Eur J Clin Microbiol Infect Dis (2009) 28:215–220
DOI 10.1007/s10096-008-0610-7

BRIEF REPORT

Nationwide surveillance of antimicrobial resistance among *Enterobacteriaceae* 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: 30 April 2008 / Accepted: 25 July 2008 / Published online: 21 August 2008

Abstract To determine the antimicrobial resistance profiles among clinical isolates of *Enterobacteriaceae* in Taiwanese intensive care units (ICUs), a national surveillance of antibiotic resistance among important *Enterobacteriaceae* was conducted from September 2005 through November 2005 at the ICUs of ten major teaching hospitals in Taiwan. A total of 574 *Enterobacteriaceae* isolates recovered from various clinical samples of our ICU patients were submitted for in vitro test. Minimum inhibitory concentrations (MICs) of these isolates to 18 antimicrobial agents were determined by the broth microdilution method. The prevalences of *Enterobacteriaceae* isolates with phenotypic extended-spectrum β-lactamase (ESBL) production were 26% in *Klebsiella pneumoniae*, 16% in *Serratia marcescens,* 14% in *Escherichia coli*, and 13% in *Proteus mirabilis,* in which a significantly rising prevalence of ESBL production among *K. pneumoniae* was noted (p=0.002) when compared with a previous Taiwanese survey in 2000. Hetero-

S.-S. Jean
Departments of Intensive Care Units 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
Departments of Internal Medicine,
National Cheng-Kung University Hospital,
Tainan, Taiwan

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

geneous resistance to various fluoroquinolones was found among our *Enterobacteriaceae* isolates, except for *Entetrobacter cloacae.* Emergence of ertapenem-resistant isolates of *E. coli*, *K. pneumoniae*, *E. cloacae*, and *S. marcescens* was noted. Gradually increasing rates of drug-resistant *Enterobacteriaceae* were noted in Taiwanese ICUs. Periodic surveillance of the evolutionary trend of antimicrobial resistance among ICU isolates is crucial for starting appropriately empirical antimicrobial therapy in the future.

Antimicrobial resistance is an increasing threat in hospitalized patients experiencing sepsis caused by *Enterobacteriaceae*, and it has resulted in increased illnesses, mortality, and healthcare costs, particularly in patients admitted to intensive care units (ICUs) [1, 2]. National programs about monitoring the trends of endemic resistance and comparing the data with those of other countries are warranted to guide optimal empirical antibiotics for selected infections. Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART), initiated in 2000, is a nationwide programme in Taiwan designed to monitor antimicrobial resistance among clinically important bacteria.

From September 2005 through November 2005, the ICU wards of ten major teaching hospitals in different regions of Taiwan were involved in this study. A total of 574 non-duplicated isolates (one isolate per patient) of *Enterobacteriaceae* were collected. Identification of species was performed with conventional biochemical methods and the Vitek system (bioMérieux Vitek, St Louis, MO, USA). The isolates that were recovered from various clinical specimens included *Escherichia coli* (160 isolates), *Klebsiella pneumoniae* (162 isolates), *Enterobacter cloacae* (75 isolates), *Serratia marcescens* (68 isolates), *Citrobacter freundii* (12 isolates), *Morganella morganii* (33 isolates), and *Proteus mirabilis* (64 isolates). Antimicrobial susceptibility testing was performed using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [3]. A total of 18 antimicrobial agents (Table 1) were tested. Reference strains *E. coli* ATCC 25922, *K. pneumonia*e ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for each batch of MIC tests. Susceptibility categories of these isolates were determined based upon CLSI MIC breakpoints, except for moxifloxacin, isepamicin, and tigecycline in that their MIC breakpoints are not available [4].

For phenotypic identification of extended-spectrum β-lactamase (ESBL) production for *E. coli*, *K. pneumoniae*, and *P. mirabilis*, CLSI guidelines using the confirmatory disk diffusion methods were applied [4]. For other *Enterobacteriaceae* species, ESBL production was defined based on the MICs of ceftazidime, ceftriaxone, or cefepime that were equal to or greater than 2 μg/ml. If the MIC of cefepime in the presence of clavulanic acid (10 μg) was at least eight-fold less than that of cefepime, the isolate was regarded to have ESBL production as previously described [5].

