METHODS USED IN NEW ZEALAND DIAGNOSTIC LABORATORIES TO IDENTIFY AND REPORT EXTENDED-SPECTRUM β-LACTAMASE-PRODUCING ENTEROBACTERIACEAE

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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*. ESBL-producing organisms are often multiresistant to several other classes of antibiotics. These organisms are becoming increasing prevalent in New Zealand, particularly in the Auckland area.

The accurate detection of ESBL-producing organisms is essential to ensure the selection of appropriate antibiotic treatment. The detection of ESBL-producing organisms is complicated by the fact that some of these organisms can test as susceptible to third- and fourth-generation cephalosporins and monobactams when standard susceptibility testing breakpoints are applied. In addition, the sensitivity and specificity of tests to detect ESBLs can vary with the cephalosporin tested. Detection of ESBLs in members of the Enterobacteriaceae that commonly possess AmpC β-lactamase, such as *Enterobacter*, *Serratia* and *Citrobacter freundii*, can be particularly problematic.

The aims of this study were (1) to record the methods being used in diagnostic microbiology laboratories in New Zealand to identify and report ESBL-producing organisms, and (2) to assess the most commonly used methods using a panel of ESBL-positive Enterobacteriaceae recently referred to ESR.

In August 2003, 47 hospital and community microbiology laboratories in New Zealand were asked to complete a questionnaire on their testing and reporting procedures for ESBL-producing organisms. There was a 98% response rate and 38 laboratories indicated that they tested for ESBLs.

Aztreonam (6 mg/L) blood agar was the most commonly used selective medium for the isolation of ESBL-producing organisms directly from clinical specimens. Many laboratories used or adapted routine susceptibility testing procedures to screen isolates for ESBL production. Multiresistance, a pattern of second-generation cephalosporin resistance but co-amoxiclav susceptibility, synergy between a second-generation cephalosporin and clavulanate, and the Clinical and Laboratory Standards Institute (CLSI) initial screen disc test were all commonly used methods of screening for ESBLs. Several laboratories sequentially used more than one method. The CLSI confirmatory disc test and the double-disc synergy (Jarlier) test were the most commonly used methods of confirming ESBL production.

The most commonly used methods were assessed using a panel of ESBL-positive *E. coli* (75), *Klebsiella* (33), and other Enterobacteriaceae (29), including *Enterobacter*, *Serratia* and *C. freundii*. Nine *K. oxytoca* that were hyperproducers of K1 (KOXY) β-lactamase were also included in the test panel. Approximately half of the test panel of isolates were also tested in the Vitek 1 and Vitek 2 automated systems.

continued

SUMMARY *continued*

The main findings of the methods' assessment were:

- Aztreonam blood agar had poor sensitivity for ESBL-positive *E. coli* and *Klebsiella*, as only 63% and 58%, respectively, grew on this agar.
- A pattern of cephalosporin resistance and co-amoxiclav susceptibility was not a sensitive screen for ESBL-positive *E. coli* and *Klebsiella*, as 31% of *E. coli* and 18% of *Klebsiella* were co-amoxiclav resistant.
- Testing for synergy between second-generation cephalosporins (cefuroxime and cefaclor) and co-amoxiclav was not a sensitive screen for ESBL-producing organisms, especially Enterobacteriaceae other than *E. coli* and *Klebsiella*, as there was relatively poor synergy between these cephalosporins and clavulanic acid.
- In the CLSI initial screen disc test, ceftazidime had poor sensitivity compared to cefotaxime, ceftriaxone, cefpodoxime and aztreonam for ESBL-positive *E. coli* and *Klebsiella*.
- In the CLSI confirmatory disc test, which specifies the use of both cefotaxime and ceftazidime discs with and without clavulanic acid, all ESBL-positive *E. coli* and *Klebsiella* were confirmed.
- Similarly, in the double-disc synergy (Jarlier) test using cefotaxime and ceftazidime, and a distance of 20 mm (centre-to-centre) between the cephalosporin discs and the co-amoxiclav disc, all ESBL-positive *E. coli* and *Klebsiella* were confirmed.
- In the double-disc synergy test, a distance of 20 mm between the cephalosporin discs and the co-amoxiclav disc was superior to a distance of 30 mm.
- There was some gain in using a fourth-generation cephalosporin to detect ESBLs in organisms, such as *Enterobacter*, *Serratia* and *C. freundii*, which usually also possess AmpC β-lactamase. The gain was smaller in the CLSI confirmatory disc test using cefpirome combination discs than in the double-disc synergy test using cefepime.
- In all cases where a comparison could be made, the results with the double-disc synergy test, performed at a disc spacing of 20 mm, were as good as or better than the CLSI confirmatory disc test.
- The Vitek 2 performed better than the Vitek 1 in detecting ESBLs in organisms such as *Enterobacter*, *Serratia* and *C. freundii*.
- K1-positive *K. oxytoca* isolates often tested as ESBL positive with antibiotics other than ceftazidime.

These results indicate that most laboratories in New Zealand use sensitive methods to confirm ESBL production. However, the methods used for initial screening of clinical specimens and isolates are less sensitive, which suggests ESBLs may often go undetected. This is especially likely to be the case with those isolates that test as susceptible to cephalosporins in standard susceptibility tests. This is a concern as such isolates have been associated with cephalosporin and monobactam treatment failure.

When an ESBL was confirmed, most (82%) laboratories correctly reported the isolate as resistant to all cephalosporins and monobactams, if they reported susceptibility to these agents at all. Almost all laboratories reported the isolation of an ESBL-producing organism to their infection control services.

RECOMMENDATIONS

- Aztreonam (6 mg/L) blood agar should not be used to screen clinical samples for ESBL-producing Enterobacteriaceae, as it does not support the growth of ESBL-positive isolates with relatively low aztreonam MICs.

- Co-amoxiclav susceptibility or resistance, along with cephalosporin resistance, should not be used as a screen for ESBL-positive *E. coli* or *Klebsiella*, as co-amoxiclav susceptibility is variable among these organisms.

- Synergy between second-generation cephalosporins and co-amoxiclav should not be used to screen for ESBLs, especially in Enterobacteriaceae other than *E. coli* and *Klebsiella*.

- If only one disc is used in the Clinical and Laboratory Standards Institute (CLSI) initial screen disc test, either cefotaxime, ceftriaxone or cefpodoxime should be used. Ceftazidime should not be used.

- Both cefotaxime and ceftazidime should be used in the CLSI confirmatory disc test and double-disc synergy (Jarlier) test to confirm ESBLs in *E. coli* and *Klebsiella*. When testing for ESBLs in other Enterobacteriaceae, these tests should be extended to include a fourth-generation cephalosporin.

- The double-disc synergy test should be performed at 20 mm, not 30 mm.

- *K. oxytoca* isolates that test positive for ESBLs with cephalosporins other than ceftazidime but negative with ceftazidime should be considered possible K1 hyperproducers, rather than ESBL producers.

