Isolation of Imipenem-Resistant *Enterobacter* Species: Emergence of KPC-2 Carbapenemase, Molecular Characterization, Epidemiology, and Outcomes[†]

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The prevalence of isolation of imipenem-resistant *Enterobacter* (IRE) strains is rising, with potential serious consequences in terms of patients' outcomes and general care. The study objective was to define the various epidemiological aspects of the isolation of these strains in comparison to cases of isolation of imipenem-susceptible *Enterobacter* (ISE) strains. Molecular analysis of IRE strains included genotyping and defining the presence of carbapenemases. We conducted a matched retrospective case-control study of patients hospitalized from April 2003 to December 2006. Each IRE case was matched with an ISE case by age and source of isolation. A multivariate analysis using conditional logistic regression was performed to compare the two patient groups. There were 33 cases of IRE isolations during the study period. Twenty isolates were analyzed and found to belong to three distinct pulsotypes. Cell extracts of all of these isolates hydrolyzed imipenem. PCR and sequencing revealed that these isolates harbored a KPC-2 gene. In multivariate analysis, a high invasive-device score ($P = 0.02$) remained a predictor of IRE isolation. The mortality in the IRE group was 33%, compared to 9% among controls. Being an IRE case was significantly associated with increased mortality after controlling for confounders in a multivariate model (odds ratio, 8.3 ± 8.6 ; 95% confidence interval, 1.07 to 64; $P = 0.043$). Resistance to imipenem due to *bla*_{KPC-2} among *Enterobacter* isolates has occurred in several clones in Tel Aviv, affecting particularly patients with multiple invasive devices compared to ISE controls. IRE infections are associated with increased mortality. Enhanced measures to control the hospital spread of IRE are warranted.

Enterobacter species are among the most common causes of gram-negative health care-associated infections (12, 20, 24, 28, 30, 35), causing 8% of nosocomial bacteremia cases, and are the second most common gram-negative pathogens causing pneumonia in patients admitted to intensive care units (ICUs) (19, 24, 28, 30). In addition, in recent years, they are an increasing cause of community-acquired infections as well (30).

Resistance to a variety of broad-spectrum antimicrobials among *Enterobacter* strains, including β -lactams, is frequently encountered (20, 26, 35). Moreover, the emergence of resistance to extended-spectrum cephalosporins occurs often during therapy (8). *Enterobacter* isolates contain a chromosomal group 1 β -lactamase (AmpC), which, when expressed in large quantities by either the induction or the selection of derepressed mutants, can hydrolyze expanded-spectrum cephalosporins, penicillins, and monobactams (29, 30). Resistance to β -lactams is usually mediated through β -lactamase production or an alteration of porins, although other mechanisms such as target site modifications (e.g., in penicillin binding proteins) and drug efflux pumps have also been reported (35). Risk factors for *Enterobacter* infections and for extended-spectrum cephalosporin-resistant *Enterobacter* infections have been extensively investigated in the past (11, 13, 20, 21, 30). These resistant pathogens pose a major therapeutic challenge and are

associated with treatment failures, which lead to increased mortality, morbidity, and cost (10, 20, 28, 30, 33).

Carbapenems are the most potent β -lactam antibiotics (2, 14, 27) and are the most reliable β -lactams for the treatment of serious infections caused by resistant *Enterobacter* strains (14, 27, 28, 33, 35). The carbapenem group is considered the class of last resort against resistant *Enterobacteriaceae*. Thus, the recent reports on resistance to carbapenems among *Enterobacter* isolates are of great concern (2, 12, 26, 33–35). In general, carbapenem resistance may be mediated by three major mechanisms: (i) the hyperproduction of a β -lactamase with weak carbapenem-hydrolyzing activity (such as AmpC-type cephalosporinase or an extended-spectrum β -lactamase) combined with decreased drug permeability through the outer membrane (i.e., outer membrane porin loss or hyperproduction of efflux pumps), (ii) a decreased affinity of the penicillin binding proteins that constitute target proteins for carbapenems, and (iii) carbapenem-hydrolyzing β -lactamase production (26, 34, 35). In the United States, an increase in the occurrence of carbapenem-resistant *Enterobacter* strains has been observed: Intensive Care Antimicrobial Resistance Epidemiology reports for the years 1998 to 2004 found pooled means of 0.5 to 1% imipenem resistance among *Enterobacter* strains causing infections, with 10% of the hospitals belonging to the project reporting proportions higher than 3.8% in ICUs (24). Despite these troubling trends and the importance of this issue from both clinical and public health perspectives, epidemiological studies are still lacking.