Among these *Enterobacteriaceae* isolates, the most common source (45.1%) was respiratory tract, 14.6% were from patients with bloodstream infection, and 7.8% from other sterile sites (pleural effusion, ascites, cerebrospinal fluid, and synovial fluid). *E. coli* (27.9%) and *K. pneumoniae* (28.2%) were the two predominant bacteria of all *Enterobacteriaceae*.

The results of antimicrobial susceptibilities for the isolates are shown in Table 1. With the exception of *E. cloacae* and *C. freundii*, cefmetazole retained acceptable in vitro activities (>75% susceptibilities) against the other isolates. Ceftazidime and ceftriaxone exhibited good activities against *Enterobacteriaceae* isolates tested except *E. cloacae* and *C. freundii*. Cefepime had rather low resistant rates (<10%) for all isolates tested. Piperacillin-tazobactam displayed fair (60–80%) susceptibility against *E. cloacae*, *C. freundii,* and *S. marcescens*. In contrast, all carbapenems exhibited excellent activities against all isolates tested. However, eight *K. pneumoniae* isolates, two *E. cloacae* isolates, and two *S. marcescens* isolates were not susceptible to ertapenem. Additionally, one isolate of *K. pneumoniae* with intermediate susceptibility to imipenem and meropenem was noted. Of note, levofloxacin has better in vitro activity than ciprofloxacin against all these isolates. The MIC_{90} levels of moxifloxacin was two-fold higher than those of levofloxacin for most of our enterobacterial isolates. Netilmicin and isepamicin showed similar in vitro susceptibilities to amikacin, and these three agents had remarkably better in vitro activity than gentamicin. All of the isolates tested, except the *P. mirabilis* isolates (MIC_{90}, 32 μg/ml), were inhibited by 2 μg/ml of tigecycline.

It is noteworthy that in this survey *K. pneumoniae* (26%), *S. marcescens* (16%), *E. coli* (14%), and *P. mirabilis*

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 574 clinical *Enterobacteriaceae* isolates recovered from patients treated at ICUs of ten major teaching hospitals in Taiwan in 2005

Antimicrobial agent	MIC (μg/ml)			% for indicated agent		
	Range	MIC_{50}	MIC_{90}	S	I	R
Escherichia coli (160)						
Cefazolin	1– >128	4	>128	66	3	31
Cefmetazole	0.25– >128	1	64	86	3	11
Ceftriaxone	<0.03– >128	0.06	64	78	7	15
Ceftazidime	<0.03– >128	0.12	32	83	2	15
Cefepime	<0.03– 128	0.06	4	94	1	5
Piperacillin-tazobactam	<0.03– >128	2	32	89	5	6
Cefoperazone-sulbactam	≤0.03– >64	1	32	–[a]	–	–
Imipenem	<0.03–4	0.25	0.25	100	0	0
Meropenem	<0.03–8	0.03	0.06	99	1	0
Ertapenem	<0.03– >32	0.03	0.25	98	0	2
Ciprofloxacin	<0.03– >128	0.25	64	68	1	31
Levofloxacin	<0.03– >32	0.25	16	71	2	27
Moxifloxacin	<0.03– >32	0.5	32	–	–	–
Gentamicin	0.25– >128	1	64	64	4	32
Amikacin	0.5– >128	2	4	98	0	2
Netilmicin	0.25– >128	1	4	99	0	1
Isepamicin	0.5– >128	1	2	–	–	–
Tigecycline	0.12–4	0.25	0.5	–	–	–
Klebsiella pneumoniae (162)						
Cefazolin	0.5– >128	2	>128	68	1	31
Cefmetazole	0.12– >128	1	128	85	1	14
Ceftriaxone	<0.03– >128	0.06	128	78	5	17
Ceftazidime	<0.03– >128	0.25	128	79	3	18
Cefepime	<0.03– >128	0.06	16	88	2	10
Piperacillin–tazobactam	<0.03– >128	4	>128	80	4	16
Cefoperazone–sulbactam	0.06– >64	0.5	64	–	–	–
Imipenem	0.12–8	0.25	0.5	99	1	0
Meropenem	<0.03–8	0.03	0.06	99	1	0
Ertapenem	<0.03– >32	0.03	1	95	0	5
Ciprofloxacin	<0.03– >128	0.06	128	73	2	25
Levofloxacin	<0.03– >32	0.06	32	79	2	19
Moxifloxacin	<0.03– >32	0.12	32	–	–	–
Gentamicin	0.06– >128	0.5	>128	73	4	23
Amikacin	0.25– >128	1	>128	86	2	12
Netilmicin	0.5– >128	1	>128	85	3	12
Isepamicin	0.06– >128	0.5	>128	–	–	–
Tigecycline	0.12–16	1	2	–	–	–
Enterobacter cloacae (75)						
Cefazolin	2– >128	>128	>128	1	4	95
Cefmetazole	16– >128	>128	>128	1	4	95
Ceftriaxone	<0.03– >128	4	128	58	19	23
Ceftazidime	0.06– >128	2	>128	53	0	47
Cefepime	<0.03–16	0.12	4	96	4	0
Piperacillin–tazobactam	0.5– >128	4	128	74	13	13
Cefoperazone–sulbactam	<0.03– >64	4	64	–	–	–
Imipenem	0.25–0.5	0.5	0.5	100	0	0
Meropenem	<0.03–0.25	0.06	0.12	100	0	0
Ertapenem	<0.03–4	0.12	2	97	3	0
Ciprofloxacin	<0.03–128	0.06	4	84	3	13
Levofloxacin	<0.03– >32	0.06	4	89	3	8
Moxifloxacin	<0.03– >32	0.12	8	–	–	–
Gentamicin	0.12– >128	0.5	128	68	0	32