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 advent of penicillin saw the rapid emergence of resistance in *Staphylococcus aureus* due to a plasmid-encoded β-lactamase or penicillinase. This β-lactamase quickly spread to most clinical isolates of *S. aureus*. 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 increased 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. ESBLs are inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam. They are usually derived from earlier, narrow-spectrum β-lactamases and differ from the parent enzyme by a few point mutations, which confer an extended spectrum of activity. The parent enzymes include the TEM, SHV and OXA families of β-lactamases. More recently another family of ESBLs, the CTX-M types, has emerged and these ESBLs are becoming increasingly common.[1]

Over 150 different ESBLs have been described.[2] 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.[3] The fact that ESBLs are plasmid-mediated poses an additional infection control problem as the genetic determinants can be readily transferred to other strains and bacterial species.

ESBL-producing organisms are increasing in New Zealand, particularly in the Auckland area. 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. However, from 2001 the number of confirmed isolates started to increase markedly, with 83 in 2001 and 389 in 2004.[4] There has been an ongoing outbreak of an ESBL-producing *E. coli* strain in Hawkes Bay Hospital since 2001.[4,5] Susceptibility data collated from hospital and clinical laboratories throughout New Zealand indicate that, in 2003, 0.4% of *E. coli* from bacteraemias, 0.9% of urinary *E. coli* and 2.9% of *Klebsiella* were resistant to cefotaxime or ceftriaxone. It is likely that the majority of these resistant isolates were ESBL producers.[6]

The accurate detection of ESBL-producing organisms is essential to ensure the selection of appropriate antibiotic treatment. The detection of ESBL producers is complicated by the fact that some of these organisms can appear susceptible when the standard cephalosporin susceptibility testing breakpoints are applied. In addition, the sensitivity and specificity of tests to detect ESBLs can vary with the cephalosporin tested. Detection of ESBLs in members of the Enterobacteriaceae that commonly possess AmpC β-lactamase, such as *Enterobacter*, *Serratia* and *Citrobacter freundii*, can be particularly problematic as the production of AmpC β-lactamase, especially when derepressed, can mask the detection of ESBL.

The aims of this study were two-fold: (1) to record the methods being used in diagnostic microbiology laboratories in New Zealand to identify and report ESBL-producing organisms, and (2) to assess the most commonly used methods using a panel of ESBL-positive Enterobacteriaceae recently referred to ESR.

2 METHODS

2.1 Questionnaire on the Methods Used by New Zealand Diagnostic Laboratories to Identify and Report ESBL-producing Organisms

In August 2003, all hospital and community laboratories in New Zealand, which perform microbiological testing, were sent a questionnaire (see Appendix) about the methods that they use to screen for, confirm and report ESBL-producing organisms. The questionnaire covered:

- direct screening of clinical specimens, including methods and when such screening is applied;
- screening isolates, including methods and which isolates are screened;
- methods used to confirm ESBL production, including which organisms they are used for;
- reporting of a confirmed ESBL producer's susceptibility to cephalosporins, monobactams, cephamycins, β-lactam/β-lactamase combinations and second-line antibiotics;
- notifying a confirmed ESBL producer to infection control staff.

2.2 Assessment of the Methods Commonly Used to Identify ESBLs in New Zealand

2.2.1 Test Panel of Isolates

A test panel of 146 isolates, which were referred to ESR in 2002 and 2003, and confirmed as producers of an ESBL or hyperproducers of K1 (KOXY) β-lactamase, was compiled. This panel of isolates was used to assess the methods most commonly used by laboratories in New Zealand to identify ESBLs. The identity and β-lactamase status of the 146 test isolates is shown in Table 1.

Table 1. Identity and β-lactamase Status of the Test Panel Isolates

	Number of isolates (number tested in Vitek trial)				
	Total	**ESBL with or without AmpC β-lactamase**	**ESBL only**	**ESBL + AmpC**	**K1 β-lactamase**
E. coli	75 (39)	75 (39)	68 (36)	7[1] (3)	
K. pneumoniae	29 (14)	29 (14)	29 (14)		
K. oxytoca	13 (7)	4 (2)	4 (2)		9 (5)
Enterobacter species	23 (12)	23 (12)		23 (12)	
C. freundii	4 (2)	4 (2)	1 (0)	3 (2)	
Escherichia hermannii	1 (0)	1 (0)	1 (0)		
Serratia fonticola	1 (1)	1 (1)		1 (1)	
Total	146 (75)	137 (70)	103 (52)	34 (18)	9 (5)

[1] These 7 ESBL-positive *E. coli* were cefoxitin resistant. These isolates' resistance to cefoxitin indicates that they may also produce AmpC β-lactamase, either following the acquisition of a plasmid with an *AmpC* gene or due to a mutation affecting the regulation of the organism's own *AmpC* gene, which is usually 'switched off' in *E coli*. However, it is possible that the cefoxitin resistance may be due to another mechanism, such as a change in the outer membrane protein affecting permeability to β-lactams.

In the results section, the 'ESBL positive' category includes both isolates that produce an ESBL only and those that also have AmpC β-lactamase. The 29 isolates of *Enterobacter*, *C. freundii*, *E. hermannii* and *S. fonticola* were grouped and termed 'Other Enterobacteriaceae'.

Only one isolate of the Hawkes Bay ESBL-positive *E. coli* outbreak strain was included in the test panel. Seven isolates of an Auckland ESBL-positive *E. coli* outbreak strain were included. Otherwise consecutive, non-duplicate isolates were included until sufficient numbers of each species were obtained. The number of each species included in the test panel was proportional to the species distribution among ESBL-producing Enterobacteriaceae referred to ESR.

The ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca* included in the test panel were identified as ESBL producers by the Clinical and Laboratory Standards Institute (CLSI) confirmatory tests (disc and microbroth dilution), using cefotaxime and ceftazidime.

The ESBL-positive *Enterobacter*, *C. freundii*, *E. hermannii* and *S. fonticola* included in the test panel were identified as ESBL producers by the double-disc synergy (Jarlier) test using cefpodoxime, cefotaxime, ceftazidime and cefepime discs. Co-production of AmpC β-lactamase was assumed on the basis of cefoxitin resistance.

The *K. oxytoca* hyperproducers of the K1 β-lactamase included in the test panel were identified by being more resistant/less susceptible to aztreonam than to ceftriaxone, less susceptible to ceftriaxone than cefotaxime, and fully susceptible to ceftazidime. In a double-disc synergy test using aztreonam, ceftriaxone, cefotaxime and ceftazidime discs, these isolates usually showed some clavulanate synergy with aztreonam, ceftriaxone and cefotaxime but not ceftazidime.

2.2.2 Growth on Aztreonam Sheep Blood Agar

The ability of the test isolates to grow on aztreonam sheep blood agar (AztBA) containing 6 mg/L of aztreonam was tested. AztBA was obtained from Fort Richard Laboratories. An inoculum of approximately 10^4 colony-forming units was applied to the surface of the agar in a 5-8 mm diameter spot using a multipoint inoculator. The plates were incubated at 35°C for 16-20 hours. When judging whether an isolate had grown on the agar, a single colony or a faint haze was disregarded.

