PREVALENCE OF EXTENDED-SPECTRUM β-LACTAMASES AMONG URINARY *ESCHERICHIA COLI* AND *KLEBSIELLA* IN NEW ZEALAND IN 2006

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SUMMARY

Extended-spectrum β-lactamases (ESBLs) confer resistance to third- and fourth-generation cephalosporins and monobactams, in addition to the earlier generation cephalosporins. ESBLs are most common in *Klebsiella pneumoniae* and *Escherichia coli*, but do occur in other Enterobacteriaceae and in *Pseudomonas aeruginosa*.

ESBLs are being increasingly identified in many parts of the world and are now very prevalent in several countries in the Asia-Pacific region. The last national prevalence survey conducted in 2000 identified no ESBLs among urinary *Klebsiella* and 0.1% (2/1760) among urinary *E. coli* in New Zealand. Data from other ESR surveillance systems indicates that ESBLs have increased markedly in New Zealand since 2000. The aim of this survey was to estimate the current prevalence of ESBLs among urinary *E. coli* and *Klebsiella*.

Thirty-eight laboratories, representing approximately 86% of hospital and community microbiology laboratories in New Zealand, participated in the survey. Depending on their cephalosporin susceptibility testing practices, laboratories referred either (1) all urinary *E. coli* and *Klebsiella* isolated during the 4-week survey period in early 2006; (2) isolates intermediate or resistant to the lowest generation cephalosporin tested; (3) isolates that were positive in the CLSI initial screen disc test with a sensitive substrate; or (4), in the case of two very experienced laboratories, isolates that had been confirmed as ESBL producers. Referred isolates were screened and tested for ESBLs by the CLSI screening and confirmatory methods.

A total of 87 ESBL-producing isolates were identified: 56 *E. coli* and 31 *Klebsiella*, equating to prevalence rates of 0.7% and 4.2%, respectively. The majority of the ESBL-producing *E. coli* (78.6%) and *Klebsiella* (58.6%) were reported to be from community-acquired urinary tract infections.

The highest rates of ESBL-producing *E. coli* and *Klebsiella* occurred in Hawkes Bay where notably 40.0% of urinary *Klebsiella* isolated during the survey period were ESBL producers. ESBL-producing *Klebsiella* were also identified in Auckland, Wellington and Canterbury. ESBL-producing *E. coli* were less prevalent but more widespread and, in addition to Hawkes Bay, were identified in Auckland, Waikato, MidCentral, Hutt, Capital and Coast, Nelson-Marlborough, Canterbury and Otago. No ESBL-producing isolates were identified in 9 of the 18 district health board areas. The areas from which ESBL-producing isolates were referred included two areas where neither the local hospital nor community laboratory routinely tested urinary *E. coli* and *Klebsiella* for ESBLs.

The results of this survey show that, at least among urinary *E. coli* and *Klebsiella*, the prevalence of ESBLs has increased significantly ($P \leq 0.05$) in New Zealand since 2000; the prevalence varies considerably throughout the country; and ESBLs are already established as community-acquired pathogens.

RECOMMENDATIONS

- All *E. coli* and *Klebsiella* isolated from sterile sites should be screened for ESBLs using a sensitive substrate(s). Laboratories in areas where ESBL-producing *E. coli* and *Klebsiella* have been isolated should also routinely screen isolates from non-sterile sites. In other areas, laboratories should either routinely screen *E. coli* and *Klebsiella* from non-sterile sites or perform periodic surveys to monitor the current prevalence of ESBLs among local isolates. This recommendation is consistent with the guidelines for the control of multidrug-resistant organisms currently being developed by the Ministry of Health.

- The 87 ESBL-producing isolates identified in this survey should be typed by pulsed-field gel electrophoresis and the ESBL types should be identified by PCR and sequencing. This further investigation would provide information on the extent of any clonal spread of particular strains of ESBL-producing *E. coli* and *Klebsiella* and would indicate the variety of ESBL types occurring in New Zealand.

- To assist in the ongoing identification of outbreaks of ESBL-producing organisms and the strains involved, laboratories should refer isolates to ESR for typing whenever they detect an increase in isolations or suspect the transmission of an ESBL-producing organism.

1 INTRODUCTION

The production of β-lactamase enzymes is the most common mechanism of bacterial resistance to β-lactam antibiotics, such as the penicillins and cephalosporins. These enzymes catalyse the hydrolysis of the β-lactam ring of the antibiotic molecule thereby destroying the antimicrobial activity of the antibiotic. The first plasmid-mediated β-lactamase in gram-negative bacteria, TEM-1, was described in the early 1960s.

Over the last 20 years many new β-lactam antibiotics, specifically designed to resist known β-lactamases, have been developed. However, almost invariably new β-lactamases have emerged to combat each new class of β-lactams.

Plasmid-mediated, extended-spectrum β-lactamases (ESBLs) emerged in gram-negative bacilli in Europe in the 1980s. ESBLs, so named because of their extended spectrum of activity, confer resistance to third- and fourth-generation cephalosporins (eg, ceftriaxone, cefotaxime, ceftazidime, cefepime and cefpirome) and monobactams (eg, aztreonam), in addition to the earlier generation cephalosporins and penicillins.