Table 1 (continued)

Antimicrobial agent	MIC (μg/ml)			% for indicated agent		
	Range	MIC_{50}	MIC_{90}	S	I	R
Amikacin	0.5– >128	1	8	97	0	3
Netilmicin	0.25– >128	1	4	98	0	2
Isepamicin	0.5– >128	1	2	–	–	–
Tigecycline	0.5–8	1	1	–	–	–
Serratia marcescens (68)						
Cefazolin	32– >128	>128	>128	0	0	100
Cefmetazole	4– >128	16	128	76	9	15
Ceftriaxone	0.06– >128	4	>128	70	9	21
Ceftazidime	0.12– >128	1	8	90	1	9
Cefepime	0.04–64	0.25	16	84	13	3
Piperacillin-tazobactam	1– >128	8	64	63	34	3
Cefoperazone–sulbactam	0.25– >64	8	>64	–	–	–
Imipenem	0.12–2	0.5	0.5	100	0	0
Meropenem	<0.03–2	0.03	0.12	100	0	0
Ertapenem	<0.03–16	0.12	0.5	97	0	3
Ciprofloxacin	0.06–128	2	32	43	16	41
Levofloxacin	0.06– 32	1	8	66	9	25
Moxifloxacin	0.06– >32	2	16	–	–	–
Gentamicin	0.5– >128	8	>128	47	10	43
Amikacin	1– >128	2	>128	87	0	13
Netilmicin	0.5– >128	2	>128	85	0	15
Isepamicin	0.5– >128	2	>128	–	–	–
Tigecycline	1–8	2	2	–	–	–
Citrobacter freundii (12)						
Cefazolin	2– >128	128	>128	33	0	67
Cefmetazole	0.5–128	32	128	25	25	50
Ceftriaxone	0.06–64	0.25	32	59	33	8
Ceftazidime	0.25–128	0.5	128	50	0	50
Cefepime	<0.03–1	0.06	1	100	0	0
Piperacillin–tazobactam	2– >128	4	>128	67	0	33
Cefoperazone–sulbactam	0.5– >64	1	>64	–	–	–
Imipenem	0.12–0.5	0.25	0.5	100	0	0
Meropenem	<0.03–0.06	0.03	0.06	100	0	0
Ertapenem	<0.03–1	0.03	1	100	0	0
Ciprofloxacin	<0.03–8	0.25	4	75	0	25
Levofloxacin	<0.03–2	0.25	2	100	0	0
Moxifloxacin	0.12–8	0.5	4	–	–	–
Gentamicin	0.12– >128	0.5	128	67	0	33
Amikacin	0.5–8	1	4	100	0	0
Netilmicin	0.5–8	1	4	100	0	0
Isepamicin	0.5–1	1	1	–	–	–
Tigecycline	0.5–1	0.5	1	–	–	–
Morganella morganii (33)						
Cefazolin	>128	>128	>128	0	0	100
Cefmetazole	4–64	8	16	97	0	3
Ceftriaxone	<0.03–128	0.03	4	94	0	6
Ceftazidime	0.12– >128	0.25	4	91	0	9
Cefepime	<0.03–2	0.03	0.25	100	0	0
Piperacillin–tazobactam	0.12– >128	0.5	2	97	0	3
Cefoperazone–sulbactam	1–64	2	8	–	–	–
Imipenem	0.06–0.25	0.25	0.25	100	0	0
Meropenem	0.06–0.12	0.12	0.12	100	0	0
Ertapenem	<0.03–0.25	0.03	0.06	100	0	0
Ciprofloxacin	<0.03–32	1	8	55	24	21

