Eur J Clin Microbiol Infect Dis (2009) 28:1013–1017
DOI 10.1007/s10096-009-0727-3

BRIEF REPORT

Nationwide surveillance of antimicrobial resistance among *Haemophilus influenzae* and *Streptococcus pneumoniae* in intensive care units in Taiwan

S.-S. Jean · P.-R. Hsueh · W.-S. Lee · H.-T. Chang · M.-Y. Chou · I.-S. Chen · J.-H. Wang · C.-F. Lin · J.-M. Shyr · W.-C. Ko · J.-J. Wu · Y.-C. Liu · W.-K. Huang · L.-J. Teng · C.-Y. Liu

Received: 10 September 2008 / Accepted: 25 February 2009 / Published online: 12 March 2009

Abstract A nationwide susceptibility surveillance of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates collected from patients treated at the intensive care units (ICUs) of ten Taiwanese major teaching hospitals was conducted from September 2005 through November 2005. High rates of resistance (intermediate/resistant) of *S. pneumoniae* to penicillin (85% resistance), ceftriaxone (46%/20%), and cefepime (43%/15%) by meningitis criteria, and in contrast, non-susceptibilities (intermediate/resistant) to penicillin (0%/0%), ceftriaxone (20%/0%) and cefepime (15%/0%) by non-meningitis criteria were noted (p values < 0.05) by the Clinical and Laboratory Standards Institute 2008. Resistant rate of *S. pneumoniae* to azithromycin was also high (63%). *S. pneumoniae* isolates were significantly more susceptible to ertapenem (87%) than to imipenem (39%) and meropenem (44%) (p values < 0.05). Rates of non-susceptibilities of *H. influenzae* isolates to ampicillin and cefaclor were high (55% and 45%, respectively). No β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* isolates were found. Imipenem has a notably higher MIC_{90} value (8 μg/ml) for *H. influenzae* than that of the other two carbapenems.

S.-S. Jean
Departments of Intensive Care and Internal Medicine,
Min-Sheng General Hospital,
Taoyuan County, Taiwan

P.-R. Hsueh (✉)
Departments of Laboratory Medicine and Internal Medicine,
National Taiwan University Hospital,
National Taiwan University Medical College,
7 Chung-Shan South Road,
100 Taipei, Taiwan
e-mail: hsporen@ntu.edu.tw

W.-S. Lee
Department of Internal Medicine,
Taipei Municipal WanFang Hospital,
Taipei, Taiwan

H.-T. Chang
Department of Internal Medicine, Far Eastern Memorial Hospital,
Taipei County, Taiwan

M.-Y. Chou
Department of Internal Medicine,
Cheng Hsin Rehabilitation Medical Center,
Taipei, Taiwan

I.-S. Chen
Department of Internal Medicine, Cardinal Tien Hospital,
Taipei County, Taiwan

J.-H. Wang
Department of Internal Medicine,
China Medical College Hospital,
Taichung, Taiwan

C.-F. Lin
Department of Internal Medicine,
Taichung Veterans General Hospital,
Taichung, Taiwan

J.-M. Shyr
Department of Clinical Pathology,
Taichung Veterans General Hospital,
Taichung, Taiwan

W.-C. Ko
Department of Internal Medicine,
National Cheng-Kung University Hospital,
Tainan, Taiwan

Tigecycline showed good in vitro activities against these two respiratory pathogens. High rates of resistance among isolates of *S. pneumoniae* and *H. influenzae* continue to exist in the ICUs of Taiwan.

Streptococcus pneumoniae and *Haemophilus influenzae* are the two most frequently encountered, community-acquired pathogens leading to lower respiratory tract infections [1]. Consequently, their resistances are of worldwide concern. The Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) was initiated in 2000. This nationwide programme was designed to longitudinally monitor the antimicrobial resistance of clinically important bacteria. A SMART survey, conducted in 2000, revealed that 58% non-susceptibility to penicillin and 33% non-susceptibility to cefotaxime among *S. pneumoniae* strains evaluated were documented according to the meningitis guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) in 2000 [2].