Study objectives were to examine the mechanisms of resistance of imipenem-resistant *Enterobacter* (IRE) strains in our institution and to evaluate the predictors for imipenem resis-

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tance among patients from whom *Enterobacter* strains were isolated. In addition, we aimed to examine the direct impact of imipenem resistance on the outcomes of patients from whom *Enterobacter* strains were isolated.

MATERIALS AND METHODS

Setting. The Tel Aviv Sourasky Medical Center (TASMC) is a 1,100-bed tertiary care teaching hospital comprised of 45 wards, with almost 100,000 admissions and 90,000 microbiological cultures processed annually. Hospital computerized databases record all isolations of IRE.

Enterobacter isolates were identified to the species level via the Vitek-2 system (bioMérieux, Hazelwood, MO). Antibiotic susceptibility testing was performed using the Vitek 2 AST GN09 card, and susceptibilities to imipenem and meropenem were confirmed by both disk diffusion and Etest (AB Biodisk, Solna, Sweden). All isolates were processed according to the Clinical and Laboratory Standards Institute (CLSI) criteria (9). IRE isolates were routinely stored at -70°C for further workup.

Infection control practices during the study period. Throughout the study period, isolation precautions for the duration of the hospital stay were advised for patients from whom IRE was isolated. In selected wards, e.g., neonatal ICU and pediatric wards, nursing staff were dedicated to treat IRE patients only. In all cases, less than 24 h after the identification of a new IRE case, ward personnel were called by infection control practitioners to confirm adherence to contact isolation. In almost all cases, isolation was done in a single-patient room and included a sign bearing the words, "contact isolation," single-use gowns and gloves for patient contact, and alcohol-based hand disinfectant. Periodic surveys revealed that the supplies required for contact isolation were present over 90% of the time; however, compliance was not recorded systematically.

Study design. A retrospective, matched, case-control study was initiated, including all available patients from whom IRE was isolated from a clinical specimen (i.e., not surveillance) at the TASMC, from 1 April 2003 to 31 December 2006. Each patient was included in the study only once, at the first episode of IRE isolation. Adult as well as pediatric patients were eligible for inclusion. Controls were matched to cases in a 1:1 ratio according to age group and the source of their clinical culture (blood, urine, respiratory, and line-associated sources, etc.) and were randomly selected from patients from whom imipenem-susceptible *Enterobacter* (ISE) strains were isolated who were hospitalized during the same time intervals as case patients. Random selection was performed by applying an automatic random number-generating function to the list of eligible controls.

Epidemiologic data collection and definitions. Epidemiologic data were collected via chart review using a uniform electronic questionnaire. Parameters assessed for each patient were as follows: (i) demographics, including age and sex; (ii) site of infection acquisition (an isolate was considered to be hospital acquired if the culture was drawn >48 h after admission or if the patient was hospitalized in the preceding month); (iii) infection versus colonization (infection was defined if the isolation was accompanied by clinical features compatible with systemic inflammatory response syndrome) (4); (iv) spectrum of in vitro resistance to cefuroxime, ceftriaxone, ceftazidime, piperacillin-tazobactam, ciprofloxacin, levofloxacin, gentamicin, amikacin, meropenem, and colistin; (v) infection site, including blood, urine, and respiratory tract, etc.; (vi) nosocomial environmental exposures, including months from last hospitalization, presence in an ICU during current hospitalization, months since last ICU stay, hospital ward at the time of culture, and previous long-term care facility residence; (vii) chronic comorbidities and substance abuse, including calculation of the Charlson comorbidity score (7); (viii) status and prognosis at admission, including functional status, level of consciousness, and severity of illness defined according to the McCabe score (3); (ix) surgery and/or invasive procedures before and after isolation; (x) use of chronic invasive devices; (xi) immunosuppressive states, including courses (of at least 5 days) of glucocorticoids in the preceding month; (xii) laboratory data, including renal function, nutritional status (serum albumin level), and liver function tests; (xiii) exposure to antimicrobials before admission, before isolation, during the *Enterobacter* isolation, and after the isolation; (xiv) hours to initiation of appropriate antimicrobial therapy (appropriateness was defined according to in vitro test susceptibilities); and (xv) outcomes, including in-hospital mortality, functional status at discharge for those who lived, discharge disposition (home, long-term care facility, or other hospital), and length of stay.

Analysis of mechanism of resistance and molecular epidemiology. The IRE isolates that were available for genotyping were classified into genetic clusters using pulsed-field gel electrophoresis. Bacterial DNA was prepared and cleaved with 20 U *SpeI* endonuclease (New England Biolabs, Boston, MA) as previously

described (28). Electrophoresis was performed in a 1% agarose gel (BMA Products, Rockland, ME) prepared and run in $0.5\times$ Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio-Rad Laboratories, Richmond, CA). The initial switch time was 10 s, the final switch time was 45 s, and the run time was 23 h at 6 V/cm. Gels were stained in ethidium bromide, destained in distilled water, and then photographed (GelDoc 2000; Bio-Rad). DNA restriction patterns were analyzed, compared, and interpreted according to criteria established previously by Tenover et al. (32).