ESBLs are inhibited in vitro by β-lactamase inhibitors such as clavulanic acid and tazobactam. Some ESBLs are derived from earlier, broad-spectrum β-lactamases (eg, the TEM, SHV and OXA enzyme families) and differ from the parent enzyme by a few point mutations, which confer an extended spectrum of activity. More recently another family of ESBLs, the CTX-M types, has emerged and these ESBLs are becoming increasingly common.[1,2]

Over 150 different ESBLs have been described.[3] ESBLs have been reported worldwide in many different genera of Enterobacteriaceae and in *Pseudomonas aeruginosa*. However, they are most common in *Klebsiella pneumoniae* and *Escherichia coli*. ESBL-producing organisms are often multiresistant to several other classes of antibiotics, as the plasmids with the genes encoding ESBLs often carry other resistance determinants. Initially ESBL-producing organisms were usually isolated from nosocomial infections, but these organisms are now also being isolated from community and rest home patients.[4,5] The fact that ESBLs are plasmid-mediated poses an additional infection control problem as the genetic determinants can readily transfer to other strains and bacterial species.

In standard antimicrobial susceptibility tests, ESBL-producing organisms may demonstrate only intermediate resistance or even test susceptible to cephalosporins and yet such isolates are associated with cephalosporin and monobactam treatment failure.[6,7] Therefore it is important to identify ESBL producers and report them as resistant to all cephalosporins and monobactams.

Before 2000, ESBLs appeared to be uncommon among Enterobacteriaceae in New Zealand. In a 1993 national survey of antimicrobial susceptibility among urinary *E. coli*, all 444 isolates included in the survey were susceptible to ceftriaxone (MICs ≤0.25 mg/L).[8] A 2000 survey of urinary *E. coli* and *Klebsiella*, found no ESBLs among *Klebsiella* and 0.1% among *E. coli*.[9]

Up until August 2005, diagnostic laboratories were requested to refer all probable ESBL-producing Enterobacteriaceae to ESR. Between the years 1996 and 2000, a maximum of 35 ESBL-producing Enterobacteriaceae were referred and confirmed in any one year.[10] From 2001 the number of ESBL-producing Enterobacteriaceae referred to ESR started to increase markedly,

with 83 in 2001 and 737 in 2005.[10] Most of this increase was due to increased isolations in the Auckland and Hawkes Bay areas. Middlemore Hospital, Auckland, reported 0.8% of *E. coli* and 2.6% of *K. pneumoniae* isolated between 2001 and mid-2004 were ESBL producers, with over seven times more isolations in 2004 than 2001.[11]

Susceptibility data collated from hospital and clinical laboratories throughout New Zealand indicates that in 2005 0.8% of *E. coli* from bacteraemia, 0.8% of urinary *E. coli* and 1.6% of *Klebsiella* from bacteraemia were resistant to cefotaxime or ceftriaxone.[12] It is likely that the majority of these resistant isolates were ESBL producers.

The aim of this survey was to obtain data on the current prevalence of ESBLs among urinary *E. coli* and *Klebsiella* throughout New Zealand. Despite the recent increase in isolations of ESBL-producing Enterobacteriaceae in some parts of the country, the overall prevalence was considered likely to be low. Therefore, for this survey, where available, cephalosporin-resistant urinary *E. coli* and *Klebsiella* were collected. Urinary isolates were selected, as the majority of ESBL-producing isolates referred to ESR have been from this site.

2 METHODS

2.1 Participating laboratories and isolate collection

In order to plan the survey, in particular the sample collection period, sample size and specific organisms to be collected, all hospital and community microbiology laboratories in New Zealand were sent a questionnaire to ascertain:

- whether the laboratory was able to participate in the survey
- the approximate number of urinary *E. coli* and *Klebsiella* isolated per week
- whether urinary *E. coli* and *Klebsiella* were tested for cephalosporin susceptibility
- if cephalosporin susceptibility was tested, which cephalosporins were tested
- if cephalosporin susceptibility was tested, the approximate number of cephalosporin-resistant urinary *E. coli* and *Klebsiella* isolated per week.

The sample collection period was set at four weeks, with the majority of participating laboratories referring urinary *E. coli* and *Klebsiella* isolated between 3 and 30 April 2006. Some laboratories used a later 4-week collection period in May or June 2006.

Depending on their cephalosporin susceptibility testing of urinary *E. coli* and *Klebsiella*, participating laboratories were categorised into four groups:

Group 1 Laboratories not routinely testing any cephalosporin susceptibility either referred all urinary *E. coli* and *Klebsiella* isolated during the 4-week collection period or referred the first 100 *E. coli* and the first 50 *Klebsiella*, whichever target was reached sooner. If the latter target was used, the laboratory reported how many urinary *E. coli* and *Klebsiella* were isolated during the first week of the collection period to allow the results to be adjusted to represent the standard 4-week collection period.

