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Original Article

In vitro susceptibilities of aerobic and facultative anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections at a medical center in Taiwan: results of the Study for Monitoring Antimicrobial Resistance Trends (SMART) 2002-2006

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Background and purpose: The Study for Monitoring Antimicrobial Resistance Trends (SMART) was initiated to monitor the in vitro antimicrobial susceptibility of aerobic and facultative anaerobic Gram-negative bacilli (GNB) isolated from patients with intra-abdominal infections (IAI). This report summarizes the SMART data from 1 of the study centers from 2002 to 2006.

Methods: 492 Gram-negative isolates were collected from 482 patients with IAI. Susceptibilities of these isolates to 12 antimicrobial agents were determined using the broth microdilution method.

Results: *Enterobacteriaceae* comprised 68.3% of the isolates (n = 336). The 4 main species were *Klebsiella* spp. (n = 129; 26.2%), *Escherichia coli* (n = 122; 24.8%), *Enterobacter* spp. (n = 36; 7.3%), and *Aeromonas hydrophila* (n = 35; 7.1%). The commonest glucose non-fermentative GNB were *Acinetobacter baumannii* (n = 46; 9.3%) and *Pseudomonas aeruginosa* (n = 35; 7.1%). Extended-spectrum β-lactamase (ESBL) production was detected in 70 *Enterobacteriaceae* isolates (70/336; 21%). The ESBL phenotype was exhibited by 23% of *Klebsiella pneumoniae*, 26% of *E. coli*, and 19% of *Enterobacter* spp. The highest rate of ESBL production was found in 2005 for *E. coli* (38%) and in 2003 for *Klebsiella* spp. (38%) and *Enterobacter* spp. (40%). The incidence of ESBL-producing isolates declined in 2005 and 2006. Low susceptibility rates of *E. coli* isolates to ciprofloxacin (58%) and levofloxacin (64%) were noted. Ertapenem (99%), imipenem (99%), and amikacin (94%) were the most potent agents against *Enterobacteriaceae* spp.

Conclusion: Continuous surveillance is crucial to monitor the trend of antimicrobial resistance patterns among GNB isolated from IAI.

Key words: beta-Lactamases; Drug resistance, bacterial; *Enterobacteriaceae*; Infections

Introduction

Several national and international surveillance programs have been initiated because of the global problem of antimicrobial resistance in bacteria [1-5]. The aims of these studies are to monitor resistance trends and provide guidance for the selection of empiric antibiotics. The Study for Monitoring Antimicrobial Resistance Trends (SMART) was initiated in 2002. The study is a global surveillance program to longitudinally monitor the in vitro antimicrobial susceptibility of aerobic and facultative anaerobic Gram-negative bacilli (GNB) isolates from patients with intra-abdominal infections (IAI).

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Results from the SMART program in 2003 [6] and 2004 [7] revealed that the Asia-Pacific region had the lowest susceptibility rates among the 5 global regions studied. Susceptibility rates to all tested antibiotics were generally lower in Asian countries than those in the Oceania region [8]. This report summarizes the antimicrobial susceptibility patterns of aerobic and facultative anaerobic GNB isolates from patients with IAI at the National Taiwan University Hospital (NTUH), a 2000-bed tertiary teaching hospital located in Taipei in northern Taiwan, from 2002 to 2006. Potential resistance trends over time were also detected.

Methods

Setting and bacterial isolates

From 2002 to 2006, the SMART program documented 492 consecutive and non-duplicate aerobic and facultative anaerobic GNB isolates from patients with IAI at the NTUH. Isolates recovered from clinical specimens of patients with IAI, including tissue, fluid, and deep wound cultures obtained intraoperatively, and fluid from paracentesis or percutaneous aspiration of abscesses, were included in the study. Isolates from abdominal drains or drainage bottles, stools, superficial wounds, or perirectal abscesses were excluded. Bacteria were identified by the conventional methods used in the clinical microbiology laboratories.