2.2.3 Antibiotic Susceptibility and Resistance Patterns

The susceptibility of the test isolates to amikacin, aztreonam, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin, co-amoxiclav, co-trimoxazole, gentamicin, meropenem, nitrofurantoin, tetracycline, tobramycin and trimethoprim was determined by agar dilution according to CLSI methodology and interpretive standards.[7,8]

Multiresistance was defined as, in addition to cephalosporin and monobactam resistance, resistance to three or more of the following antibiotic classes: co-amoxiclav, meropenem, ciprofloxacin, aminoglycosides (gentamicin, tobramycin and/or amikacin), folate pathway inhibitors (co-trimoxazole and/or trimethoprim), nitrofurantoin and tetracycline.

2.2.4 CLSI Initial Screen Disc Test

The performance of the test isolates in the CLSI ESBL initial screen disc test was assessed using aztreonam, cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone discs.[8]

2.2.5 CLSI Phenotypic Confirmatory Disc Test

The performance of the test isolates in the CLSI ESBL phenotypic confirmatory disc test, using cefotaxime and ceftazidime discs with and without clavulanic acid, was assessed.[8] Supplemental testing with cefpirome 30 μg discs and cefpirome-clavulanic acid 30/10 μg discs was performed for the *Enterobacter, C. freundii, E. hermannii* and *S. fonticola* isolates. A ≥4 mm increase in cefpirome zone diameter when tested in combination with clavulanic acid versus when tested alone was considered to demonstrate the presence of an ESBL.[9]

2.2.6 CLSI Phenotypic Confirmatory Microbroth Dilution Test

The performance of the test isolates in the CLSI ESBL phenotypic confirmatory microbroth test, using cefotaxime and ceftazidime with and without clavulanic acid, was assessed.[8]

2.2.7 Double-disc Synergy (Jarlier) Test

The performance of the test isolates in the double-disc synergy test, using a modification of the method of Jarlier,[10] was assessed.

Three Mueller-Hinton agar plates were inoculated with a suspension of the test isolate adjusted to a 0.5 McFarland standard according to the standard CLSI disc diffusion susceptibility testing methodology.[11] On one plate, a ceftazidime 30 μg disc and cefotaxime 30 μg disc were placed either side of a co-amoxiclav 20/10 μg disc at a distance of 20 mm (centre-to-centre). On the second plate, a cefpodoxime 10 μg disc and cefepime 30 μg disc were placed either side of a co-amoxiclav disc at a distance of 20 mm. On the third plate, a cefaclor 30 μg disc and cefuroxime 30 μg disc were placed either side of a co-amoxiclav disc at a distance of 20 mm.

A further two Mueller-Hinton plates were inoculated with a 1:10 dilution of a suspension of the test isolate adjusted to a 0.5 McFarland standard. On one plate, a ceftazidime disc, cefotaxime disc, cefpodoxime disc and cefepime disc were placed above, below and either side of a co-amoxiclav disc at a distance of 30 mm (centre-to-centre). On the second plate, a cefaclor disc and cefuroxime disc were placed either side of a co-amoxiclav disc at a distance of 30 mm.

Synergy between the clavulanate from the co-amoxiclav disc and cephalosporins was indicated by a characteristic augmentation of the cephalosporin inhibition zone adjacent to the co-amoxiclav disc, or a small elliptical zone ('keyhole') between the cephalosporin disc and co-amoxiclav disc.

If the result was indeterminate at distances of 20 and 30 mm, the test was repeated with a distance of 25 mm (centre-to-centre) between the cephalosporin discs and the co-amoxiclav disc, using an inoculum adjusted to a 0.5 McFarland standard.

2.2.8 Vitek 1 and Vitek 2 Automated Systems

A test panel of 75 isolates was compiled from the full test panel of 146 isolates. This was done by randomly selecting approximately every second isolate of each species. The isolates were tested at Wellington Hospital, Capital and Coast Health District Health Board, on a Vitek 1 automated system using the GNS424 card, and at Middlemore Hospital, Counties Manukau District Health Board, on a Vitek 2 using the ASTN041 card. Both cards include tests for ESBL production.

2.2.9 Estimating the Sensitivity of the ESBL Screening and Confirmatory Tests

The sensitivity of each ESBL screening and confirmatory test was estimated as the percentage of the ESBL-positive test isolates that tested positive in the test.

3 RESULTS

3.1 Methods Used by New Zealand Diagnostic Laboratories to Identify ESBLs

The questionnaire was sent to 47 laboratories, and responses were received from 46 (97.9%). The non-respondent was a small primary-level hospital laboratory.

Among the 46 respondents, eight (17.4%) indicated that they did not screen or test for ESBLs at all. These eight laboratories comprised four community laboratories, three primary-level hospital laboratories and one secondary-level hospital laboratory. Two of the eight laboratories indicated that they were currently assessing whether to introduce ESBL screening.

Among the 38 respondents that did test for ESBLs, 11 noted that they had never identified an ESBL-producing organism, two noted that they had only rarely identified an ESBL-producing organism, and one noted that they had identified less than five.

3.1.1 Methods Used to Directly Screen Clinical Specimens

Among the 38 laboratories that did test for ESBLs, 12 indicated that they screened clinical specimens. The methods used and the specimens screened are shown in Table 2.

Table 2. Methods Used to Screen Clinical Specimens for ESBLs

		Number of laboratories
Media		
	Aztreonam (6 mg/L) blood agar	7
	Gentamicin disc on aztreonam blood agar	1
	MacConkey agar + 1 mg/L cefotaxime and MacConkey agar + 1 mg/L ceftazidime	1
	Cefpodoxime disc on coliform chromogenic agar	1
	Cefotaxime and ceftazidime discs on CNA (colistin and nalidixic acid) blood agar and Orientation chromogenic agar	1
	VACC (vancomycin, amphotericin B, ceftazidime and clindamycin) agar	1
Specimens screened[1]		
	Faecal/rectal swabs	5
	Wound/skin swabs	3
	Urines	3
	Ear swabs	1
	Tracheal aspirates	1
	Environmental swabs	1
	Not specified	4
Circumstances in which specimens are screened [2]		
	During outbreaks or enhanced surveillance of areas where ESBLs have been isolated	3
	Urines, tracheal aspirates and wounds from ICU patients	1
	When requested	1
	Not specified	8

[1] Five labs indicated that ≥2 specimen types were screened.
[2] One lab indicated two circumstances in which specimens would be screened.

3.1.2 Methods Used to Screen Isolates

The methods that laboratories used to screen isolates for ESBL production are shown in Table 3. Fourteen laboratories indicated that they used two or more methods of screening. Some of these laboratories indicated that the methods were used sequentially, for example, isolates with certain resistance profiles would be further tested either in the CLSI initial screening tests or for cephalosporin-co-amoxiclav synergy.