Group 2 Laboratories routinely testing susceptibility to a first- and/or second-generation cephalosporin referred all urinary *E. coli* and *Klebsiella* with intermediate or full resistance to the lowest generation cephalosporin tested during the 4-week collection period. These laboratories also reported the total number of urinary *E. coli* and *Klebsiella* isolated during the same four weeks.

Group 3 Laboratories whose only routine cephalosporin susceptibility testing was with one of the third-generation cephalosporins, cefotaxime, ceftriaxone and/or cefpodoxime, referred all urinary *E. coli* and *Klebsiella* that met the CLSI ESBL screening criteria for these cephalosporins during the 4-week collection period.[13] These laboratories also reported the total number of urinary *E. coli* and *Klebsiella* isolated during the same four weeks.

Two laboratories only tested ceftazidime susceptibility. As ceftazidime is not a sensitive screening substrate for the ESBLs currently prevalent in New Zealand, these two laboratories were requested to refer all urinary *E. coli* and *Klebsiella*

isolated during the 4-week collection period.[14]

Group 4 Two laboratories referred only confirmed ESBL-producing urinary *E. coli* and *Klebsiella* isolated during the 4-week collection period. Both these laboratories are very experienced in the identification of ESBL-producing Enterobacteriaceae. These two laboratories also reported the total number of urinary *E. coli* and *Klebsiella* isolated during the same four weeks.

Clinical data collected with each referred isolate included patient name/laboratory code, gender, age, isolation site, and whether the isolate was considered hospital- or community-acquired. Hospital-acquired isolates were defined as isolates from in-patients who had been admitted at least 48 hours earlier. Community-acquired isolates were defined as isolates from specimens referred from general practitioners, rest homes, hospital outpatient clinics, accident and emergency units, or from hospital in-patients within 48 hours of admission.

For the geographic analysis, district health board (DHB) boundaries were used. As the patient's place of residence was not usually known, the location of the referring diagnostic laboratory was used to assign cases to DHBs. The three Auckland district health boards (Waitemata, Auckland and Counties Manukau) and the two Canterbury district health boards (Canterbury and South Canterbury) were combined for these analyses.

2.2 Screening and confirmation of ESBL production

Isolates from the laboratories that referred all urinary *E. coli* and *Klebsiella* (ie, Group 1 laboratories) were screened for ESBLs by the CLSI initial screen disc test using cefotaxime, ceftazidime, cefpodoxime and aztreonam discs.[13] Isolates that screened positive with any of the four substrates were then tested by the CLSI ESBL phenotypic confirmatory disc test,[13] and for cefoxitin resistance.

All isolates from all other laboratories (ie, Group 2, 3 and 4 laboratories) were tested for ESBL production by the CLSI ESBL phenotypic confirmatory disc test, and for cefoxitin resistance.[13]

Any isolates that were cefoxitin intermediate or resistant were also tested for ESBL production using cefepime and cefpirome, in a double-disc synergy test and a combination clavulanate disc test as previously described.[14] Cefoxitin resistance indicates that an isolate is possibly a producer of AmpC β-lactamase which can mask ESBL production in the standard CLSI ESBL confirmatory tests. Unlike ESBLs, AmpC β-lactamase does not confer resistance to fourth-generation cephalosporins. Therefore, the use of fourth-generation cephalosporins, such as cefepime and cefpirome, should facilitate the detection of ESBLs in organisms that also produce AmpC β-lactamase.[15]

Isolates were assumed to be the species they were referred as unless they gave incompatible tests results. In which case they were fully identified by ESR's Enteric Reference Laboratory. Any isolates not confirmed as *E. coli* or *Klebsiella* were eliminated from the survey.

2.3 Statistical analysis

Statistical analyses were performed with SAS software v.9.1.2 (SAS Institute Inc, Cary, NC, USA). The chi-square test or Fisher's exact test, as appropriate, were used to determine the significance of any observed differences. An associated P value ≤0.05 was used to indicate that a difference was significant.

3 RESULTS

3.1 Isolates and data submitted for the survey

Thirty-eight (86.4%) of the 44 clinical microbiology laboratories invited to participate in the survey did so (Appendix 1 and Table 1).

Seven laboratories did not routinely test urinary *E. coli* and *Klebsiella* for susceptibility to any cephalosporin. These laboratories included six hospital laboratories and one community laboratory. Another two hospital laboratories reported that they only tested susceptibility to ceftazidime, which is not considered a sensitive substrate for ESBL detection in New Zealand.[14] These nine laboratories referred all urinary *E. coli* and *Klebsiella* they isolated during the 4-week survey period or referred the first 100 *E. coli* and the first 50 *Klebsiella*, whichever target was reached sooner (Table 1, Group 1).

Twenty-two laboratories tested the susceptibility of urinary *E. coli* and *Klebsiella* to a first-generation cephalosporin (cephalothin or cefaclor). One laboratory tested susceptibility to a second-generation cephalosporin (cefuroxime). Seven of the 22 laboratories that tested first-generation cephalosporin susceptibility also tested second- and/or third-generation cephalosporin susceptibility. These laboratories referred all isolates that were intermediate or resistant to the lowest-generation cephalosporin tested (Table 1, Group 2).