From September 2005 through November 2005, a total of 85 non-duplicated clinical isolates, including 54 *S. pneumoniae* and 31 *H. influenzae* isolates, recovered from the ICU patients of ten major Taiwanese teaching hospitals, were included. Each isolate was obtained from one individual patient. Minimum inhibitory concentrations (MICs) were determined by using the agar dilution method [3], and interpreted by the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI) 2008 [4]. A total of 23 antimicrobial agents were tested (Table 1). Reference strains including *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, and *Escherichia coli* ATCC 35218 were used as quality control strains for each batch of MIC tests. The *H. influenzae* isolates were tested for β-lactamase production using the cefinase disk.

The majority of the isolates were cultured from the respiratory tract (80% for *S. pneumoniae* and 97% for *H. influenzae*, respectively). The results of antimicrobial susceptibilities are shown in Table 1. For *S. pneumoniae* isolates, CLSI 2008 meningitis interpretative criteria were used (the susceptible and resistant MIC interpretative breakpoints for penicillin were ≤0.06 and ≥0.12 μg/ml, respectively, and the susceptible, intermediate, and resistant MIC breakpoints, for ceftriaxone and cefepime [4] were ≤0.5, 1, and ≥2 μg/ml, respectively). High rates of non-susceptibility for penicillin (85%), ceftriaxone (66%) and cefepime (57%) were demonstrated for ICU *S. pneumoniae* isolates. However, using the non-meningitis interpretative criteria (MIC interpretative breakpoints for susceptible, intermediate and resistant, respectively, were ≤2, 4, and ≥ 8 μg/ml for penicillin and ≤1, 2, and ≥4 μg/ml for ceftriaxone and cefepime [4]) in CLSI 2008, penicillin, ceftriaxone and cefepime showed high rates of susceptibility (100% to penicillin, 80% to ceftriaxone, and 85% to cefepime) for *S. pneumoniae*.

H. influenzae strains exhibited a high rate (55%) of resistance to ampicillin. All isolates intermediate or resistant to ampicillin were positive for β-lactamase production, indicating the absence of β-lactamase-negative ampicillin-resistant (BLNAR) isolates. The susceptibilities of *H. influenzae* isolates to cefaclor, cefuroxime, cefixime, cefpodoxime, cefotaxime and amoxicillin-clavulanate were 55%, 90%, 100%, 100%, 100% and 100%, respectively. Azithromycin (MIC_{90} value, 4 μg/ml) showed significantly better in vitro activity against *H. influenzae* than clarithromycin (MIC_{90} value, 32 μg/ml; $p=0.0177$ [statistical method]).

Levofloxacin and moxifloxacin retained good activities against *S. pneumoniae* and *H. influenzae* isolates, but two (6.5%) of the *H. influenzae* strains exhibited non-susceptibilities to fluoroquinolones (MIC values of the two isolates for ciprofloxacin/levofloxacin/moxifloxacin were 8/4/8 and 4/4/4 μg/ml, respectively). Isolates of *S. pneumoniae* and *H. influenzae* exhibited low MIC_{90} values (0.03 and 0.25 μg/ml, respectively) to tigecycline. Ertapenem showed better activity than imipenem and meropenem (susceptibilities of 87%, 39%, 44%, respectively; p values < 0.05) against *S. pneumoniae*, and the imipenem MIC_{90} level (8 μg/ml) for *H. influenzae* was notably higher (64-fold) than that of the other two carbapenems (MIC_{90} value, 0.12 μg/ml).

J.-J. Wu
School of Medical Technology,
National Cheng-Kung University College of Medicine,
Tainan, Taiwan

Y.-C. Liu
Department of Clinical Pathology,
Kaohsiung Veterans General Hospital,
Kaohsiung, Taiwan

W.-K. Huang
Department of Internal Medicine,
Kaohsiung Veterans General Hospital,
Kaohsiung, Taiwan

L.-J. Teng
School of Medical Technology,
National Taiwan University Hospital,
National Taiwan University College of Medicine,
Taipei, Taiwan

C.-Y. Liu
Department of Internal Medicine,
Taipei Veterans General Hospital,
Taipei, Taiwan

