
Cefuroxime	<0.016–0.5	0.125	0	0	0.063–8	4	32	1	2–32	4	1	99
Ceftriaxone	<0.016–0.25	0.063	0	0	0.032–2	1	60	1	1–4	2	93	7
Cefpirome	<0.016–2	0.125	NA	NA	0.063–2	1	NA	NA	0.5–2	2	NA	NA
Azithromycin	0.5–>256	>256	6	61	8–>256	>256	0	100	0.5–>256	>256	0	99
Clarithromycin	<0.016–>256	>256	2	61	0.5–>256	>256	1	99	0.032–>256	>256	1	97
TMP-SMX	0.063–>32	4	3	9	0.063–>32	>32	35	38	0.25–>32	16	43	42
Ofloxacin	1–4	2	9	0	0.5–4	2	3	0	0.5–>32	1	0	1

[a] I, intermediate; R, resistant.
[b] NA, not applicable.

diate and -resistant isolates (Table 2). Among *S. pneumoniae* isolates intermediate to penicillin, 61% were nonsusceptible (intermediate, 60%; fully resistant, 1%) to ceftriaxone. However, among *S. pneumoniae* isolates fully resistant to penicillin, 86% were intermediate to amoxicillin, all were nonsusceptible (intermediate, 93%; fully resistant, 7%) to ceftriaxone, and nearly all (97 to 99%) were fully resistant to cefuroxime and macrolides.

Table 3 shows the incidence of antimicrobial resistance to *S. pneumoniae* and *H. influenzae* in the five hospitals. The incidence of penicillin-nonsusceptible *S. pneumoniae* isolates ranged from 67 to 84%; the highest rate of full resistance was found in CGMH (38%). The highest rates of TMP-SMX-nonsusceptible *S. pneumoniae* and *H. influenzae* isolates were both observed in VGH-Kaohsiung, and the lowest rate of macrolide-nonsusceptible *H. influenzae* was observed in NTUH. β-Lactamase production in *H. influenzae* isolates ranged from 40% in NTUH to 71% in VGH-Kaohsiung. The incidence of macrolide resistance was higher in the central region than in the other two regions.

Comparison of our results with reported resistance rates of the three species demonstrated by recent surveillance data from other countries showed significantly higher rates in Taiwan of penicillin, extended-spectrum cephalosporins, TMP-SMX, and macrolide resistance among *S. pneumoniae*, as well as significantly higher rates of β-lactamase production and macrolide resistance among *H. influenzae* isolates (1, 4, 5, 15, 16). However, the prevalence of β-lactamase production among *M. catarrhalis* isolates was uniform among isolates from Taiwan (95.7%), the United States (92.0%), and Canada (93.0%) (5). The major concern is the continuing upsurge in Taiwan of *S. pneumoniae* isolates nonsusceptible to penicillin (from 61% in 1996–1997 to 76% in 1998–1999) and to clarithromycin (89% in 1996–1997 to 95% in 1998–1999) and of *H. influenzae* isolates nonsusceptible to azithromycin (from 4.8% in 1994–1995 to 31% in 1998–1999) and to TMP-SMX (from 33.8% in 1994–1995 to 52% in 1998–1999) (8, 11). Moreover, this study is the first to report β-lactamase-negative and amoxicillin-resistant *H. influenzae* isolates in Taiwan, though such organisms have been reported in the rest of the world (11, 14). However, a decrease in the rate of nonsusceptibility to TMP-SMX for *S. pneumoniae* was found (87% in 1996–1997 to 65% in 1998–1999) (8).

Clinical isolates of *S. pneumoniae* resistant to newer fluoroquinolones with notable activity against gram-positive bacteria are rare, and this has been demonstrated to be due to mutations in the quinolone resistance-determining regions of the DNA gyrase and topoisomerase IV genes (9, 13). In the pres-

TABLE 3. Comparison of antimicrobial resistance to *S. pneumoniae* and *H. influenzae* isolates recovered from five major teaching hospitals in Taiwan

Teaching hospital	*S. pneumoniae*					*H. influenzae*			
	No. of isolates	% Nonsusceptible[b] to[c]:				No. of isolates	% Nonsusceptible to:		
		PCN (I/R)[d]	CRO	AZ/CLA	TMP-SMZ		β-Lactamase (+)	AZ/CLA	TMP-SMX
NTUH	88	47/22	51	91/83	51	63	40	22/24	46
CGMH	48	46/38	65	96/92	69	54	63	44/44	52
VGH-Taichung	69	61/23	59	99/99	59	66	64	41/67	52
NCKUH[a]	30	47/20	43	93/90	60	62	47	29/32	47
VGH-Kaohsiung	32	53/25	60	94/94	81	56	71	20/34	66
Total	267	76	56	94/95	65	301	56	31/34	52

[a] NCKUH, National Cheng-Kung University Hospital, Tainan.
[b] Nonsusceptible includes intermediate and resistant isolates.
[c] AZ, azithromycin; CLA, clarithromycin; CRO, ceftriaxone; PCN, penicillin.
[d] I, intermediate; R, resistant.

ent study, one isolate of multidrug-resistant *S. pneumoniae* was also resistant to the five quinolones tested, which had MICs remarkably higher than those reported previously. Further study will be performed to elucidate the resistance mechanism of the isolate.

In conclusion, our data not only present a general view of the incidence of resistance in recent isolates of three major respiratory tract pathogens in Taiwan but also emphasize the increasing incidence of penicillin and macrolide resistance in *S. pneumoniae* and macrolide resistance in *H. influenzae* isolates. Increases in resistance, together with remarkable geographical variations in resistance patterns, make local and ongoing antimicrobial susceptibility surveillance crucial in establishing and/or modifying guidelines for the empiric treatment of respiratory tract infections caused by these three pathogens.

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