Four laboratories screened urinary *E. coli* and *Klebsiella* for ESBLs by the CLSI initial screen disc test using cefotaxime, ceftriaxone and/or cefpodoxime. These laboratories referred all isolates that were screen positive (Table 1, Group 3).

Two laboratories referred all confirmed ESBL-producing urinary *E. coli* and *Klebsiella* (Table 1, Group 4).

All Group 2, 3 and 4 laboratories provided data on the total number of urinary *E. coli* and *Klebsiella* isolated in their laboratory during the 4-week survey period to provide denominator data to calculate prevalence rates (Table 1).

Table 1. Source of survey isolates

Laboratory category[1]	**Number of laboratories**	**Number of isolates referred**		**Total number of urinary *E. coli* and *Klebsiella* isolated during the 4-week collection period**[2]	
		E. coli	***Klebsiella***	***E. coli***	***Klebsiella***
Group 1	9	448[3]	73	628[3]	73
Group 2	23	301	38	6891	580
Group 3	4	6	2	916	56
Group 4	2	7	9	272	37
total	38	762	122	8707	746

[1] refer to Methods section 2.1 for a description of the laboratory categories

[2] these total isolation numbers provided the denominator for the calculation of prevalence rates

[3] one laboratory referred only the first 100 urinary *E. coli* isolated during the 4-week collection period, but is estimated to have isolated 280 urinary *E. coli* over the whole 4-week period.

Of the six laboratories that did not participate, two were very small hospital laboratories that

advised that they isolated very few urinary isolates. The remaining four non-participating laboratories were community laboratories in Whangarei, Wanganui, Hawkes Bay and Christchurch.

3.2 Prevalence of ESBLs

A total of 87 ESBL-producing isolates were identified among the isolates submitted: 56 *E. coli* and 31 *Klebsiella*.

One of the ESBL-producing *E. coli* was identified among the isolates from the laboratory that only referred the first 100 urinary *E. coli* isolated during the 4-week survey period. As these 100 isolates represented only one-third of the total number of urinary *E. coli* that the laboratory would isolate during a 4-week period, two additional ESBL-producing *E. coli* were allowed for when estimating prevalence. Accordingly the prevalence of ESBLs among urinary *E. coli* was calculated to be 0.7% (58/8707) and 4.2% (31/746) among *Klebsiella* (Table 2).

Six (6.7 %) of the ESBL-producing isolates – all *E. coli* – were identified among isolates from three of the seven laboratories that did not routinely test urinary *E. coli* and *Klebsiella* for susceptibility to any cephalosporin.

3.3 Comparison of ESBL prevalence in 2006 and 2000

A comparison of the results of this survey with those of the 2000 survey[9] shows that there has been a significant increase in the prevalence the ESBLs in both urinary *E. coli* and *Klebsiella* (Table 2).

Table 2. Comparison with the 2000 survey results

	ESBL prevalence (number/number tested)		**P value**
	2006	**2000**	
E. coli	0.7% (58/8707)	0.1% (2/1760)	0.0051
Klebsiella	4.2% (31/746)	0% (0/189)	0.0044

3.4 Patient age distribution

The majority of the 86 patients with an ESBL-producing organism, and for whom age was reported, were middle aged or older: 57.0% were ≥65 years old and another 22.9% were between the age of 50 and 64 years.

3.5 Hospital and community distribution of ESBL-producing organisms

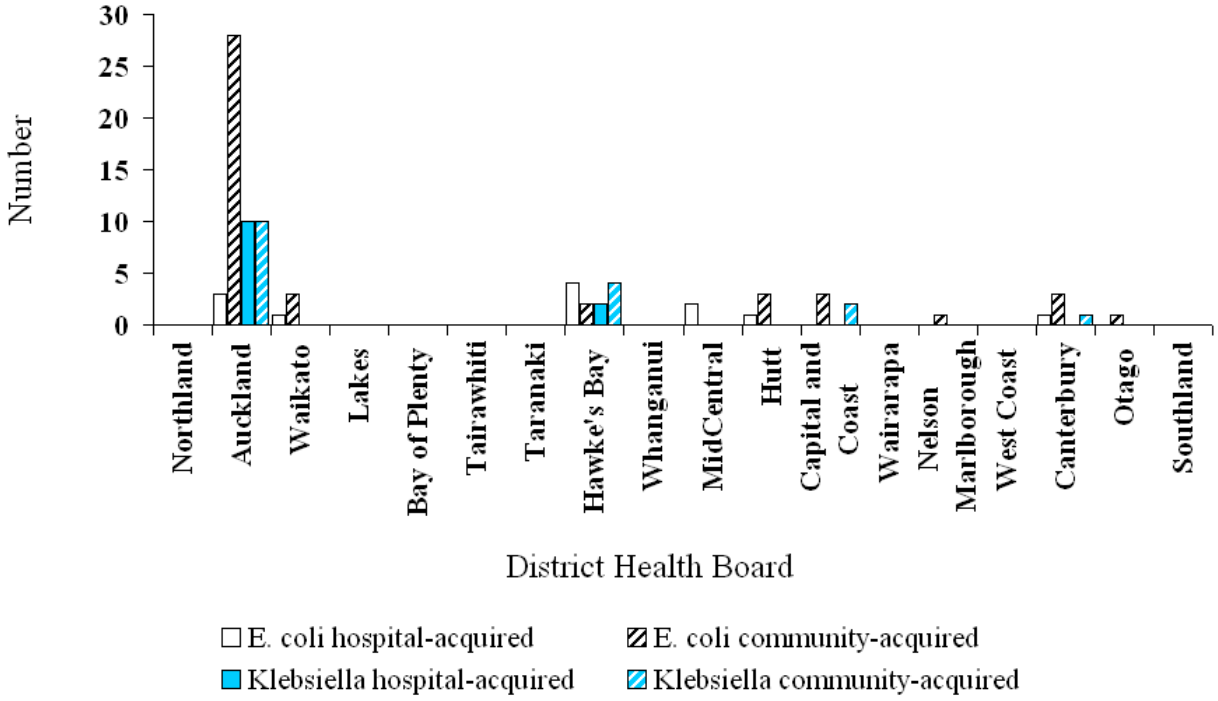
Among the 85 ESBL-producing isolates for which source was reported, 71.8% (61) were categorised as community-acquired: 78.6% (44 of 56) of the *E. coli* and 58.6% (17 of 29) of the *Klebsiella*. The difference between the species in the proportion that were community-acquired was not highly significant (P = 0.0527). As data on the source of all urinary *E. coli* and *Klebsiella* isolated during the 4-week survey period was not collected, the prevalence of ESBLs among community-acquired versus hospital-acquired isolates could not be estimated.