β -Lactamases were analyzed using isoelectric focusing on crude enzyme preparations from sonicated cell cultures of IRE isolates grown on tryptic soy broth (Biolife Italiana, Milan, Italy). Isoelectric focusing was performed according to methods described previously by Matthew and Harris (23) using an electrophoresis system apparatus on prepared polyacrylamide gel electrophoresis plates (pH 3.5 to 9.5; Amersham Biosciences, Buckinghamshire, United Kingdom). β -Lactamase activity was revealed with nitrocefin (0.5 mg/ml; Calbiochem-Novabiochem Corp., San Diego, CA), and pIs were determined by running β -lactamases with known pIs in parallel as controls. Screening for the production of metallo- β -lactamase was done by disk approximation tests using EDTA and 2-mercapto- β -lactamase activity (1). Identification of the carbapenemase genes was determined by PCR using specific primers designed for identifying known class A β -lactamase genes including *bla*_{KPC}, *bla*_{SME}, *bla*_{IMI}, and *bla*_{NMC} (6).

PCR conditions were as follows: 15 min at 95°C and 35 cycles of 1 min at 94°C , 2 min at 68°C , and 3 min at 72°C , followed by an extension step of 10 min at 72°C . PCR were performed with Hot-StarTaq DNA polymerase (Qiagen, Hilden, Germany), and the resulting PCR products were analyzed in a 1% agarose gel. Full-length *bla*_{KPC} PCR products were ligated into a pGEM-T Easy PCR cloning vector and transformed into competent cells of *Escherichia coli* JM109 according to the manufacturer's instructions (Promega, Madison, WI). Sequencing of cloned genes was performed by using SP6 and T7 promoter primers. Sequences were analyzed with an ABI Prism 3100 genetic analyzer (PE Biosystems), using DNA sequencing analysis software and 3100 data collection software, version 1.1. The nucleotide acid and the deduced protein sequences were analyzed and compared using software available via the Internet at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

Statistical analysis. A matched analysis was conducted to compare cases and controls in order to reflect the study design. Continuous variables were compared between groups by paired *t* test. Categorical variables were compared by χ^2 test for matched pairs. All statistical analyses were conducted using SPSS (version 13.0; SPSS Inc., Chicago, IL) and STATA (version 9.0; STATA Corp., College Station, TX). In order to adjust for confounders and to identify the independent predictors for IRE isolation and for in-hospital mortality, multivariate analyses were performed by using conditional (fixed-effect) logistic regression. For model building, parameters with *P* values of ≤ 0.1 between groups in the univariate analysis were entered into the model, and parameters with *P* values of ≤ 0.05 were retained in the model. Variables with *P* values of ≤ 0.05 in the multivariable analysis were considered to be statistically significant.

RESULTS

Thirty-three patients with IRE in clinical culture were identified at the TASMC during the study years; all were included in the epidemiological analysis. Twenty of the IRE strains were available for further study. One of the strains was identified as being *Enterobacter aerogenes* and therefore was not genotyped, and another was untypeable. The 18 genotyped strains were distributed among three distinct clones: 11 belonged to clone A, 5 belonged to clone B, and 2 belonged to clone C.

All IRE isolates were resistant to ertapenem. Twenty-one isolates (64%) were resistant to meropenem, four (12%) were intermediate (MIC, 8 $\mu\text{g/ml}$), and eight (24%) were reported as being susceptible to meropenem but with an elevated MIC of 4 to 6 $\mu\text{g/ml}$.

Cell extracts of five representative IRE strains showed imipenem hydrolysis activity, indicating the production of a β -lactamase with carbapenemase activity. Phenotypic screening for metallo- β -lactamase was negative for all 20 IRE strains. Two distinct β -lactamase bands were observed in the IRE strains, focusing at pI 5.4 (corresponding to *bla*_{TEM}) and at pI 6.7

(corresponding to *bla*_{KPC}). PCR screening for specific carbapenem-hydrolyzing β -lactamases and sequencing revealed the presence of *bla*_{KPC-2} in all 20 IRE strains, corresponding to the β -lactamase that focused at pI 6.7. All other screening PCRs using primers specific for known class A carbapenemases were negative.

The 33 IRE cases were distributed in multiple wards and services, with three apparent small time and space clusters: during June to July 2004, three cases affected the neonatal ICU (Y. Carmeli, G. Grisaru-Soen, A. Leavitt, M. Schwaber, S. Dolberg, and S. Navon-Venezia, presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2006); during April 2006, three cases occurred in a medical ward; and during October 2006, two cases occurred in a pediatric ICU. All cases were investigated by the hospital infection control team at the time of occurrence to determine whether they belonged to a cluster and to find a common source, but except for the small clusters listed above, no investigation yielded a clear link between the cases (data not shown).