Table 1 Antimicrobial susceptibilities of *S. pneumoniae* and *H. influenzae* isolates recovered from patients treated at the intensive care units of ten major teaching hospitals in Taiwan in 2005

Antimicrobial agent	MIC (μg/ml)			No. (%) of isolates[a]		
	Range	MIC_{50}	MIC_{90}	S	I	R
S. pneumoniae (54)						
Penicillin	0.03–2	1	2	8 (15)	NA[c]	46 (85)[b]
				54 (100)	0 (0)	0 (0)[c]
Ceftriaxone	0.03–2	1	2	18 (33)	25 (46)	11 (20)[b]
				43 (80)	11 (20)	0 (0)[c]
Cefepime	0.03–2	1	2	23 (43)	23 (43)	8 (15)[b]
				46 (85)	8 (15)	0 (0)[c]
Imipenem	0.03–1	0.25	0.5	21 (39)	32 (59)	1 (2)
Meropenem	0.03–2	0.5	0.5	24 (44)	27 (50)	3 (6)
Ertapenem	0.03–8	1	2	47 (87)	6 (11)	1 (2)
Azithromycin	0.03– >128	8	>128	18 (33)	2 (4)	34 (63)
Levofloxacin	0.06–16	1	2	52 (96)	0 (0)	2 (4)
Moxifloxacin	0.03–4	0.12	0.25	52 (96)	1 (2)	1 (2)
Vancomycin	0.03–0.5	0.25	0.5	54 (100)	0 (0)	0 (0)
Linezolid	0.06–1	0.5	1	54 (100)	0 (0)	0 (0)
Telithromycin	0.03–0.25	0.03	0.06	54 (100)	0 (0)	0 (0)
Tigecycline	0.03	0.03	0.03	NA	NA	NA
H. influenzae (31)						
Ampicillin	0.25–128	2	64	14 (45)	2 (7)	15 (48)
Amoxicillin	0.25–128	4	64	NA	NA	NA
Amoxicillin-clavulanate	0.25–4	1	4	31 (100)	NA	0 (0)
Cefaclor	4–64	8	64	17 (55)	6 (19)	8 (26)
Cefuroxime	0.5–8	1	4	28 (90)	3 (10)	0 (0)
Cefixime	0.03–0.5	0.06	0.12	31 (100)	NA	NA
Cefpodoxime	0.03–0.25	0.12	0.25	31 (100)	NA	NA
Cefotaxime	0.03	0.03	0.03	31 (100)	NA	NA
Imipenem	0.12–16	1	8	26 (84)[d]	NA	NA
Meropenem	0.03–0.5	0.06	0.12	31 (100)	NA	NA
Ertapenem	0.03–0.25	0.06	0.12	31 (100)	NA	NA
Azithromycin	1–4	2	4	31 (100)	NA	NA
Clarithromycin	1–128	16	32	10 (32)	16 (52)	5 (16)
Ciprofloxacin	0.03–8	0.03	0.25	29 (94)[e]	NA	NA
Levofloxacin	0.03–4	0.03	0.12	29 (94)[e]	NA	NA
Moxifloxacin	0.03–8	0.03	0.12	29 (94)	NA	NA
TMP-SMX	1–>128	16	128	11 (35)	6 (19)	14 (45)
Tigecycline	0.12–0.5	0.25	0.25	NA	NA	NA

MIC minimum inhibitory concentration, *S* susceptible, *I* intermediate, *R* resistant, *NA* not available, *TMP-SMX* trimethoprim-sulfamethoxazole

[a] Some interpretative MIC breakpoints of susceptibility categories were not available (NA) [4]

[b] For *S. pneumoniae* isolates, the rates of susceptibilities to penicillin, ceftriaxone, and cefepime were calculated as percentages by meningitis criteria (the susceptible and resistant MIC interpretative breakpoints for penicillin were ≤0.06 and ≥0.12 μg/ml, respectively, and the susceptible, intermediate, and resistant MIC breakpoints, for ceftriaxone and cefepime were ≤0.5, 1, and ≥2 μg/ml, respectively) [4]