3.6 Geographic distribution of ESBLs

The largest number of ESBL-producing isolates were referred from the Auckland area (31 *E. coli* and 20 *Klebsiella*), where community-acquired ESBL-producing *E. coli* were the most common isolates (28) (Figure 1). Other areas from which ESBL-producing isolates were referred were Hawkes Bay (7 *E. coli* and 8 *Klebsiella*), Canterbury (5 *E. coli* and 1 *Klebsiella*), Capital and Coast (3 *E. coli* and 2 *Klebsiella*), Waikato (4 *E. coli*), Hutt (4 *E. coli*), MidCentral (2 *E. coli*), Nelson-Marlborough (1 *E. coli*), and Otago (1 *E. coli*).

No ESBL-producing *E. coli* or *Klebsiella* were identified among isolates from 9 of the 18 DHB areas: Northland, Lakes, Bay of Plenty, Tairawhiti, Taranaki, Whanganui, Wairarapa, West Coast and Southland (Figure 1). As noted in Section 3.1, the community laboratories in Northland and Whanganui did not participate in this survey.

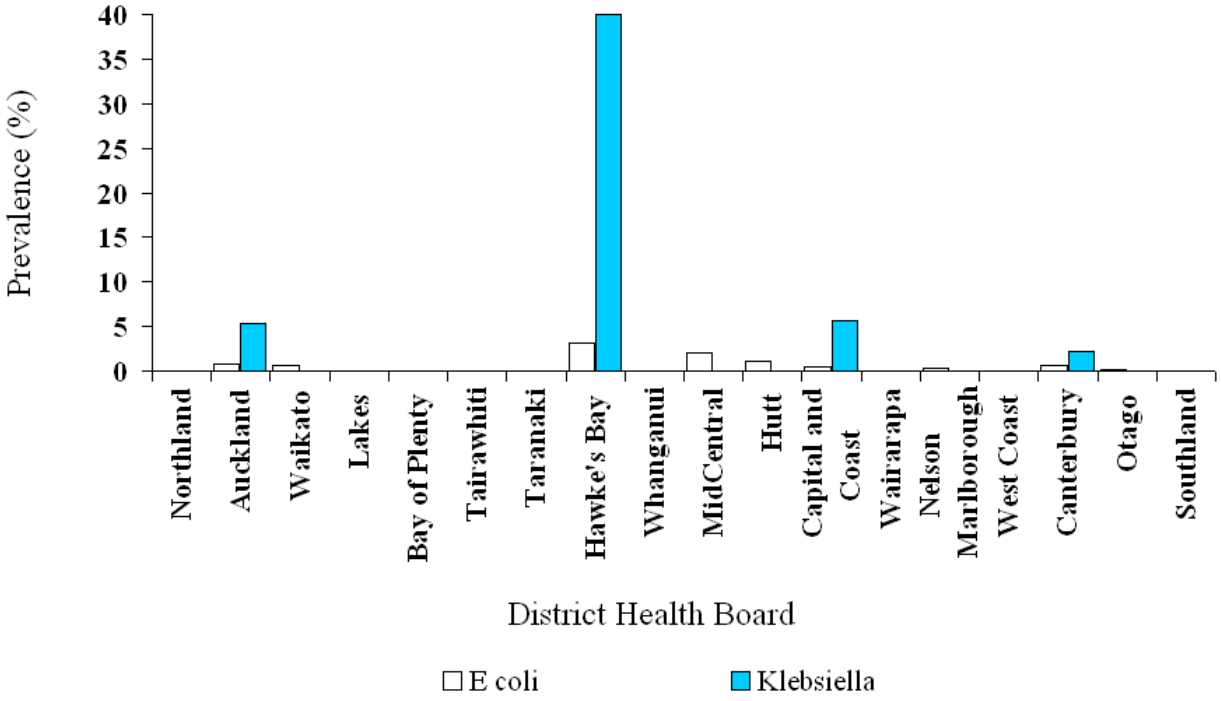
Figure 1. Frequency of ESBL-producing urinary *E. coli* and *Klebsiella* by district health board area