Of the 66 *Enterobacter* isolates (33 from the case patients and 33 from their matched controls), 56 were identified as being *Enterobacter cloacae*, nine were *E. aerogenes*, and one was *Enterobacter gergoviae*. In eight of the matched pairs, the *Enterobacter* isolation originated from the blood, eight were from urine, seven were from respiratory secretions, six were from skin and soft tissue (including surgical sites), two were from central venous catheters, one was from the middle ear, and one was from peritoneal fluids. Twenty-four of the matched pairs were adults (median age, 74.5 years; range 27 to 94 years), four were children, and five were neonates. Five of the IRE patients (15%) had previous clinical isolations of ISE prior to the isolation of the resistant strain.

The strains of the IRE group were multidrug resistant and expressed enhanced resistance to various antibiotics in comparison to the ISE group, including ceftriaxone (33 versus 14, respectively; $P < 0.001$), piperacillin-tazobactam (33 versus 8, respectively; $P < 0.001$), gentamicin (30 versus 6, respectively; $P < 0.001$), and ciprofloxacin (6 versus 1, respectively; $P = 0.05$).

Table 1 depicts the univariate matched analysis of predictors differentiating between cases and controls. A multivariate model was constructed to adjust for confounding variables. To account for interactions between various variables related to invasive devices, a dichotomized procedure score was constructed. Patients with a high procedure score included those who were intubated, patients who had a central venous line and a urinary catheter, or patients who had a central venous line and a nasogastric tube. The only independent predictor in the multivariate analysis was a high procedure score (odds ratio [OR], 4.93; 95% confidence interval [CI], 1.3 to 18.6; $P = 0.02$), and an immunosuppressive state almost reached significance in the multivariate model (OR, 3.23; 95% CI, 0.9 to 11.6; $P = 0.07$).

In the univariate analysis, as depicted in Table 2, being an IRE case was significantly associated with worse outcomes: increased mortality (OR, 5; 95% CI, 1.09 to 22.8; $P = 0.038$), morbidity as expressed by the need for intubation (OR, 3.25; 95% CI, 1.05 to 10; $P = 0.04$), worsening of functional status at discharge compared to that at admission (OR, 3.33; 95% CI,

1.43 to 10; $P = 0.001$), and the need to be discharged to an intermediate or long-term care facility (OR, 3.03; 95% CI, 1.43 to 5; $P = 0.006$). Lengths of hospital stay before and after the isolation did not differ between groups. A multivariate analysis controlling for confounding was performed only for in-hospital mortality. Being an IRE case remained independently associated with increased in-hospital mortality in multivariate analyses (OR, 8.3 ± 8.6 ; 95% CI, 1.07 to 64; $P = 0.043$). The other factors that were significantly associated with increased mortality in the model were a high procedure score at the time of isolation ($P = 0.003$), a low McCabe score ($P = 0.008$) (5), and ICU stay preceding the isolation ($P = 0.036$).

There were 11 patients who received appropriate empirical therapy within 48 h among the IRE group, versus 21 among the ISE group ($P = 0.027$; 95% CI, 0.09 to 0.87). Overall, treatment was delayed for a mean of 32.9 ± 6.4 h for IRE patients, versus 12 ± 4.6 h for the ISE group ($P = 0.033$; 95% CI, 1.001 to 1.035). The delay in effective therapy did not affect the in-hospital mortality (six versus eight patient deaths, respectively; $P = 0.64$) but was found to increase the length of hospitalization among survivors (34 ± 3.2 days versus 26.5 ± 5.4 days, respectively; $P = 0.02$).

DISCUSSION

Carbapenem resistance among the *Enterobacteriaceae* is an emerging phenomenon of great clinical and public health importance. While carbapenem resistance among *Klebsiella* species isolates has been studied (6; M. Schwaber, S. Klarfeld-Lidji, S. Navon-Venezia, D. Schwartz, A. Leavitt, and A. Carmeli, presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2007), no clinical or epidemiological studies have thus far focused on carbapenem resistance in *Enterobacter* spp. In the present study, we aimed to understand the clinical, epidemiological, and molecular characteristics of imipenem resistance among *Enterobacter* spp. isolated in a single institution.