[c] For *S. pneumoniae* isolates, the rates of susceptibilities to penicillin, ceftriaxone, and cefepime were calculated as percentages by non-meningitis criteria (the MIC interpretative breakpoints for susceptible, intermediate and resistant were, respectively, ≤2, 4, and ≥ 8 μg/ml for penicillin and ≤1, 2, and ≥4 μg/ml for ceftriaxone and cefepime) [4]

[d] Five isolates of *H. influenzae* were not susceptible to imipenem

[e] Two isolates of *H. influenzae* were not susceptible to ciprofloxacin, levofloxacin, or moxifloxacin

Table 2 Differences in the prevalences of antibiotic resistance of penicillin- non-susceptible *S. pneumoniae* (PNSSP) in 2000, penicillin-resistant *S. pneumoniae* (PRSP) (evaluated by the meningitis criterion of CLSI 2008) in 2005, and the third-generation cephalosporins (cefotaxime [CTX], ceftriaxone [CFO])-non-susceptible *S. pneumonia* (CTX- or CFO-NSSP) isolates from the Taiwanese intensive care units between 2000 and 2005 with ampicillin-resistant (Amp-R) and β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* isolates between 2003 and 2005

Resistant bacteria (y)	% (no. of resistant isolates/no. of isolates tested)	*P* value
S. pneumoniae		
PNSSP or PRSP		
2000 (PNSSP)	58 (12/24)	
2005 (PRSP)	85 (46/54)	0.009
CTX- or CFO-NSSP		
2000	33 (8/24)	
2005	67 (36/54)	0.006
H. influenzae		
Amp-R		
2003	60 (36/60)	
2005	55 (17/31)	0.636
BLNAR isolates		
2003	8.3 (5/60)	
2005	0 (0/31)	0.100

Compared to the data of previous surveillances, significantly increasing prevalences of penicillin-resistant *S. pneumoniae* (PRSP, evaluated by the meningitis criterion of CLSI 2008) and the third-generation cephalosporins (cefotaxime, ceftriaxone)-non-susceptible *S. pneumoniae* (by using meningitis criteria; $p=0.009$ and 0.006, respectively) between 2000 [2] and 2005 in Taiwanese ICUs were noted. Also, a decline in the rate of BLNAR (evaluated by chi-square test; $p=0.100$) for *H. influenzae* between 2003 [1] and 2005 (Table 2) was seen.

In this Taiwanese ICU surveillance, the markedly high prevalence of penicillin resistance (meningitis criteria) for ICU *S. pneumoniae* was noted. Because of the rapidly rising prevalence of PRSP, which also have a high likelihood of exhibiting co-resistance to non-β-lactam antimicrobials [5], institution of a stricter control policy for the administration of β-lactams in Taiwanese ICUs is mandatory. No BLNAR isolate in this survey was found, which is different from that of a previous Taiwanese study [1].

In this study, imipenem and meropenem showed poorer in vitro activities against *S. pneumoniae* isolates than that of ertapenem. Hilliard et al. demonstrated that *S. pneumoniae* strains with intermediate susceptibility to imipenem and meropenem were likely (>80% probability) to be susceptible to ertapenem [6], and the susceptibilities of amoxicillin-clavulanate and ceftriaxone to *S. pneumoniae* were well-correlated with that of ertapenem [6], which were consistent with our findings on the similarity between the susceptibility of ceftriaxone (by non-meningitis criterion) and ertapenem to pneumococci. Besides, our *H. influenzae* isolates had a significantly lower imipenem susceptibility (84%) than that of the other two carbapenems (p values < 0.05). Different entry routes of imipenem from meropenem [7] and decreased imipenem affinity to penicillin-binding protein in mutated *H. influenzae* isolates [8] were the presumed mechanisms resistant to imipenem. Notably, our *H. influenzae* isolates displayed remarkable differences in their susceptibilities to azithromycin and clarithromycin. However, the clinical applicability of this MIC data suggestive of the superiority of azithromycin to clarithromycin is controversial because the synergistic effect of the 14-OH metabolite of clarithromycin is not routinely tested in the MIC determination of clarithromycin [9]. The tigecycline MIC_{90} value for our *S. pneumoniae* is lower than that of a previous global study [10], and similar to that of the same global survey for *H. influenzae* [10].