Hawkes Bay had the highest prevalence rate of ESBL-producing *Klebsiella* where they accounted for 40.0% of urinary *Klebsiella* isolated (Figure 2). ESBL-producing *Klebsiella* were identified in three other areas and the prevalence rates in these areas were: Wellington, 5.7%; Auckland, 5.3%; and Canterbury, 2.1%.

ESBL-producing *E. coli* were less prevalent but more widespread, and were identified in Hawkes Bay (3.2%), MidCentral (2.1%), Hutt (1.2%), Canterbury (0.7%), Auckland (0.7%), Waikato (0.6%), Capital and Coast (0.4%), Nelson-Marlborough (0.4%) and Otago (0.2%).

Figure 2. Prevalence of ESBL-producing urinary *E. coli* and *Klebsiella* by district health board area



3.7 Susceptibility phenotype

In the CLSI phenotypic confirmatory disc test, all ESBL-producing isolates demonstrated an ESBL with cefotaxime but only 79.8% with ceftazidime.

Among the isolates tested for cefoxitin resistance, 36 *E. coli* and 5 *Klebsiella* were cefoxitin resistant. These isolates included one ESBL-producing *E. coli* and one ESBL-producing *Klebsiella.* This cefoxitin resistance indicates these isolates may produce AmpC β-lactamase.

4 DISCUSSION

This is the third national survey of ESBL prevalence among urinary *E. coli* and *Klebsiella*, with earlier surveys in 1993 and 2000.[8,9] A comparison of the results of this current survey with those of the 2000 survey, which used a similar methodology, indicates that ESBLs have increased significantly in both *E. coli* and *Klebsiella* in New Zealand in recent years. While the prevalence of ESBLs in urinary *Klebsiella* (4.2%) was higher than that in *E. coli* (0.7%), as *E. coli* is the more common urinary pathogen, greater numbers of ESBL-producing *E. coli* were identified.

The ESBL prevalence rate among urinary *E. coli* found in this survey is very similar to the 0.8% third-generation cephalosporin resistance estimated from susceptibility data collected from diagnostic laboratories throughout New Zealand in 2005.[12] There is no similar comparable data for third-generation cephalosporin resistance among urinary *Klebsiella*, as only susceptibility data for *Klebsiella* from bacteraemia is collected from diagnostic laboratories. In 2005, 1.6% of these *Klebsiella* were resistant to third-generation cephalosporins compared with the 4.2% ESBL prevalence among the urinary *Klebsiella* tested for this survey.

It is difficult to make valid international comparisons of the prevalence of ESBLs, because of variations in study designs. In addition, any published data from other countries is inevitably at least two years old. These limitations notwithstanding, the prevalence of ESBLs in urinary *E. coli* and *Klebsiella* found in this survey appears to be similar to that in Australia, Europe and the United States. A 2004 survey in Australia reported ESBLs in 1.2% of *E. coli* and 5.4% of *Klebsiella* isolated from a variety of infection sites.[16] A PEARLS study conducted in 2001-2002 reported ESBLs in 1.4% of *E. coli* and 5.2% of *K. pneumoniae* in Northern European countries.[17] The latest available NNIS data from the United States, which only records third-generation cephalosporin resistance, reported 1.3% resistance in *E. coli* and 5.8% resistance in *Klebsiella* from non-ICU hospital in-patients for the 1998-2004 period.[18] In contrast, the latest SENTRY survey in the Asia-Pacific region showed that ESBLs are very prevalent in many countries in the region. The highest rates were in China, Hong Kong, the Philippines and Singapore, and the lowest rates in Japan and Australia. New Zealand did not participate in this survey.[19]

Originally ESBLs were most commonly reported to be a hospital-based problem. However, there are now numerous reports that ESBLs are becoming common among community-acquired pathogens, especially *E. coli*.[4,5] In particular, the CTX-M family of ESBLs appears to be associated with community-acquired infections.[20,21,22,23] It is unfortunate that the survey method we used does not enable us to calculate and compare the prevalence of ESBLs among community-acquired and hospital-acquired isolates. However, our results show that both ESBL-producing *E. coli* and *Klebsiella* are probably already established in the community in several areas of New Zealand (Figure 1).