During the 45 months of the study period, 33 patients were affected, but most cases did not cluster in time and space, and no common source was found. Molecular epidemiology revealed that the isolates belonged to three distinct clones and that all isolates carried *bla*_{KPC-2}. This suggests that resistance to imipenem among *Enterobacter* sp. isolates is through the spread of a single enzyme-encoding gene by pauciclonal dissemination, which in most cases could not be linked to a patient-to-patient transmission event or to a common source. This phenomenon may be explained by the relatively rare detection of clinical cases; i.e., the hidden reservoir of carriers may be much larger than the detected proportion. However, during 2007, we screened 390 hospitalized patients for rectal carriage of IRE using both conventional microbiologic methods (selective medium containing 1 μ g/ml of imipenem) and PCR for *bla*_{KPC-2}, and none of the screened patients was positive (our unpublished data). Therefore, other possible explanations include a common source that has not yet been detected, such as equipment, disposable items, or food; a colonized health care worker who serves multiple wards and is an intermittent transmitter; or a reservoir in the community and transfer of IRE strains into the hospital, which manifest as

TABLE 1. Univariate matched analysis of isolation of IRE strains versus isolation of ISE strains

Parameter ^a	Value for group		P value
	IRE (n = 33)	ISE (n = 33)	
General			
Adult mean age (yr) ± SD	70.7 ± 15.4	71.5 ± 16.5	0.5
No. of female patients	11	13	0.4
No. of patients with colonization without infection	8	12	0.26
No. of patients with hospital-acquired infection	32	26	0.07
Mean no. of days admitted before culture ± SD	12 ± 1.8	8.5 ± 1.5	0.15
No. of patients with hospitalization in past 6 mo	16	17	0.47
Mean no. of mo from last hospitalization ± SD	13.2 ± 5.6	17 ± 7	0.34
No. of patients in long-term care facility residency	5	9	0.26
Mean McCabe score at admission ^b ± SD	2.1 ± 0.1	2.3 ± 0.13	0.15
No. of patients with independent functional status at admission	7	9	0.2
No. of patients with reduced consciousness at admission	19	10	0.032
Mean albumin level (mg/liter) at admission ± SD	30.3 ± 1.4	32.9 ± 1.3	0.14
No. of patients with ICU stay preceding culture^c	21	14	0.07
No. of patients with surgery preceding isolation during current hospitalization	17	16	0.8
No. of patients who underwent invasive procedure^d preceding isolation during current hospitalization	26	14	0.02
No. of patients intubated at isolation	22	11	0.02
No. of patients with central line at isolation	21	9	0.01
No. of patients with urinary catheter at isolation	26	14	0.01
No. of patients with nasogastric tube at isolation	22	8	0.01
No. of patients harboring other resistant organisms^e	17	9	0.04
No. of patients with previous isolations of other organisms^f	21	13	0.07
Department			
No. of patients in department of:			
Medicine	11	14	0.4
Surgery	4	7	0.32
Adult ICU	9	3	0.06
Pediatric/neonate ICU	9	6	0.38
Antimicrobial			
No. of patients admitted on antibiotics	7	7	0.74
No. of patients who received antibiotics during current hospitalization prior to isolation	32	30	0.34
No. of patients who received carbapenems during current hospitalization prior to isolation	11	3	0.04
No. of patients who received β-lactams during current hospitalization prior to isolation	27	24	0.37
No. of patients who received minoglycosides during current hospitalization prior to isolation	8	8	1.0
No. of patients who received fluoroquinolones during current hospitalization prior to isolation	2	2	1.0
No. of patients who received macrolides during current hospitalization prior to isolation	6	1	0.09
Background			
No. of patients with background of:			
Immunosuppressive states^g	18	10	0.05
Substance abuse	7	5	0.5
Surgery in the past yr	21	17	0.35
ICU in the past yr	25	15	0.02
Invasive procedure^d in the past 6 mo	29	20	0.03
Chronic tracheotomy	4	3	0.7
Permanent central line catheter	2	2	1.0
Permanent urinary catheter	3	3	1.0
Chronic dialysis	1	2	0.57
Permanent gastrostomy	4	4	1.0
External fixator	2	4	0.4
Cardiovascular disease	20	12	0.04
Pulmonary disease	9	10	0.76
Liver disease	1	4	0.21
Renal disease	10	8	0.6
Diabetes	8	5	0.32
Neurological disease	18	16	0.59
Posttransplantation	1	1	1.0
Malignant disease (present and past)	12	10	0.59
Charlson comorbidity ^h			
Mean weighted index of comorbidity ± SD	5.5 ± 0.5	4.6 ± 0.6	0.28
Mean combined condition and age-related score ± SD	8.3 ± 0.7	7.3 ± 0.6	0.28
Mean % estimated 10-yr survival ± SD	11 ± 5.7	16 ± 6.3	0.3

^a Parameters that were inserted into the multivariate model are shown in boldface type.^b See reference 3.^c ICU stay preceding index isolation during current hospitalization.^d Endoscopy of any kind, urinary catheterization, any percutaneous procedure (central line insertion, external biliary drainage, and thoracocentesis, etc.), tracheotomy, and more.^e Clinical isolation in the past year of one of the following organisms: ceftazidime-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, or extended-spectrum- β -lactamase-producing *Enterobacteriaceae*.^f Additional isolations of other organisms in the index hospitalization prior to the isolation of an *Enterobacter* strain.^g Glucocorticoid course (≥ 5 days) in the past month, chemotherapy in the past 3 months, radiotherapy in the past 3 months, human immunodeficiency virus or AIDS, neutropenia at culture, and status posttransplantation.^h See reference 7.