Table 3. Methods Used to Screen Isolates for ESBLs

		Number of laboratories
Resistance profiles		24
	Multiresistance or unusual resistance patterns	13
	Cefuroxime/cefaclor resistance with co-amoxiclav susceptibility	6
	Cefuroxime/cefaclor resistance with co-amoxiclav resistance	2
	First-generation cephalosporin resistance	2
	Second-generation cephalosporin resistance	2
	Ampicillin resistance with co-amoxiclav resistance	1
	First-generation cephalosporin resistance with co-amoxiclav susceptibility	1
	Third-generation cephalosporin resistance	1
Other ESBL screens		15
	Cefaclor-co-amoxiclav synergy[1]	9
	Cefuroxime-co-amoxiclav synergy[1,2]	6
	Cephalothin-co-amoxiclav synergy (urines only)[2]	1
	Cefotaxime-co-amoxiclav synergy	1
	Ceftazidime-co-amoxiclav synergy	1
	Aztreonam-co-amoxiclav synergy	1
	Aztreonam blood agar	1
CLSI initial screening tests[3]		14
	Disc	12
	Dilution	2
Other methods		
	Vitek/Microscan flagging	4
	Clinical treatment failure	1

[1] Four labs indicated that they tested either cefuroxime or cefaclor synergy with co -amoxiclav. These four labs are counted in both the categories.

[2] One lab indicated that cephalothin-co-amoxiclav synergy was used for urinary isolates and cefuroxime-co-amoxiclav synergy for isolates from other sites. This lab is counted in both categories.

[3] The antibiotics used in the CLSI tests were specified as cefotaxime and ceftazidime (4 labs); cefpodoxime (3); cefotaxime, ceftriaxone, ceftazidime and aztreonam (2); cefotaxime, ceftriaxone and aztreonam (1); ceftriaxone and ceftazidime (1); cefotaxime (1); ceftazidime (1); and ceftriaxone (1).

Table 4. Isolate Groups Screened for ESBLs

		Number of laboratories
	Enterobacteriaceae	16
	E. coli and *Klebsiella*	6
	Gram-negatives	5
	Coliforms	3[1]
	E. coli	1[2]
	Not specified	7

[1] One of the three labs further specified that 'significant' coliforms were screened.
[2] The lab that specified that they only tested *E. coli* was from the Hawkes Bay area where there was a current outbreak of an ESBL-producing *E. coli*.

The CLSI screening tests are recommended specifically for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *Proteus mirabilis* only. Among the 14 laboratories that used the CLSI screening tests, eight reported that they used the test for all Enterobacteriaceae, four used it for *E. coli* and *Klebsiella* only, one for gram-negative organisms, and one for 'significant coliforms'.

Many laboratories did not specify the circumstances in which isolates were screened for ESBL production. However, of those that did, the most commonly screened isolates were those from invasive disease and other infections that may be treated with a third-generation cephalosporin.

3.1.3 Confirmation Methods

Thirty-one of the 38 laboratories that screened for ESBLs undertook confirmatory testing. The remaining seven laboratories referred possible ESBL-producing isolates on to another laboratory for confirmation.

Table 5. Methods Used to Confirm ESBLs

Confirmation method		Number of laboratories[1]
	CLSI confirmatory disc test	19
	Double-disc synergy (Jarlier) test	13
	Etest	2
	Other	1

[1] Four labs used more than one method.

The CLSI confirmatory disc test is recommended specifically for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* only. Sixteen laboratories used this test for all Enterobacteriaceae, but two of these 16 laboratories also used the double-disc synergy test.

Table 6. Antibiotics Used in the CLSI and Double-disk Synergy ESBL Confirmatory Methods

Confirmation method	Number of laboratories
CLSI confirmatory disc test[1]	19
Ceftazidime	17
Cefotaxime	17
Cefpodoxime	5
Cefepime	2[2]
Double-disc synergy (Jarlier)[3,4]	13
Ceftazidime	13
Ceftriaxone	11
Cefotaxime	10
Cefepime	4
Cefuroxime	1
Aztreonam	2

[1] The antibiotics used in the CLSI test were used in the following combinations: cefotaxime and ceftazidime (10 labs); cefotaxime, ceftazidime and cefpodoxime (3); cefotaxime (1); ceftazidime (1); cefotaxime and cefpodoxime (1); ceftazidime and cefpodoxime (1); cefotaxime, ceftazidime and cefepime (1); and cefotaxime, ceftazidime, cefpodoxime and cefepime (1).

[2] Only used for Enterobacteriaceae other than *E. coli* and *Klebsiella.*

[3] The antibiotics used in the Jarlier test were used in the following combinations: cefotaxime, ceftazidime and ceftriaxone (4 labs); cefotaxime, ceftazidime, ceftriaxone and aztreonam (3); ceftazidime, ceftriaxone, cefepime and aztreonam (2); cefotaxime and ceftazidime (1); cefotaxime, ceftazidime and cefepime (1); ceftazidime, ceftriaxone and cefepime (1); and cefotaxime, ceftazidime, ceftriaxone, aztreonam and cefuroxime (1).

[4] The distance between the clavulanate-containing disc and the cephalosporin/monobactam discs varied as follows: 20 and 30 mm (3 labs); 20, 25 and 30 mm (2); 25 and 30 mm (2); 20 mm (2); 15 mm (1); 30 mm (1); 20 and 25 mm (1); and 25 mm and adjustment depending on actual zone diameters (1). Overall, one lab tested at a distance of 15 mm, eight at 20 mm, six at 25 mm and eight at 30 mm.

3.2 Reporting and Further Testing of ESBL-producing Organisms in New Zealand Diagnostic Laboratories

3.2.1 Reporting Cephalosporin and Aztreonam Susceptibility

Thirty-four laboratories answered the question about how they reported cephalosporin and aztreonam susceptibility. Twenty-eight (82.4%) laboratories standardly reported ESBL-producing organisms as resistant to all cephalosporins and aztreonam. One laboratory indicated that their reporting was variable "depending on a number of factors". Three laboratories did not test or report susceptibility to these antimicrobials, as they were community laboratories. The remaining two laboratories did not have a standard reporting procedure, as they had not identified any ESBL-producing organisms.

3.2.2 Reporting Cephamycin Susceptibility

Thirty-one laboratories answered the question about how they reported susceptibility to cephamycins, such as cefoxitin and cefotetan. Six (19.4%) laboratories reported the actual result obtained in cephamycin susceptibility tests. One laboratory standardly reported ESBL-producing organisms as cephamycin resistant. One laboratory indicated that their reporting was

variable "depending on a number of factors". However, the majority (67.7%) of laboratories either did not test or did not report cephamycin susceptibility. The remaining two laboratories did not have a standard reporting procedure, as they had not identified any ESBL-producing organisms.

3.2.3 Reporting of Susceptibility to β-lactam/β-lactamase Inhibitor Combinations

Twenty-nine laboratories answered the question about how they reported susceptibility to β-lactam/β-lactamase inhibitor combinations. Nine (31.0%) laboratories reported the actual result obtained in susceptibility tests. Eight standardly reported ESBL-producing organisms as resistant to β-lactam/β-lactamase inhibitor combinations, while three standardly reported ESBL producers as susceptible to these combinations. One laboratory indicated that their reporting was variable "depending on a number of factors". Five laboratories did not report susceptibility to these combinations. The remaining three laboratories did not have a standard reporting procedure, as they had not identified any ESBL-producing organisms.