CTX-M ESBLs have been identified in New Zealand, but their prevalence and the relative prevalence of other ESBL types is unknown. CTX-M-15 has been identified as the ESBL in two *E. coli* outbreak strains and two *K. pneumoniae* outbreak strains (ESR unpublished observations). One of these *E. coli* strains was associated with an outbreak in the community in the Auckland area.[24] In order to further describe the epidemiology of ESBLs in New Zealand, the ESBL-producing isolates identified in this survey should be further investigated to determine the range of strains circulating and the variety of ESBL types that are occurring. Information from such an investigation should indicate the extent of clonal spread of particular strains of ESBL-producing *E. coli* and *Klebsiella*. We therefore recommend that all ESBL-producing isolates identified in this survey are typed by pulsed-field gel electrophoresis and that the ESBLs

types are identified by PCR and sequencing. We also recommend that to assist in the ongoing identification of outbreaks of ESBL-producing organisms and the strains involved, laboratories should refer isolates to ESR for typing whenever they detect an increase in isolations or suspect the transmission of an ESBL-producing organism.

Hawkes Bay had the highest prevalence of both ESBL-producing *E. coli* (3.2%) and *Klebsiella* (40.0%), with the rate in *Klebsiella* being seven times higher than anywhere else (Figure 2). There has been an outbreak of a CTX-M-15-producing *E. coli* strain in Hawkes Bay Hospital since 2001 and at the end of 2005 an outbreak of CTX-M-15-producing *K. pneumoniae* was identified.[10,25]

There was considerable variation throughout the country, with no ESBL-producing isolates being identified during the survey period among isolates from half the 18 DHB areas. ESBL-producing *Klebsiella* were identified among isolates from just 4 of the 18 DHB areas, with rates above the national average in three areas: Hawkes Bay, Capital and Coast, and Auckland. ESBL-producing *E. coli* were more widespread than *Klebsiella* and were identified in nine DHB areas with rates above the national average in three areas: Hawkes Bay, MidCentral and Hutt. Unfortunately, the laboratories serving both the community and hospitals in two of these areas, MidCentral and Hutt, reported that they did not routinely test urinary *E. coli* and *Klebsiella* for susceptibility to any cephalosporin.

The guidelines for the control of multidrug-resistant organisms in New Zealand, currently being developed by the Ministry of Health, recommend that all Enterobacteriaceae isolated from sterile sites should be screened for ESBLs. The Guidelines also recommend that isolates from other (non-sterile) sites, for example, urine and superficial swabs, be screened for ESBLs. At least one of the CLSI ESBL screening substrates, but preferably not ceftazidime alone, should be included as part of routine susceptibility testing.[13] Alternatively, laboratories need to consider periodic surveys to monitor the current prevalence of ESBLs among local, non-invasive isolates. Similarly, guidelines from the United Kingdom, where ESBLs have become common among community-acquired *E. coli*, also recommend including an indicator cephalosporin in the first-line panel for all urinary tract infection isolates.[26]

The results we obtained in the CLSI phenotypic confirmatory disc test support the CLSI recommendation to use both cefotaxime and ceftazidime in this test, as 20.2% of isolates would not have been identified as ESBLs if only ceftazidime was used. The inferior sensitivity of ceftazidime to detect ESBLs currently occurring in New Zealand was also found in an earlier methods study.[14]

Perhaps an unexpected result from this survey was the number of cefoxitin-resistant isolates, especially among *E. coli*. Thirty-six cefoxitin-resistant *E. coli* were identified among the same pool of screened isolates from which 56 ESBL producers were identified. There are at least three possible mechanisms of cefoxitin resistance in *E. coli.* These include Amp C β-lactamase production following either the acquisition of a plasmid with the bla_{ampC} gene or a mutation resulting in the upregulation of the organism's own chromosomal bla_{ampC} which usually produces only very small amounts of AmpC β-lactamase. Alternatively, the cefoxitin resistance may be due to a change in the outer membrane protein affecting permeability to β-lactams.

This survey has at least three limitations. First, using intermediate resistance or resistance to a first- or second-generation cephalosporin as an initial screen may lack sensitivity. In an earlier study, we found that 30.3% of ESBL-producing *Klebsiella* and 2.7% of *E. coli* tested as cefuroxime susceptible in routine susceptibility tests.[14] However, 22 of the 23 laboratories actually submitted isolates intermediate or resistant to a first-generation cephalosporin

(cephalothin or cefaclor) rather than a second-generation cephalosporin. The sensitivity of first-generation cephalosporins to detect ESBLs is not known.

Second, as already mentioned above, due to the denominator data available, we could not compare the prevalence of ESBLs among hospital-acquired isolates with that among community-acquired isolates. While the majority (71.8%) of the ESBL-producing isolates identified were reported to have been community acquired, it is also very likely that the majority of urinary *E. coli* and *Klebsiella* isolated are from community-acquired infections. Third and finally, as ESBL-producing Enterobacteriaceae may be associated with outbreaks, a point-prevalence study such as this survey will be affected by any outbreaks occurring in a hospital or area.

5 REFERENCES

1 Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004; 48: 1-14.

2 Livermore DM, Woodford N. The β-lactamase threat in Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter*. Trends Microbiol 2006; 14: 413-20.

3 Amino acid sequences for TEM, SHV and OXA extended-spectrum and inhibitor resistant ß-lactamases. Available at http://www.lahey.org/studies/webt.asp.