TABLE 2. Univariate matched analysis between outcomes of cases of isolation of IRE strains versus cases of isolation of ISE strains

Parameter	Value for group		P value
	IRE (n = 33)	ISE (n = 33)	
No. of patients with worsening functional status compared to that at admission	11	3	0.001
No. of patients discharged to long-term care facility ^a	5	0	0.006
No. of patients who underwent surgery postisolation	6	3	0.27
No. of patients who underwent invasive procedure ^b postisolation	14	4	0.02
No. of patients who were intubated postisolation	18	9	0.04
No. of patients who underwent dialysis postisolation ^c	7	0	0.08
Mean no. of consecutive days on antibiotics after isolation $\pm$ SD	14 $\pm$ 4.9	21.7 $\pm$ 13	0.65
No. of patients admitted to ICU after infection	16	10	0.09
Mean no. of hospital days after infection (excluding dead) $\pm$ SD	17 $\pm$ 3.5	23.6 $\pm$ 5.7	0.6
Mean no. of hospital days after infection (not excluding dead) $\pm$ SD	18.2 $\pm$ 3.4	1.9 $\pm$ 5.2	0.56
In-hospital mortality	11	3	0.038

^a Does not include patients who were residents of a long-term care facility prior to the index hospitalization.

^b Endoscopy of any kind, urinary catheterization, any percutaneous procedure (central line insertion, external biliary drainage, and thoracocentesis, etc.), tracheotomy, and more.

^c Includes only cases not treated with dialysis before the index infection.

infection only late during hospitalization due to the opportunistic nature of the organism.

In addition, we examined the factors that independently predicted imipenem resistance among patients from whom *Enterobacter* spp. were isolated and found that multiple invasive devices (with an immunocompromised state almost reaching significance as well), but not recent use of antibiotics in general or of specific classes of antibiotics, predicted imipenem resistance. These observations also support the molecular epidemiology findings that the dissemination of imipenem resistance among *Enterobacter* isolates is related primarily to transmission rather than to antibiotic-associated selection pressure leading to the perpetuation of de novo mutations.

As our analysis compared patients with IRE to patients with susceptible isolates (ISE), the results allow us to predict who among all patients from whom *Enterobacter* sp. strains were isolated will have an imipenem-resistant strain. They do not, however, imply causality, as the study did not compare patients with IRE to the source population, i.e., all hospitalized patients (15–18, 31).

The epidemiology of IRE infections differs from that observed in our hospital for imipenem-resistant *E. coli* infections, in which each of the four isolates reported belonged to different clones, and although all of those isolates also carried *bla*_{KPC-2}, plasmids among isolates differed (25). The epidemiology of IRE infections also differed from that of *Klebsiella pneumoniae* carrying *bla*_{KPC-2}, in which multiple clones were detected (22). All the isolates carrying *bla*_{KPC-2} (*E. coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp.) in this investigation did not cluster in time and space, and no dissemination pattern was revealed by epidemiological investigation. This finding is in contrast to the epidemiology of the infections caused by *Klebsiella* spp. carrying *bla*_{KPC-3}, where a single clone was responsible for all cases, clearly implicating patient-to-patient transmission (22).

Ertapenem susceptibility testing detected all 33 IRE isolates, while meropenem susceptibility testing misclassified 24% of the isolates as being susceptible. Imipenem and meropenem are often used as a class marker for carbapenem susceptibility (9). Our results suggest that in the era of emerging carbapenem resistance, this may no longer be an acceptable strategy,

as it may lead to major errors in classifying resistant organisms as susceptible, which in turn may have important clinical and infection control implications.

When patient outcomes were examined, we found that isolations of IRE were independently associated with increased mortality, even after strictly controlling for various confounders. This is not a surprising finding, as carbapenems are the class of last resort to treat multidrug-resistant infections due to the *Enterobacteriaceae*, and indeed, IRE isolates were multidrug resistant. Thus, treatment of these patients was delayed, and effective therapy was often not possible. Similar deleterious effects on patient outcomes have been observed with other imipenem-resistant isolates of the *Enterobacteriaceae* (5; M. Schwaber, S. Klarfeld-Lidji, S. Navon-Venezia, D. Schwartz, A. Leavitt, and A. Carmeli, presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2007).