In conclusion, high penicillin non-susceptibility and marked differences concerning the susceptibilities of Taiwanese ICU *S. pneumoniae* isolates for penicillin and cefotaxime/ceftriaxone through the evaluation by meningitis and non-meningitis criteria were clearly documented. Imipenem and meropenem showed high non-susceptibilities to pneumococci. With regards to Taiwanese *H. influenzae* strains, ampicillin, cefaclor, clarithromycin and trimethoprim-sulfamethoxazole showed poor activities by in vitro susceptibility data, and the susceptibility of imipenem was inferior to that of the other carbapenems. Alarmingly, higher β-lactam-non-susceptible rates (meningitis criteria) among *S. pneumoniae* isolates and the persistently high prevalence of ampicillin- non-susceptible *H. influenzae* isolates in comparison with prior Taiwanese data were noted. Tigecycline and respiratory fluoroquinolones are promising agents with potent activity against both *S. pneumoniae* and *H. influenzae* isolates. However, prudent use of these agents to prevent the potential emergence of resistance is warranted.

References

1. Lau YJ, Hsueh PR, Liu YC, Shyr JM, Huang WK, Teng LJ, Liu CY, Luh KT (2006) Comparison of in vitro activities of tigecycline with other antimicrobial agents against *Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis* in Taiwan. Microb Drug Resist 12:130–135. doi:10.1089/mdr.2006.12.130
2. Hsueh PR, Liu YC, Yang D, Yan JJ, Wu TL, Huang WK, Wu JJ, Ko WC, Leu HS, Yu CR, Luh KT (2001) Multicenter surveillance of antimicrobial resistance of major bacterial pathogens in intensive care units in 2000 in Taiwan. Microb Drug Resist 7:373–382. doi:10.1089/10766290152773383

3. CLSI (2005) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th edn. Approved standard M7-A6. Clinical and Laboratory Standards Institute, Wayne, PA, USA
4. CLSI (2008) Performance standards for antimicrobial susceptibility testing, 15th informational supplement. Supplemental tables M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA, USA
5. Whitney CG, Farley MM, Hadler J, Harrison LH, Lexau C, Reingold A, Lefkowitz L, Cieslak PR, Cetron M, Zell ER, Jorgensen JH, Schuchat A, Active Bacterial Core Surveillance Program of the Emerging Infections Program Network (2000) Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N Engl J Med 343:1917–1924. doi:10.1056/NEJM200012283432603
6. Hilliard NJ, Johnson CN, Armstrong SH, Quarles S, Waites KB (2002) In vitro activity of ertapenem (MK-0826) against multidrug resistant *Streptococcus pneumoniae* compared with 13 other antimicrobials. Int J Antimicrob Agents 20:136–140. doi:10.1016/S0924-8579(02)00129-2
7. Yourassowsky E, Van der Linden MP, Crokaert F (1991) Antibacterial effects of meropenem and imipenem against *Haemophilus influenzae*. Chemotherapy 37:38–42. doi:10.1159/000114345
8. Cerquetti M, Giufrè M, Cardines R, Mastrantonio P (2007) First characterization of heterogeneous resistance to imipenem in invasive nontypeable *Haemophilus influenzae* isolates. Antimicrob Agents Chemother 51:3155–3161. doi:10.1128/AAC.00335-07
9. Hardy DJ, Swanson RN, Rode RA, Marsh K, Shipkowitz NL, Clement JJ (1990) Enhancement of the in vitro and in vivo activities of clarithromycin against *Haemophilus influenzae* by 14-hydroxy-clarithromycin, its major metabolite in humans. Antimicrob Agents Chemother 34:1407–1413
10. Hoban DJ, Bouchillon SK, Johnson BM, Johnson JL, Dowzicky MJ, Tigecycline Evaluation and Surveillance Trial (TEST Program) Group (2005) In vitro activity of tigecycline against 6792 Gram-negative and Gram-positive clinical isolates from the global Tigecycline Evaluation and Surveillance Trial (TEST Program, 2004). Diagn Microbiol Infect Dis 52:215–227. doi:10.1016/j.diagmicrobio.2005.06.001