Table 1 (continued)

Antimicrobial agent	MIC (μg/ml)			% for indicated agent		
	Range	MIC_{50}	MIC_{90}	S	I	R
Levofloxacin	<0.03–8	0.5	8	82	3	15
Moxifloxacin	0.06–32	2	16	–	–	–
Gentamicin	0.05– >128	2	128	61	0	39
Amikacin	0.5–4	1	2	100	0	0
Netilmicin	0.25– 8	1	4	100	0	0
Isepamicin	0.5–8	1	4	–	–	–
Tigecycline	1–8	2	2	–	–	–
Proteus mirabilis (64)						
Cefazolin	4– >128	8	>128	59	13	28
Cefmetazole	1–16	2	4	100	0	0
Ceftriaxone	<0.03–128	<0.03	8	95	2	3
Ceftazidime	<0.03–32	0.12	0.5	97	0	3
Cefepime	0.06–16	0.12	4	98	2	0
Piperacillin–tazobactam	0.25–16	0.5	1	100	0	0
Cefoperazone–sulbactam	0.5–32	2	16	–	–	–
Imipenem	<0.03–0.12	0.06	0.12	100	0	0
Meropenem	<0.03–0.12	0.06	0.06	100	0	0
Ertapenem	<0.03–0.12	<0.03	0.03	100	0	0
Ciprofloxacin	<0.03–64	0.5	32	60	6	34
Levofloxacin	0.06– >32	0.5	16	64	11	25
Moxifloxacin	0.25– >32	4	32	–	–	–
Gentamicin	0.5– >128	16	>128	42	3	55
Amikacin	1– >128	4	8	91	0	9
Netilmicin	0. 5– >128	4	16	90	6	4
Isepamicin	2– >128	8	16	–	–	–
Tigecycline	2–>32	16	32	–	–	–

S susceptible, *I* intermediate, *R* resistant

[a] Dash (–) implies that interpretive MIC breakpoints were not available by the CLSI 2005 [4]

(13%) were the four leading pathogens with the highest rates of ESBL production. None of our *C. freundii* isolates exhibited ESBL-producing phenotype.

This 2005 multicenter study regarding the antimicrobial susceptibilities of *Enterobacteriaceae* disclosed three important points. First, persistently high rates of ESBL phenotype were found among our *K. pneumoniae* and *E. coli* isolates. In comparison with the data in 2000 [1], a 2.4-fold increase in the prevalence rate of ESBL phenotype was found among *K. pneumoniae* isolates ($p=0.002$, by chi-square test). Second, high percentages (>10%) of ESBL phenotype were also found in our *S. marcescens* and *P. mirabilis* isolates. Third, carbapenem-resistant *Enterobacteriaceae* isolates have emerged in ICUs in Taiwan.