We conclude that resistance to imipenem due to KPC-2 among *Enterobacter* isolates has occurred in several clones in Tel Aviv, although the spread of these genes and clones is still not well explained. According to our investigation, the isolation of IRE strains affects mostly immunocompromised patients with multiple invasive devices, and the infections are associated with increased mortality. Further studies to better define the spread of these strains are required, and enhanced measures to control the spread of IRE are warranted.

REFERENCES

1. Arakawa, Y., N. Shibata, K. Shibayama, H. Kurokawa, T. Yagi, H. Fujiwara, and M. Goto. 2000. Convenient test for screening metallo- β -lactamase-producing gram-negative bacteria by using thiol compounds. *J. Clin. Microbiol.* 38:40–43.
2. Aubron, C., L. Poirel, R. J. Ash, and P. Nordmann. 2005. Carbapenemase-producing *Enterobacteriaceae*, U.S. rivers. *Emerg. Infect. Dis.* 11:260–264.
3. Bion, J. F., S. A. Edlin, G. Ramsay, S. McCabe, and I. M. Ledingham. 1985. Validation of a prognostic score in critically ill patients undergoing transport. *Br. Med. J.* 291:432–434.
4. Bone, R. C., R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. Schein, W. J. Sibbald, et al. 1992. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 101:1644–1655.
5. Bratu, S., S. Brooks, S. Burney, S. Kochar, J. Gupta, D. Landman, and J. Quale. 2007. Detection and spread of *Escherichia coli* possessing the plasmid-borne carbapenemase KPC-2 in Brooklyn, New York. *Clin. Infect. Dis.* 44:972–975.
6. Bratu, S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D.