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) of the isolates were determined using the broth microdilution method with customized microtiter plates (Dade MicroScan, Inc., Sacramento, CA, USA) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [9], and susceptibility was interpreted based on the CLSI breakpoints [10]. Twelve antimicrobial agents were tested: ertapenem, imipenem, cefepime, cefotaxime, ceftriaxone, ceftazidime, cefoxitin, ampicillin-sulbactam, piperacillin-tazobactam, amikacin, ciprofloxacin, and levofloxacin. *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains.

A modified CLSI method [10] was used to identify extended-spectrum β-lactamase (ESBL) production in *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. If the ceftazidime, ceftriaxone or cefepime MIC was ≥2 μg/mL, the MIC of cefepime was compared with the MIC of cefepime plus clavulanic acid (10 μg). ESBL production was defined as a ≥8-fold decrease in the cefepime MIC when tested in combination with clavulanic acid compared with no clavulanic acid. *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. confirmed to produce ESBLs were designated as resistant to ceftazidime, ceftriaxone, and cefepime regardless of their MIC results.

Statistical analysis

95% confidence intervals (CIs) were calculated for differences using the method of Miettinen and Nurminen for each comparison [11]. No statistical adjustment was made for computing multiple CIs.

Results

There were 492 GNB isolated from 482 patients during the study period. *Enterobacteriaceae* comprised

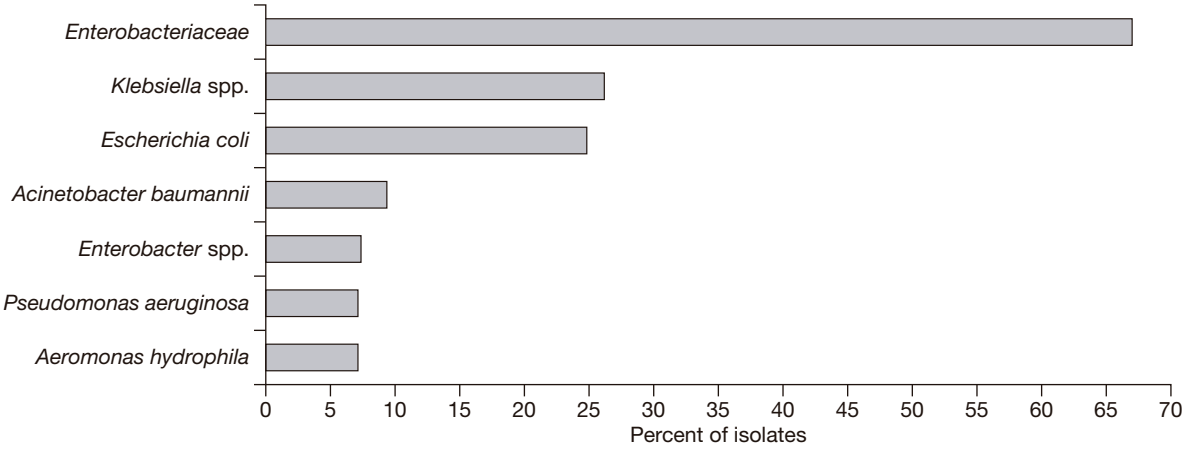

Fig. 1. Distribution of 6 major pathogens among 492 facultative anaerobic Gram-negative bacilli from patients with intra-abdominal infections at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