3.2.4 Additional Antimicrobial Susceptibility Testing and/or Reporting for Multiresistant ESBL-producing Organisms

Twenty-five laboratories answered the question about which additional antibiotics were tested and/or reported when an ESBL-producing organism is multiresistant. There were a wide variety of responses. However, the most common additional antibiotics tested or reported are shown in Table 7.

Table 7. Additional Antibiotics Tested or Reported when an ESBL-producing Organism is Multiresistant

Antibiotic	Number of laboratories
Gentamicin	8
Amikacin [1]	8
Tobramycin	5
Aminoglycoside[2]	2
Ciprofloxacin	6
Fluoroquinolone[2]	3
Norfloxacin	1
Imipenem	10
Meropenem	4
Ertapenem	1
Carbapenem[2]	1
Piperacillin/tazobactam	4
Cefoxitin	4
Co-trimoxazole	4

[1] Four of the eight labs specified that they only tested amikacin susceptibility if the isolate was gentamicin resistant.
[2] Particular antibiotic of the class not specified.

3.2.5 Reporting the Isolation of an ESBL-producing Organism to the Infection Control Services

Thirty-one laboratories responded to the question about whether they reported the isolation of an ESBL-producing organism to infection control services. Most (87.1%) did advise infection control staff. One laboratory advised the clinical microbiologist. Two laboratories did not report to infection control, but both of these laboratories were community laboratories. The remaining laboratory did not have a policy on reporting to infection control services, as they had not identified any ESBL-producing organisms to date.

3.3 Assessment of the Methods Commonly Used to Identify ESBLs

3.3.1 Growth on Aztreonam Sheep Blood Agar

Table 8. Growth of ESBL-positive Enterobacteriaceae and K1-positive *K. oxytoca* on Aztreonam Sheep Blood Agar

	Percent (number) of isolates				
	ESBL positive				**K1 positive**
	E. coli n=75	***K. pneumoniae*** n=29	***K. oxytoca*** n=4	**Other Enterobacteriaceae** n=29	***K. oxytoca*** n=9
Growth on aztreonam (6 mg/L) sheep blood agar	62.7 (47)	51.7 (15)	100 (4)	93.1 (27)	100 (9)

Aztreonam sheep blood agar (AztBA) had poor sensitivity for the selection of ESBL-positive *E. coli* and *K. pneumoniae* (Table 8).

There was complete correlation between the ability to grow on AztBA, which contains 6 mg/L of aztreonam, and the aztreonam MICs. All isolates with aztreonam MICs ≥16 mg/L grew on AztBA, while none of the isolates with MICs ≤4 mg/L grew. Among seven isolates with aztreonam MICs of 8 mg/L, four did and three did not grow on AztBA. The precise aztreonam MICs of these seven isolates would be between 4 and 8 mg/L, which is consistent with their variable growth on a medium containing 6 mg/L aztreonam.

3.3.2 Antimicrobial Susceptibility

Table 9. Antimicrobial Susceptibility among ESBL-positive Enterobacteriaceae and K1-positive *K. oxytoca*

Antibiotic	Percent resistance				
	ESBL positive				K1 positive
	E. coli n=75	*K. pneumoniae* n=29	*K. oxytoca* n=4	Other Enterobacteriaceae n=29	*K. oxytoca* n=9
Cefuroxime	97.3	69.0	75.0	100	100
Aztreonam	56.0	44.8	50.0	93.1	33.3
Cefotaxime	77.3 (93.3)[1]	13.8 (75.9)	0 (25.0)	20.7 (65.5)	0
Ceftazidime	40.0 (58.7)	55.2 (55.2)	100 (100)	93.1 (93.1)	0
Co-amoxiclav	30.7[2]	17.2	25.0	93.1	44.4
Meropenem	0	0	0	0	0
Ciprofloxacin	81.3	24.1	0	17.2	0
Gentamicin	60.0	72.4	50.0	82.8	11.1
Tobramycin	65.3	41.4	25.0	72.4	11.1
Amikacin	0	0	0	0	0
Co-trimoxazole	70.7	69.0	50.0	89.7	11.1
Trimethoprim	73.3	69.0	50.0	93.1	11.1
Nitrofurantoin	1.3	24.1	25.0	20.7	0
Tetracycline	82.7	55.2	25.0	69.0	0

[1] The percentages in brackets are the resistance rates based on the microbroth dilution MICs, whereas all the other resistance rates are based on agar dilution MICs.

[2] The rate of co-amoxiclav resistance among the 68 *E. coli* isolates that produced only an ESBL (ie, excluding the 7 isolates that produced both an ESBL and AmpC) was 26.5%

There were differences between the estimates of cefotaxime and ceftazidime resistance depending on whether the MIC was determined by agar or microbroth dilution. While for this study, all MICs were determined by agar dilution, microbroth dilution MICs were also available from the CLSI phenotypic confirmatory microbroth dilution testing (see section 3.3.7). The microbroth dilution MICs were often higher than the agar dilution MICs. Where there was a major discrepancy between the two MICs, that is, an interpretation of susceptible versus resistant, the microbroth dilution MICs were repeated. The results in Table 9 are those after any repeat testing.

3.3.3 Multiresistance

Table 10. Multiresistance among ESBL-positive Enterobacteriaceae and K1-positive *K. oxytoca* to Individual Antibiotics in Addition to Cephalosporins and Monobactams

Resistant to (number of antibiotics):[1]	Percent (number)				
	ESBL positive				K1 positive
	E. coli n=75	*K. pneumoniae* n=29	*K. oxytoca* n=4	Other Enterobacteriaceae n=29	*K. oxytoca* n=9
0	2.7 (2)	10.3 (3)	50.0 (2)	0 (0)	44.4 (4)
1	4.0 (3)	0 (0)	0 (0)	6.9 (2)	44.4 (4)
2	8.0 (6)	6.9 (2)	0 (0)	3.5 (1)	0 (0)
3	10.7 (8)	20.7 (6)	0 (0)	0 (0)	11.1 (1)
4	18.7 (14)	31.0 (9)	0 (0)	3.5 (1)	0 (0)
5	13.3 (10)	17.2 (5)	50.0 (2)	24.1 (7)	0 (0)
6	26.7 (20)	10.3 (3)	0 (0)	44.8 (13)	0 (0)
7	16.0 (12)	3.5 (1)	0 (0)	17.2 (5)	0 (0)

[1] From the following antibiotics: co-amoxiclav, meropenem, ciprofloxacin, gentamicin, tobramycin, amikacin, co - trimoxazole, trimethoprim, nitrofurantoin and tetracycline.