In this study, the prevalence rates of ESBL-producing *K. pneumoniae* and *E. coli* isolates resembled those of ICU pathogens in North America [6]. Fortunately, these rates remained lower than those from Latin America and several Asian countries [5, 7]. Higher prevalence of ESBL production among our *S. marcescens* isolates than two common AmpC producers (*E. cloacae* and *C. freundii*) might be partially responsible for the higher non-susceptible rate of *S. marcescens* to cefepime.

Carbapenems are often considered as the last resort for the management of serious infections in ICUs. However, ertapenem was considered as the most vulnerably affected carbapenem agent against *K. pneumoniae* (with plasmid encoding AmpC or ESBLs or in porin-deficient isolates) [8], *E. coli* (AmpC β- lactamase production, associated with loss of both OmpC and OmpF porins) [9], and *E. cloacae* (with enhanced efflux of ertapenem) isolates [10], which was consistent with our data in terms of higher non-susceptibilities of ertapenem than others. Concerning the susceptibilities of fluoroquinolones in our study, with the exception of *E. cloacae*, the other *Enterobacteriaceae* showed heterogeneous susceptible rates to these agents. However, levofloxacin was significantly more active against important enteric GNBs than ciprofloxacin in our ICU survey results. Finally, except for *Proteus* isolates, tigecycline possessed excellent in vitro activity against

Enterobacteriaceae isolates, including phenotypic ESBL- and AmpC-producing organisms. However, because of its bacteriostatic mechanism, close monitoring of future changes in the values of tigecycline MIC for *Enterobacteriaceae* isolates is warranted.

In conclusion, the emergence of ESBL-producing *Enterobacteriaceae* other than *E. coli* and *K. pneumoniae* was found. Resistance to carbapenems is emerging, and resistance to fluoroquinolones continues to be a worrisome problem. Periodic surveillance of antimicrobial resistances among isolates from ICUs is crucial for initiation of appropriate empirical antimicrobial therapy.

References

1. Hsueh PR, Liu YC, Yang D, Yan JJ, Wu TL, Huang WK et al (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
2. Ho PL, Chan WM, Tsang KW, Wong SS, Young K (2002) Bacteraemia caused by *Escherichia coli* producing extended-spectrum β-lactamase: a case-control study of risk factors and outcomes. Scand J Infect Dis 34:567–573. doi:10.1080/00365540210147516
3. Clinical and Laboratory Standards Institute (2005) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th edn. Approved standard M7-A6. CLSI, Wayne, PA, USA
4. Clinical and Laboratory Standards Institute (2005) Performance standards for antimicrobial susceptibility testing—fifteenth informational supplement. M100-S15. CLSI, Wayne, PA, USA
5. Paterson DL, Rossi F, Baquero F, Hsueh PR, Woods GL, Satishchandran V et al (2005) In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 55:965–973. doi:10.1093/jac/dki117
6. Streit JM, Jones RN, Sader HS, Fritsche TR (2004) Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial Surveillance Programme (North America, 2001). Int J Antimicrob Agents 24:111–118. doi:10.1016/j.ijantimicag.2003.12.019
7. Gales AC, Sader HHS, Jones RN (2002) Respiratory tract pathogens isolated from patients hospitalized with suspected pneumonia in Latin America: frequency of occurrence and antimicrobial susceptibility profile: results from the SENTRY Antimicrobial Surveillance Program (1997–2000). Diagn Microbiol Infect Dis 44:301–311. doi:10.1016/S0732–8893(02)00499–6
8. Jacoby GA, Mills DM, Chow N (2004) Role of β-lactamases and porins in resistance to ertapenem and other β-lactams in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:3203–3206. doi:10.1128/AAC.48.8.3203–3206.2004
9. Mammeri H, Nordmann P, Berkani A, Eb F (2008) Contribution of extended-spectrum AmpC (ESAC) beta-lactamases to carbapenem resistance in *Escherichia coli*. FEMS Microbiol Lett 282:238–240. doi:10.1111/j.1574–6968.2008.01126.x
10. Szabó D, Silveira F, Hujer AM, Bonomo RA, Hujer KM, Marsh JW et al (2006) Outer membrane protein changes and efflux pump expression together may confer resistance to ertapenem in *Enterobacter cloacae*. Antimicrob Agents Chemother 50:2833–2835. doi:10.1128/AAC.01591–05