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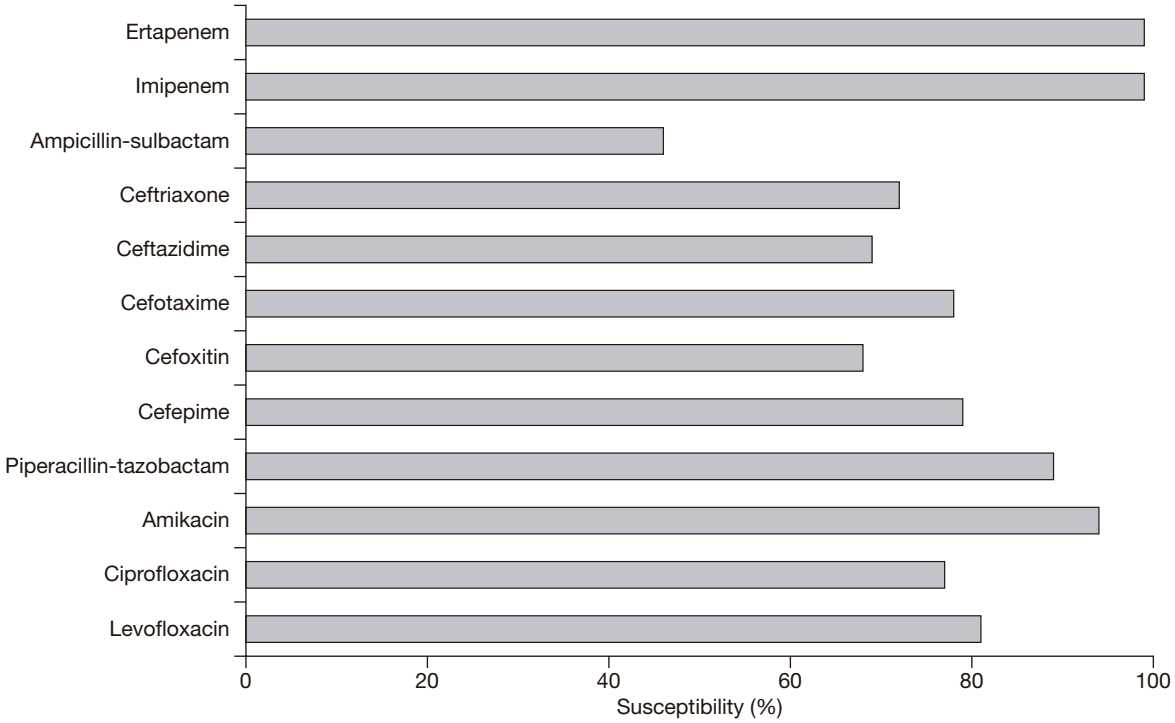

Fig. 2. Susceptibility of *Enterobacteriaceae* isolates from patients with intra-abdominal infections at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

68.3% of all isolates (n = 336). The 4 main genuses or species were *Klebsiella* spp. (n = 129; 26.2%), *E. coli* (n = 122; 24.8%), *Enterobacter* spp. (n = 36; 7.3%), and *Aeromonas hydrophila* (n = 35; 7.1%). The 2 most common glucose non-fermentative GNB were *Acinetobacter baumannii* (n = 46; 9.3%) and *P. aeruginosa* (n = 35; 7.1%) [Fig. 1].

The susceptibility rates of all *Enterobacteriaceae* isolates to 12 antimicrobial agents are shown in Fig. 2. Ertapenem (99%), imipenem (99%), and amikacin (94%) were the most active agents against *Enterobacteriaceae* in vitro. Less than 70% of *Enterobacteriaceae* isolates were susceptible to cefoxitin and ceftazidime. Less than 50% of *Enterobacteriaceae* isolates were susceptible to ampicillin-sulbactam. The susceptibility rates of *Enterobacteriaceae* to ciprofloxacin and levofloxacin were 77% and 81%, respectively. Analysis of data for 2003 to 2006 showed an annual ascending trend for susceptibility to ciprofloxacin and levofloxacin among *Enterobacteriaceae* isolates except for the year 2005 (Fig. 3). An annual trend for increasing susceptibility to fluoroquinolones among *E. coli* was also noted (Fig. 4A and Fig. 4B).