Table 11. Multiresistance among ESBL-positive Enterobacteriaceae and K1-positive *K. oxytoca* to Antibiotic Classes in Addition to Cephalosporins and Monobactams

Resistant to (number of antibiotic classes):[1]	Percent (number)				
	ESBL positive				K1 positive
	E. coli n=75	*K. pneumoniae* n=29	*K. oxytoca* n=4	Other Enterobacteriaceae n=29	*K. oxytoca* n=9
0	2.7 (2)	10.3 (3)	50.0 (2)	0 (0)	44.4 (4)
1	9.3 (7)	3.5 (1)	0 (0)	6.9 (2)	44.4 (4)
2	5.3 (4)	31.0 (9)	0 (0)	3.5 (1)	11.1 (1)
3	28.0 (21)	31.0 (9)	25.0 (1)	13.8 (4)	0 (0)
4	36.0 (27)	17.2 (5)	25.0 (1)	55.2 (16)	0 (0)
5	18.7 (14)	6.9 (2)	0 (0)	20.7 (6)	0 (0)
≥3	82.7 (62)	55.2 (16)	50.0 (2)	89.7 (26)	0 (0)

[1] From the following antibiotic classes: co-amoxiclav, meropenem, ciprofloxacin, aminoglycosides, folate pathway inhibitors, nitrofurantoin and tetracycline.

The most common antibiotic multiresistance patterns among *E. coli* were aminoglycoside / ciprofloxacin / folate pathway inhibitor / tetracycline resistance (22 isolates) and co-amoxiclav / aminoglycoside / ciprofloxacin / folate pathway inhibitor / tetracycline resistance (14 isolates). No pattern was dominant among multiresistant *K. pneumoniae*. Co-amoxiclav / aminoglycoside / folate pathway inhibitor / tetracycline resistance was the most common multiresistance pattern among isolates in the Other Enterobacteriaceae category.

3.3.4 CLSI Initial Screen Disc Test

The sensitivity of the CLSI initial screen disc test for all 137 ESBL-positive isolates; the 108 ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca*; and the 29 ESBL-positive Other Enterobacteriaceae is shown in Table 12.

Table 12. Sensitivity of CLSI Initial Screen Disc Test

Disc	CLSI screening interpretive standard (mm)	Percent positive		
		All ESBL-positive isolates n=137	**ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca*** n=108	**ESBL-positive Other Enterobacteriacea e** n=29
Cefpodoxime 10 μg	≤17	97.1	96.3	100
Cefotaxime 30 μg	≤27	98.5[1]	98.2	100
Ceftriaxone 30 μg	≤25	98.5[1]	98.2	100
Ceftazidime 30 μg	≤22	78.1	74.1	93.1
Aztreonam 30 μg	≤27	94.9	95.4	93.1

[1] There was complete correlation between the cefotaxime and ceftriaxone results.

None of the 108 ESBL-positive *E. coli*, *K. pneumoniae* or *K. oxytoca* screened negative with both cefotaxime and ceftazidime or both ceftriaxone and ceftazidime. Six of the isolates screened negative with more than one of the five antimicrobials tested: three were negative with both ceftazidime and aztreonam, one was negative with both cefpodoxime and aztreonam, one was negative with both cefotaxime and ceftriaxone, and one was negative with cefpodoxime, cefotaxime, ceftriaxone and aztreonam.

None of the 29 ESBL-positive Other Enterobacteriaceae screened negative with both cefotaxime and ceftazidime or both ceftriaxone and ceftazidime. Two isolates screened negative with both ceftazidime and aztreonam.

All nine K1-positive *K. oxytoca* screened positive with aztreonam and ceftriaxone, eight were positive with cefotaxime, five with cefpodoxime, and none with ceftazidime.

3.3.5 CLSI Initial Screen Microbroth Dilution Test

The sensitivity of the CLSI initial screen microbroth dilution test was not fully tested as only two laboratories indicated that they used it. However, as cefotaxime and ceftazidime microbroth dilution MICs were available from the CLSI phenotypic confirmatory microbroth dilution testing (see section 3.3.7), the sensitivity of this screening test with these two cephalosporins could be evaluated and is shown in Table 13. CLSI methods also provide ceftriaxone, cefpodoxime and aztreonam MIC screening breakpoints.

Table 13. Sensitivity of CLSI Initial Screen Microbroth Test

	CLSI screening MIC breakpoint (mg/L)	**Percent positive**		
		All ESBL-positive isolates n=137	**ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca*** n=108	**ESBL-positive Other Enterobacteriaceae** n=29
Cefotaxime	≥2	97.8	97.2	100
Ceftazidime	≥2	92.7	91.7	96.6

None of the 137 ESBL-positive isolates screened negative with both cefotaxime and ceftazidime.

Seven of the nine K1-positive *K. oxytoca* screened positive with cefotaxime and one was positive with ceftazidime.

3.3.6 CLSI Phenotypic Confirmatory Disc Test

Table 14. Sensitivity of CLSI Confirmatory Disc Test

Disc (30 µg) ± clavulanic acid (10 µg)	**Interpretive standard**	**Percent positive**		
		All ESBL-positive isolates n=137	**ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca*** n=108	**ESBL-positive Other Enterobacteriaceae** n=29
Cefotaxime	≥5 mm difference[1]	86.9	94.4	58.6
Ceftazidime	≥5 mm difference[1]	65.7	63.9	72.4
Cefpirome[2]	≥4 mm difference[3]	-	-	82.8
Cefotaxime and/or ceftazidime		96.4	100	82.8
Cefotaxime, ceftazidime and/or cefpirome		97.1	100	86.2

[1] CLSI interpretive standard, reference 8.
[2] Only the 29 isolates in the Other Enterobacteriaceae category were tested with cefpirome combination discs.
[3] Based on reference 9.

None of the nine K1-positive *K. oxytoca* isolates was positive in this test with either cefotaxime or ceftazidime.

3.3.7 CLSI Phenotypic Confirmatory Microbroth Dilution Test

Table 15. Sensitivity of CLSI Confirmatory Microbroth Test

	CLSI interpretive standard	Percent positive		
		All ESBL-positive isolates n=137	ESBL-positive *E. coli*, *K. pneumoniae* and *K. oxytoca* n=108	ESBL-positive Other Enterobacteriaceae n=29
Cefotaxime ± clavulanic acid	8-fold decrease in MIC	84.7	97.2	37.9
Ceftazidime ± clavulanic acid	8-fold decrease in MIC	78.1	83.3	58.6
Cefotaxime and/or ceftazidime		92.7	100	65.5

Six of the nine K1-positive *K. oxytoca* isolates were positive with cefotaxime. None was positive with ceftazidime.

3.3.8 Double-disc Synergy (Jarlier) Test

The original description of the double-disc synergy (Jarlier) method specifies placing the cephalosporin disc and the co-amoxiclav disc at a distance of 30 mm centre-to-centre.[10] All isolates were tested at distances of both 20 and 30 mm. Cefaclor and cefuroxime are second-generation antibiotics and therefore would not usually be used in this test. They were tested as several laboratories indicated that they screened for ESBLs by placing a co-amoxiclav disc adjacent to a cefaclor or cefuroxime disc on their routine antimicrobial susceptibility testing plates.