4 Pitout JDD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. J Antimicrob Chemother 2005; 56: 52-9.

5 Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control 2006; 34 Suppl 1: S20-8.

6 Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol 2001; 39: 2206-12.

7 Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, et al. International prospective study of *Klebsiella pneumoniae* bacteraemia: implications of extended-spectrum beta-lactamase production in nosocomial infections. Clin Infect Dis 2004; 39: 31-7.

8 Brett M. Survey of the antimicrobial susceptibilities of urinary isolates of *Escherichia coli*. Porirua: Institute of Environmental Science & Research; 1993.

9 Brett M. Extended-spectrum β-lactamases among urinary *Escherichia coli* and *Klebsiella* species in New Zealand in 2000. Porirua: Institute of Environmental Science & Research; 2000.

10 Extended-spectrum ß-lactamases (ESBLs) in Enterobacteriaceae confirmed in 2005. Porirua: ESR. Available at http://www.surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ESBL_2005.pdf.

11 Briggs S, Ussher J, Taylor S. Extended-spectrum beta-lactamase-producing Enterobacteriaceae at Middlemore Hospital. NZ Med J 2005; 118 (1218). Available at http://www.nzma.org.nz/journal/118-1218/1563/

12 Antimicrobial resistance data from hospital and community laboratories, 2005. Porirua: ESR. Available at http://www.surv.esr.cri.nz/PDF_surveillance/Antimicrobial/National_AR_2005.pdf.

13 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. Pennsylvania: CLSI; 2006. M100-S16.

14 Heffernan H, Woodhouse R, Blackmore T. Methods used in New Zealand diagnostic laboratories to identify and report extended-spectrum β-lactamase-producing Enterobacteriaceae. Porirua: Institute of Environmental Science & Research Ltd; November 2005. Report No. FW0598. Available at http://www.surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ESBLMethods_2005.pdf.

15 Tzelepi E, Giakkoupi P, Sofianou D, Loukova V, Kemeroglou A, Tsakris A. Detection of extended-spectrum beta-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J Clin Microbiol 2000; 38: 542-6.

16 The Australian Group on Antimicrobial Resistance. Gram-negative survey. 2004 Antimicrobial Susceptibility Report; 2005. Available at http://antimicrobial-resistance.com.

17 Bouchillon SK, Johnson BM, Hoban DJ, et al. Determining incidence of extended spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS

study 2001-2002. Int J Antimicrob Agents 2004; 24; 119-24.
18 National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control 2004; 32: 470-85.
19 Hirakata Y, Matsuda J, Miyazaki Y, et al. Regional variation in the prevalence of extended-spectrum β-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). Diagn Microbiol Infect Dis 2005; 52: 323-9.
20 Livermore DM, Hawkey PM. CTX-M: changing the face of ESBLs in the UK. J Antimicrob Chemother 2005; 56: 451-4.
21 Woodford N, Ward ME, Kaufmann ME, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother 2004; 54:735-43.
22 Pitout JD, Gregson DB, Church DL, et al. Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. J Clin Microbiol 2005; 43: 2844-9.
23 Rodriguez-Bano J, Navarro MD, Romero L, et al. Bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli* in the CTX-M era: a new challenge. Clin Infect Dis 2006; 43: 1407-14.
24 Moor CT, Roberts S, Simmons G, Briggs S, Morris AJ, Smith J, Heffernan H. Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: emergence and risk factors for infection in the community setting, Auckland, New Zealand. J Hosp Infect. In press 2006.
25 Antibiotic resistance. Extended-spectrum ß-lactamase-producing Enterobacteriaceae. LabLink 2002; 9 (1): 8-11.
26 Livermore DM, Woodford N. Guidance to diagnostic laboratories: laboratory detection and reporting of bacteria with extended-spectrum β-lactamases. London: Health Protection Agency; 2004.

APPENDIX 1: Laboratories participating in the survey:

Canterbury Health Laboratories
Diagnostic and Medical Laboratory, Auckland
Dargaville Hospital
Gisborne Hospital
Gisborne Medlab
Grey Hospital, Greymouth
Hamilton Medical Laboratory
Hamilton Pathology Laboratory
Hawkes Bay Hospital
Hutt Hospital, Lower Hutt
LabPlus, Auckland
LabCare Pathology, New Plymouth
Masterton Hospital
Medlab Central, Palmerston North
Medlab Thames
Medlab Timaru
Middlemore Hospital
Nelson Hospital
Nelson Diagnostic Laboratory
New Plymouth Medlab
North Shore Hospital
Otago Diagnostic Laboratories, Dunedin
Rotorua Hospital
Rotorua Diagnostic Laboratory
Southern Community Laboratories, Hastings, Christchurch and Dunedin
Southland Hospital
Taumarunui Hospital
Tauranga Medlab
Valley Diagnostic Laboratories, Lower Hutt
Waikato Hospital
Wairau Hospital, Blenheim
Wanganui Hospital
Wellington Hospital
Wellington Medical Laboratory
Whakatane Hospital
Whangarei Hospital