For *E. coli* isolates, ertapenem (100%), imipenem (99%), and amikacin (98%) were the most active agents. Ampicillin-sulbactam (35%), ciprofloxacin (58%), and levofloxacin (64%) had the lowest in vitro activities against these isolates.

Among *Klebsiella* spp. isolates, *K. pneumoniae* (90%) and *Klebsiella oxytoca* (10%) were most commonly isolated, and ertapenem (99%) and imipenem (99%) were the most active agents.

ESBL production was detected in 70 *Enterobacteriaceae* isolates (70/336; 21%). The ESBL phenotype was exhibited by 23% of *K. pneumoniae*, 26% of *E. coli*, and 19% of *Enterobacter* spp. (Table 1). The highest rate of ESBL production for *E. coli* was found in 2005. For *Klebsiella* spp. and *Enterobacter* spp.,

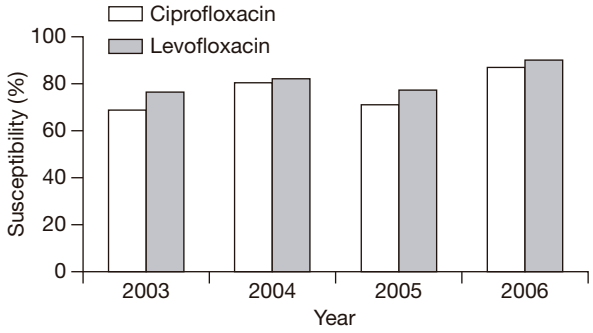

Fig. 3. Trends of susceptibility rates to ciprofloxacin and levofloxacin among *Enterobacteriaceae* isolates from patients with intra-abdominal infections at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

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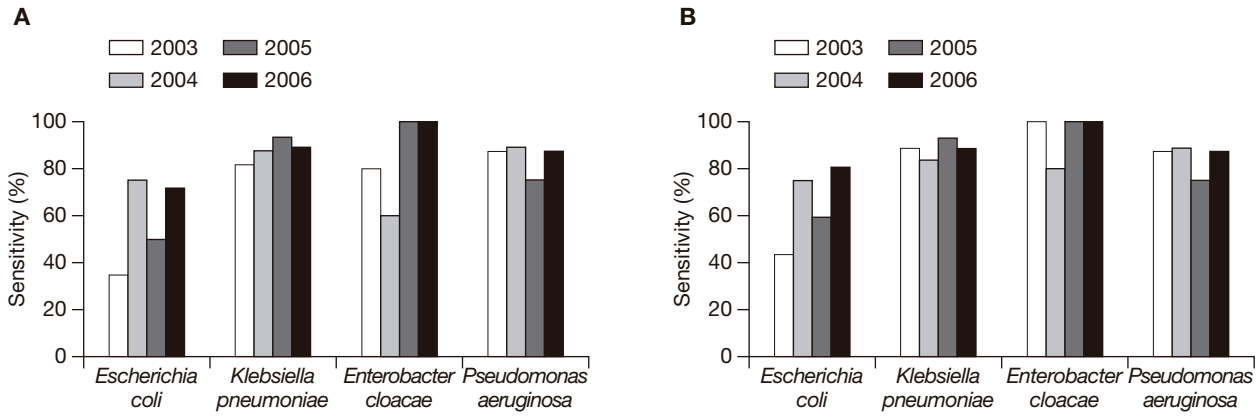

Fig. 4. Susceptibility of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates from patients with intra-abdominal infections to ciprofloxacin (A) and levofloxacin (B) at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

the highest rate of ESBL production was in 2003. The incidence of ESBL-producing isolates declined in 2005 and 2006 (Fig. 5).

Among the ESBL-producing *Enterobacteriaceae* isolates, ertapenem (97%) and imipenem (96%) were the most active agents, and fluoroquinolones (36% to 46%) and cefoxitin (55%) exhibited the poorest activities (Fig. 6). Susceptibility rates to ertapenem and imipenem were not significantly different among ESBL-producing and non-ESBL–producing *E. coli* isolates (Table 1).