The shorter distance of 20 mm was clearly superior to that of 30 mm (Table 16). Performing the test at a distance of 30 mm added no additional sensitivity, as none of the 137 ESBL-positive isolates that were negative at 20 mm were positive at 30 mm. Further testing at a distance of 25 mm, of isolates that gave indeterminate results at 20 and 30 mm, added very little increased sensitivity (Table 16). As testing at 25 mm was only performed when the result was indeterminate at 20 and 30 mm, a comparison of the sensitivity at 25 mm with that at 20 and 30 mm cannot be made.

The second-generation cephalosporins, cefaclor and cefuroxime, performed relatively poorly compared to the third- and fourth-generation cephalosporins. This result may be related to the fact that the cefaclor and cefuroxime zones of inhibition were usually very small. Among the 137 ESBL-positive isolates, 76% had no (≤6 mm) cefaclor zone of inhibition and 66% had no cefuroxime zone. Similarly the sensitivity with cefpodoxime was relatively low and 65% of the ESBL-positive isolates had no cefpodoxime zone of inhibition. In contrast only 15%, 8% and 0% of the isolates, respectively, had no cefotaxime, ceftazidime and cefepime zone of inhibition.

At disc spacings of either 20, 25 or 30 mm, all nine K1-positive *K. oxytoca* isolates tested positive with cefaclor, cefpodoxime, cefotaxime and cefepime, eight were positive with cefuroxime, and all were negative with ceftazidime.

Table 16. Sensitivity of Double-disc Synergy (Jarlier) Test

Antimicrobial	20 mm[1]		30 mm[1]		20/25/30 mm[1]
	Percent positive[2]	Positive at 20 but not 30mm	Percent positive[2]	Positive at 30 but not 20mm	Percent positive at any distance[2]
All ESBL-positive isolates (n=137)					
Cefaclor[3]	76.6	59.9	17.5	0	77.4
Cefuroxime[3]	83.9	61.3	22.6	0	83.9
Cefpodoxime	88.3	61.3	27.0	0	88.3
Cefotaxime	95.6	15.3	80.3	0	95.6
Ceftazidime	93.4	35.0	58.4	0	94.2
Cefepime	98.5	13.1	85.4	0	99.3
Cefotaxime and/or ceftazidime	97.8	11.7	86.1	0	97.8
Cefotaxime, ceftazidime and/or cefepime	100	6.6	93.4	0	100
ESBL-positive *E. coli, K. pneumoniae* and *K. oxytoca* (n=108)					
Cefaclor[3]	91.7	70.4	22.2	0	92.6
Cefuroxime[3]	90.7	70.4	20.4	0	90.7
Cefpodoxime	95.4	63.9	31.5	0	95.4
Cefotaxime	98.2	17.6	80.6	0	98.2
Ceftazidime	94.4	35.2	59.3	0	95.4
Cefepime	98.2	13.9	84.3	0	99.1
Cefotaxime and/or ceftazidime	100	13.0	87.0	0	100
Cefotaxime, ceftazidime and/or cefepime	100	6.5	93.5	0	100
ESBL-positive Other Enterobacteriaceae (n=29)					
Cefaclor[3]	20.7	20.7	0	0	20.7
Cefuroxime[3]	58.6	27.6	31.0	0	58.6
Cefpodoxime	62.1	51.7	10.3	0	62.1
Cefotaxime	86.2	6.9	79.3	0	86.2
Ceftazidime	89.7	34.5	55.2	0	89.7
Cefepime	100	10.3	89.7	0	100
Cefotaxime and/or ceftazidime	89.7	6.9	82.8	0	89.7
Cefotaxime, ceftazidime and/or cefepime	100	6.9	93.1	0	100

[1] Distance centre-to-centre between co-amoxiclav and cephalosporin discs.

[2] Doesn't include indeterminate results.

[3] Included as several labs indicated that they placed a cefaclor or cefuroxime disc adjacent to a co -amoxiclav disc on routine susceptibility testing plates to screen for ESBLs.

3.3.9 Vitek 1 and Vitek 2 Automated Systems

Seventy of the ESBL-positive isolates in the full test panel were tested in the Vitek 1 and Vitek 2 automated systems (Table 17).

Table 17. Sensitivity in the Vitek 1 and 2 Automated Systems

	Percent positive		
	All ESBL-positive isolates n=70	**ESBL-positive *E. coli, K. pneumoniae* and *K. oxytoca*** n=55	**ESBL-positive Other Enterobacteriaceae** n=15
Vitek 1[1]	82.9	94.6	40.0
Vitek 2[2]	95.7	98.2	86.7[3]

[1] The lab that tested the isolates in the Vitek 1, retested any isolates that the Vitek deemed negative using the CLSI confirmatory disc test with the addition of the cefpirome discs with and without clavulanic acid. This additional testing resulted in all isolates that only produced an ESBL and 88.9% of ESBL + AmpC producers being identified as ESBL producers.

[2] Based on final results which include the advanced expert findings.

[3] All of the ESBL-positive Other Enterobacteriaceae tested in the Vitek systems also produced AmpC β-lactamase. The Vitek 2's advanced expert system noted that 5 of the 15 isolates (2 *C. freundii*, 2 *Enterobacter* and 1 *S. fonticola*) also produced high-level or wild cephalosporinase.

Five K1-positive *K. oxytoca* isolates were tested in the Vitek1 and Vitek 2 systems, and four and three isolates, respectively, were identified as ESBL producers.

4 DISCUSSION

Most (82.6%) diagnostic laboratories in New Zealand are testing for ESBL-producing organisms. It is likely that this proportion has increased since August 2003 when laboratories were surveyed for this study.

Studies in other countries have variously reported the superior sensitivity of particular third-generation cephalosporins over others in identifying ESBLs.[12,13,14,15] There are several possible reasons for this variation. First, the relative sensitivity of the cephalosporins will depend on the local prevailing ESBL types and their substrate specificity. Second, changes in the prevailing ESBL types over time. Many earlier studies, conducted when TEM and SHV-derived ESBLs were most common, often reported ceftazidime was more useful. However, CTX-M ESBLs, which typically confer greater resistance to cefotaxime than ceftazidime, are now becoming increasingly prevalent in many countries.[16] Preliminary work on the identification of ESBL types in New Zealand at ESR indicates that two outbreak strains of ESBL-positive *E. coli* both have CTX-M-15 ESBL. Third, the range of organisms included in a study may influence the relative sensitivity of the different cephalosporins. For example, the inclusion of AmpC β-lactamase producers, such as *Enterobacter*, may alter the relative sensitivity. It is therefore important that the methods used to identify ESBLs are those that are most appropriate for the types of ESBLs that are currently prevalent in a country or area.