- Landman. 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, N.Y.: molecular epidemiology and in vitro activity of polymyxin B and other agents. *J. Antimicrob. Chemother.* **56**:128–132.
7. Charlson, M. E., P. Pompei, K. L. Ales, and C. R. MacKenzie. 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J. Chronic Dis.* **40**:373–383.
8. Chow, J. W., M. J. Fine, D. M. Shlaes, J. P. Quinn, D. C. Hooper, M. P. Johnson, R. Ramphal, M. M. Wagener, D. K. Miyashiro, and V. L. Yu. 1991. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann. Intern. Med.* **115**:585–590.
9. Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. Approved standard M100-S16. CLSI, Wayne, PA.
10. Cosgrove, S. E., K. S. Kaye, G. M. Eliopoulos, and Y. Carmeli. 2002. Health and economic outcomes of the emergence of third-generation cephalosporin resistance in Enterobacter species. *Arch. Intern. Med.* **162**:185–190.
11. Davin-Regli, A., D. Monnet, P. Saux, C. Bosi, R. Charrel, A. Barthelemy, and C. Bollet. 1996. Molecular epidemiology of *Enterobacter aerogenes* acquisition: one-year prospective study in two intensive care units. *J. Clin. Microbiol.* **34**:1474–1480.
12. De Gheldre, Y., N. Maes, F. Rost, R. De Ryck, P. Clevenbergh, J. L. Vincent, and M. J. Struelens. 1997. Molecular epidemiology of an outbreak of multidrug-resistant *Enterobacter aerogenes* infections and in vivo emergence of imipenem resistance. *J. Clin. Microbiol.* **35**:152–160.
13. Georgiadi, P. R., R. J. Hamill, C. E. Wright, J. Versalovic, T. Koeuth, D. A. Watson, and J. R. Lupski. 1995. Molecular epidemiology of infections due to Enterobacter aerogenes: identification of hospital outbreak-associated strains by molecular techniques. *Clin. Infect. Dis.* **20**:84–94.
14. Gupta, E., S. Mohanty, S. Sood, B. Dhawan, B. K. Das, and A. Kapil. 2006. Emerging resistance to carbapenems in a tertiary care hospital in north India. *Ind. J. Med. Res.* **124**:95–98.
15. Harris, A. D., Y. Carmeli, M. H. Samore, K. S. Kaye, and E. Perencevich. 2005. Impact of severity of illness bias and control group misclassification bias in case-control studies of antimicrobial-resistant organisms. *Infect. Control Hosp. Epidemiol.* **26**:342–345.
16. Harris, A. D., T. B. Karchmer, Y. Carmeli, and M. H. Samore. 2001. Methodological principles of case-control studies that analyzed risk factors for antibiotic resistance: a systematic review. *Clin. Infect. Dis.* **32**:1055–1061.
17. Harris, A. D., M. H. Samore, and Y. Carmeli. 2000. Control group selection is an important but neglected issue in studies of antibiotic resistance. *Ann. Intern. Med.* **133**:159.
18. Harris, A. D., M. H. Samore, M. Lipsitch, K. S. Kaye, E. Perencevich, and Y. Carmeli. 2002. Control-group selection importance in studies of antimicrobial resistance: examples applied to *Pseudomonas aeruginosa*, enterococci, and *Escherichia coli*. *Clin. Infect. Dis.* **34**:1558–1563.
19. Kang, C. I., S. H. Kim, W. B. Park, K. D. Lee, H. B. Kim, E. C. Kim, M. D. Oh, and K. W. Choe. 2005. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. *Antimicrob. Agents Chemother.* **49**:760–766.
20. Kang, C. I., S. H. Kim, W. B. Park, K. D. Lee, H. B. Kim, M. D. Oh, E. C. Kim, and K. W. Choe. 2004. Bloodstream infections caused by Enterobacter species: predictors of 30-day mortality rate and impact of broad-spectrum cephalosporin resistance on outcome. *Clin. Infect. Dis.* **39**:812–818.
21. Kaye, K. S., S. Cosgrove, A. Harris, G. M. Eliopoulos, and Y. Carmeli. 2001. Risk factors for emergence of resistance to broad-spectrum cephalosporins among *Enterobacter* spp. *Antimicrob. Agents Chemother.* **45**:2628–2630.
22. Leavitt, A., S. Navon-Venezia, I. Chmelnitsky, M. J. Schwaber, and Y. Carmeli. 2007. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. *Antimicrob. Agents Chemother.* **51**:3026–3029.
23. Matthew, M., and A. M. Harris. 1976. Identification of beta-lactamases by analytical isoelectric focusing: correlation with bacterial taxonomy. *J. Gen. Microbiol.* **94**:55–67.
24. National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1992 through June 2004, issued October 2004. *Am. J. Infect. Control* **32**:470–485.
25. Navon-Venezia, S., I. Chmelnitsky, A. Leavitt, M. J. Schwaber, D. Schwartz, and Y. Carmeli. 2006. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. *Antimicrob. Agents Chemother.* **50**:3098–3101.
26. Nordmann, P., and L. Poirel. 2002. Emerging carbapenemases in gram-negative aerobes. *Clin. Microbiol. Infect.* **8**:321–331.
27. Potumarthi, S., E. S. Moland, S. Juretschko, S. R. Swanzy, K. S. Thomson, and T. R. Fritzsche. 2003. NmcA carbapenem-hydrolyzing enzyme in Enterobacter cloacae in North America. *Emerg. Infect. Dis.* **9**:999–1002.
28. Schlesinger, J., S. Navon-Venezia, I. Chmelnitsky, O. Hammer-Münz, A. Leavitt, H. S. Gold, M. J. Schwaber, and Y. Carmeli. 2005. Extended-spectrum beta-lactamases among *Enterobacter* isolates obtained in Tel Aviv, Israel. *Antimicrob. Agents Chemother.* **49**:1150–1156.
29. Schwaber, M. J., S. E. Cosgrove, H. S. Gold, K. S. Kaye, and Y. Carmeli. 2004. Fluoroquinolones protective against cephalosporin resistance in gram-negative nosocomial pathogens. *Emerg. Infect. Dis.* **10**:94–99.
30. Schwaber, M. J., C. S. Graham, B. E. Sands, H. S. Gold, and Y. Carmeli. 2003. Treatment with a broad-spectrum cephalosporin versus piperacillin-tazobactam and the risk for isolation of broad-spectrum cephalosporin-resistant *Enterobacter* species. *Antimicrob. Agents Chemother.* **47**:1882–1886.
31. Sunenshine, R. H., M. O. Wright, L. L. Maragakis, A. D. Harris, X. Song, J. Hebdon, S. E. Cosgrove, A. Anderson, J. Carnell, D. B. Jernigan, D. G. Kleinbaum, T. M. Perl, H. C. Standiford, and A. Srinivasan. 2007. Multi-drug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. *Emerg. Infect. Dis.* **13**:97–103.
32. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
33. Tumbarello, M., T. Spanu, M. Sanguinetti, R. Citton, E. Montuori, F. Leone, G. Fadda, and R. Cauda. 2006. Bloodstream infections caused by extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob. Agents Chemother.* **50**:498–504.
34. Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann. 2005. Metallo- β -lactamases: the quiet before the storm? *Clin. Microbiol. Rev.* **18**:306–325.
35. Yigit, H., G. J. Anderson, J. W. Biddle, C. D. Steward, J. K. Rasheed, L. L. Valera, J. E. McGowan, Jr., and F. C. Tenover. 2002. Carbapenem resistance in a clinical isolate of *Enterobacter aerogenes* is associated with decreased expression of OmpF and OmpC porin analogs. *Antimicrob. Agents Chemother.* **46**:3817–3822.