For *P. aeruginosa* isolates, levofloxacin (86%), ciprofloxacin (83%), amikacin (91%), and imipenem (80%) had high susceptibility. A modest annual variation in susceptibility rates to ciprofloxacin and levofloxacin was also noted for *P. aeruginosa* (Fig. 4A and Fig. 4B). For *A. baumannii* isolates, imipenem (67%) was the most active agent. Low susceptibility rates (4% to 41%) were found for the other agents tested (data not shown).

Discussion

Several ongoing antimicrobial surveillance studies have provided antimicrobial resistance and epidemiological data that are useful for guiding empiric antibiotic treatment of IAI [1-5]. The SMART program data from 2002 to 2006 at the NTUH show that *Enterobacteriaceae* constituted 68% of aerobic and facultative anaerobic GNB isolated from patients with IAI and

Table 1. In vitro susceptibility rates (% susceptible) of extended-spectrum β-lactamase (ESBL)– and non-ESBL–producing University Hospital, Taipei, Taiwan, from 2002 to 2006.

Bacteria	No. of isolates (%)	Ertapenem	Imipenem	Cefepime	Cefotaxime	Cefoxitin
Escherichia coli (n = 122)						
Non-ESBL	90 (73.8)	100.0	100.0	100.0	94.7	85.6
ESBL	32 (26.2)	100.0	96.8	0	0	43.8
Difference		0	3.2	100.0	94.7	41.8
95% CI[a]		–4.1-10.7	–1.1-15.8	93.9-100	73.2-98.6	30.0-59.0
Klebsiella spp. (n = 129)						
Non-ESBL	99 (76.7)	100.0	100.0	100.0	100.0	92.9
ESBL	30 (23.3)	96.7	96.7	0	0	80.0
Difference		3.3	3.3	100.0	100.0	12.9
95% CI[a]		–0.5-16.8	–0.5-16.8	94.2-100	85.2-100	0.5-30.9
Enterobacter spp. (n = 36)						
Non-ESBL	29 (80.6)	96.6	100.0	96.6	66.7	3.4
ESBL	7 (19.4)	85.7	85.7	0	0	0
Difference		10.8	14.3	96.6	66.7	3.4
95% CI[a]		–7.1-48.8	0.9-51.8	59.8-99.4	–18.6-84.0	–32.9-17.5

[a]The 95% confidence intervals for the difference calculated as the % susceptibility rate for non-ESBL producers minus the % susceptibility rate
Abbreviation: CI = confidence interval.

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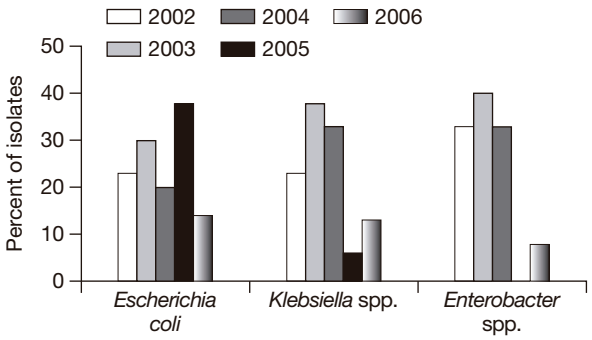

Fig. 5. Extended-spectrum β-lactamase production among isolates of *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. from patients with intra-abdominal infections at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

Klebsiella spp. (26%) were the most common isolates. These regional data are different from the worldwide data reported from the SMART study in 2004 [7], in which *E. coli* was the most common isolate (48%).

The increasing emergence of ESBL-producing *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. associated with IAI poses a major problem for empiric treatment in Taiwan. According to the 2002 to 2006 SMART data from the NTUH summarized in this report, the ESBL rates showed an increasing trend until 2004, then decreased in 2005. A possible explanation for this pattern may be related to antimicrobial control practices in the hospital. In 2004, Taiwan and the NTUH had higher rates of ESBL-producing *E. coli* than any other region of the world (Fig. 7) [7]. The prevalence of ESBL-producing organisms may also be related to differences in antibiotic susceptibilities.

E. coli showed high rates of resistance to fluoroquinolones, with 42% resistance to ciprofloxacin and 36% resistance to levofloxacin. *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa* showed resistance rates to ciprofloxacin and levofloxacin of <20%. Previous studies found an association between fluoroquinolone resistance and ESBL production [12,13]. Thus, decreasing trends of ESBL production might explain a stabilization or decrease in the rates of fluoroquinolone resistance.

The international guidelines for the empirical treatment of community-acquired complicated IAI include the 2002 Surgical Infections Society (SIS) guidelines [14], the 2003 Infectious Diseases Society of America (IDSA) guidelines [15], and 2006 guidelines for empirical therapy for complicated IAI in Asia [16]. These guidelines consistently recommend ampicillin-sulbactam for patients with IAIs of mild-to-moderate severity. However, this recommendation would be inappropriate at the NTUH because of the high resistance rates reported in this study. With respect to the fluoroquinolones, the SIS guidelines recommend ciprofloxacin only for high-risk patients [14]. However, in the IDSA guidelines, ciprofloxacin is recommended for mild-to-moderate and high-risk patients, and levofloxacin, moxifloxacin, and gatifloxacin are recommended for mild-to-moderate infections [15]. Fluoroquinolones are not recommended in the Asian

Escherichia coli, *Klebsiella* spp., and *Enterobacter* spp. isolated from patients with intra-abdominal infections at the National Taiwan

Ceftazidime	Ceftriaxone	Ampicillin-sulbactam	Piperacillin-tazobactam	Amikacin	Ciprofloxacin	Levofloxacin
92.2	94.4	31.1	85.6	100.0	73.3	58.9
0	0	0	75.0	90.6	15.6	25.0
92.2	94.4	31.1	10.6	9.4	57.7	33.9
81.0-96.2	83.3-97.6	19.6-41.3	–4.2-28.9	3.2-24.3	39.2-70.5	14.0-49.8
97.0	98.0	53.5	98.0	100.0	100.0	78.8
0	0	0	60.0	53.3	50.0	60.0
97.0	98.0	53.5	38.0	46.7	50.0	18.8
85.4-99.0	86.4-99.5	41.2-63.1	22.1-55.9	30.2-63.9	33.1-66.9	0.8-38.2
65.5	72.4	6.9	86.2	96.6	96.6	86.2
0	0	0	42.9	85.7	42.9	57.1
65.5	72.4	6.9	43.3	10.8	53.7	29.1
26.4-80.2	33.4-85.4	–29.8-22.3	7.0-73.5	–7.1-48.8	19.9-81.7	–3.2-63.8

for ESBL producers were determined using the Miettinen and Nurminen method [11].

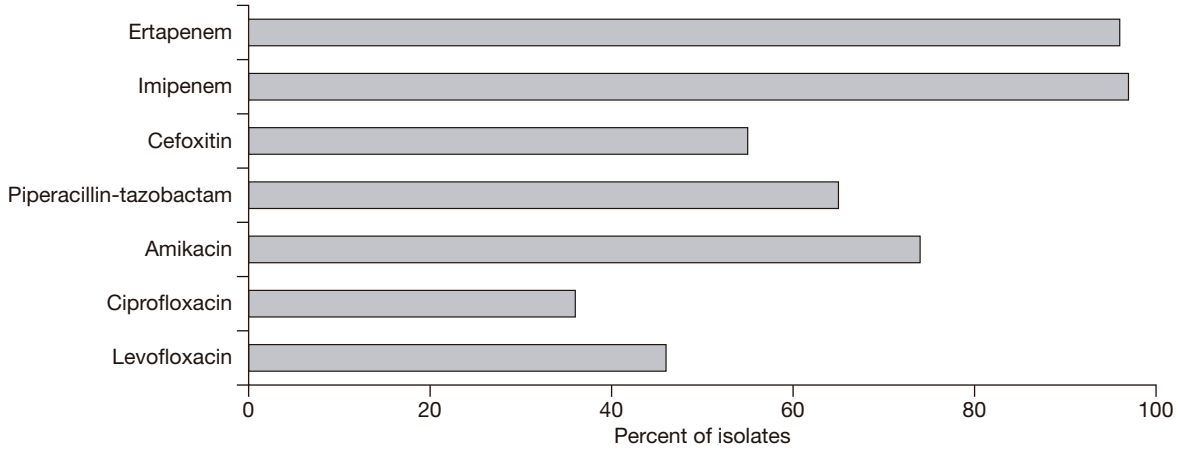

Fig. 6. Susceptibility of extended-spectrum β-lactamase–producing *Enterobacteriaceae* isolates from patients with intra-abdominal infections at the National Taiwan University Hospital, Taipei, Taiwan, from 2002 to 2006.

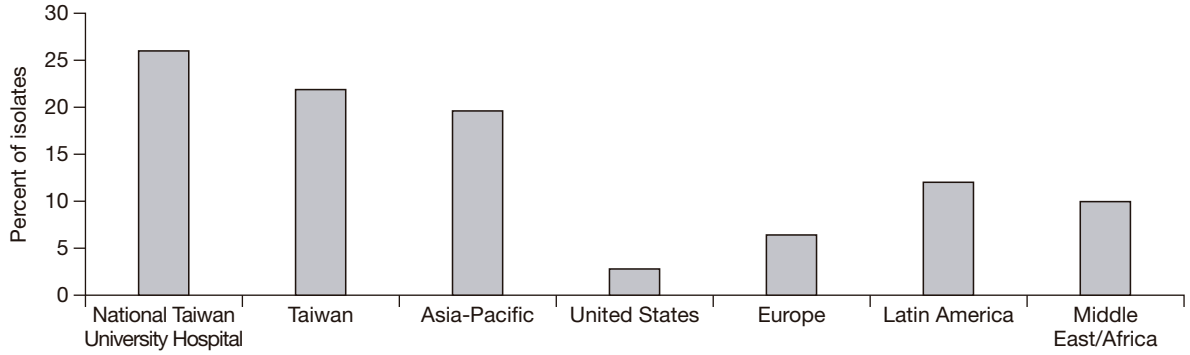

Fig. 7. Extended-spectrum β-lactamase production among *Escherichia coli* isolates from patients with intra-abdominal infections in Taiwan and other regions of the world in 2004 [7].

IAI guidelines [16]. Regional epidemiological data and fluoroquinolone resistance should be considered for the development and updating of guidelines for empirical antimicrobial therapy. The data from the NTUH summarized in this study demonstrate an annual increase in the susceptibility rate to fluoroquinolones during 2002 and 2006. An empiric regimen including levofloxacin or ciprofloxacin may be considered for patients at mild-to-moderate risk.

Two major drawbacks of this study are noted. First, resistance patterns among isolates causing nosocomial and community-acquired IAIs are not available. Second, the method used in the SMART study for screening ESBL production among *Enterobacteriaceae* was not that recommended by the CLSI. These partly limit the clinical implications of using these resistance patterns for empirical treatment of IAIs.

In conclusion, ertapenem, imipenem, and amikacin were the most consistently active agents against *Enterobacteriaceae* isolates from patients with IAIs during 2002 to 2006 at this large tertiary care university hospital located in northern Taiwan. Continuous surveillance of the general trends of antimicrobial resistance patterns among GNB isolated from IAIs is crucial to provide guidance for choosing antibiotic regimens for the empirical treatment of IAIs.

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