Sensitivity of the Most Common Screening Methods

About a quarter of laboratories at times perform direct screening of clinical specimens for ESBL-producing organisms. The most common method of screening clinical specimens was plating on aztreonam (6 mg/L) blood agar. Aztreonam blood agar is designed specifically for the isolation of gram-positive cocci and anaerobes against which aztreonam has no useful activity. The growth of aerobic gram-negative organisms is usually suppressed. But, as ESBLs confer resistance to aztreonam, in theory gram-negative organisms that produce an ESBL should grow on this medium. However, the concentration of aztreonam in the agar is markedly higher than the CLSI ESBL screening breakpoint concentration of 1 mg/L. Aztreonam blood agar had poor sensitivity for ESBL-positive *E. coli* and *Klebsiella*, with only 62.7 and 57.6%, respectively, growing under our test conditions. In contrast, 90% of the ESBL-positive *E. coli* and *Klebsiella* had aztreonam MICs ≥2 mg/L and therefore would grow at the 1 mg/L screening breakpoint concentration (data not shown).

Many laboratories, especially community laboratories, used or adapted routine susceptibility testing procedures to screen for ESBL-producing organisms. Nearly two-thirds of the laboratories reported that they used multiresistance or a particular resistance pattern as an indicator that an organism may produce an ESBL. Multiresistance, including ciprofloxacin and aminoglycoside resistance, is a frequently described attribute of ESBL-producing organisms.[12,17] The majority of the ESBL-positive *E. coli* (82.7%), *Klebsiella* (54.5%) and isolates in the other Enterobacteriaceae species category (89.7%) were multiresistant to ≥3 classes of antibiotics in addition to cephalosporins and monobactams (Table 11). ESBL-positive *E. coli* were commonly resistant to ciprofloxacin, aminoglycosides, co-trimoxazole/trimethoprim and tetracycline. No particular multiresistance pattern was dominant among the ESBL-positive *Klebsiella*.

Seven laboratories reported that they used a pattern of first- or second-generation cephalosporin resistance with co-amoxiclav susceptibility as an indicator of a possible ESBL producer. However, a perhaps unexpected finding was the amount of co-amoxiclav resistance among the

ESBL-positive *E. coli* (30.7%) and *Klebsiella (*18.2%*).* Many ESBL-producing organisms also produce the narrower-spectrum, parent TEM and SHV β-lactamases (eg, TEM-1, TEM-2 or SHV-2). Hyperproduction of these β-lactamases can result in a reduction in the effect of β-lactamase inhibitors and therefore co-amoxiclav resistance. Alternatively, ESBL producers may be co-amoxiclav resistant due to several other mechanisms, including the co-production of inhibitor-resistant OXA-1-like β-lactamases or porin loss.[18]

Another common screening method was the placement of a cefaclor or cefuroxime disc adjacent to a co-amoxiclav disc on routine susceptibility testing plates to detect clavulanate inhibition of β-lactamase. The principal of this procedure is the same as that of the double-disc synergy (Jarlier) test, and, in this test, these second-generation cephalosporins performed relatively poorly compared to the third- and fourth-generation cephalosporins. Poor specificity for ESBLs would be expected with second- and first-generation cephalosporins, but the poor sensitivity was initially surprising. However, it may be related to the fact that the cefaclor and cefuroxime zones of inhibition were usually very small. In this situation, disc spacing closer than the minimum 20 mm that we used is probably required to consistently demonstrate synergy with clavulanic acid.

The CLSI initial screen disc test was also commonly used. While this test, like the other CLSI screening and confirmatory tests, is specifically recommended for *E. coli*, *K. pneumoniae* and *K. oxytoca* (and more recently *P. mirabilis*), it showed good sensitivity for all 137 ESBL-positive isolates with cefotaxime/ceftriaxone, cefpodoxime and aztreonam discs, but was much less sensitive with ceftazidime discs (Table 12). Based on these results, we recommend that, if only one disc is used in this screening test, either cefotaxime, ceftriaxone or cefpodoxime be used. Ceftazidime alone should not be used.

Sensitivity of the Most Common Confirmatory Methods

E. coli and Klebsiella

The CLSI confirmatory disc test, which uses cefotaxime and ceftazidime discs with and without clavulanic acid, was the most common confirmatory test used. Not unexpectedly, the results in the CLSI initial screen disc test were mirrored in the CLSI confirmatory disc test. Among the ESBL-positive *E. coli* and *Klebsiella*, 94.4% were confirmed with cefotaxime but only 63.9% with ceftazidime. However, this test specifies the use of both cephalosporins, and all ESBL-positive *E. coli* and *Klebsiella* were confirmed with at least one of these two cephalosporins.

It is often claimed that the double-disc synergy (Jarlier) test is less reliable than the CLSI confirmatory disc test because it is dependent on using optimal disc spacing between the co-amoxiclav disc and the cephalosporin discs.[19] However, all ESBL-positive *E. coli* and *Klebsiella* were also confirmed by the double-disc synergy test with either cefotaxime or ceftazidime at a disc spacing of 20 mm (Table 16). A notable feature of the double-disc synergy test was the much better sensitivity of ceftazidime in this test than in the CLSI confirmatory disc test: 94.4 vs 63.9% sensitivity, respectively. The reason for this is unclear. While the double-disc synergy test performed at least as well as the CLSI confirmatory disc test in our hands, the CLSI test requires less experience to read and therefore may be more reliable for laboratories performing ESBL confirmatory tests only irregularly.

The original description of the double-disc synergy test specifies the placement of the discs

30 mm apart (centre-to-centre). Several investigators have suggested that reducing the distance between the discs can increase the sensitivity of this test. Our results confirm this, with much greater sensitivity at a spacing of 20 mm. Any isolate positive at 30 mm was also positive at 20 mm, but not vice versa. Therefore, we recommend that this test should be performed at 20 mm, rather than 30 mm. A spacing of 25 mm may also be satisfactory, but this distance was not comprehensively tested in this study.

Both Vitek automated systems performed well in identifying ESBL-positive *E. coli* and *Klebsiella*, with sensitivities of 94.6% and 98.2%, respectively, for the Vitek 1 and 2 (Table 17). Similar sensitivity has been previously reported.[20,21]

AmpC β-lactamase Producers

Detection of ESBLs in Enterobacteriaceae species that produce AmpC β-lactamase (eg, *Enterobacter*, *Serratia* and *C. freundii*) can be difficult, especially when the AmpC β-lactamase is derepressed. However, 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 are also producing AmpC β-lactamase.[22]

The test panel included 29 isolates of Enterobacteriaceae other than *E. coli* or *Klebsiella* (Table 1). Among these 29 isolates, 27 produced both AmpC β-lactamase, either inducible or derepressed, and an ESBL. Our results showed some gain in using a fourth-generation cephalosporin over the third-generation cephalosporins to detect ESBLs in the presence of AmpC β-lactamase. The gain was small in the CLSI confirmatory disc test: 86.2% sensitivity using cefotaxime, ceftazidime and cefpirome versus 82.8% sensitivity with the standard cefotaxime and ceftazidime. In the double-disc synergy test, the inclusion of cefepime resulted in all 29 ESBL-positive isolates being identified compared with 89.7% when only cefotaxime and ceftazidime were used. As different fourth-generation cephalosporins were used in the two tests, the difference in sensitivity may be a reflection of a difference between the two cephalosporins rather than a difference between the two test methods.

The Vitek 2 automated system, using its advanced expert system, performed better than the Vitek 1 in identifying ESBLs in AmpC β-lactamase producers. The ability of the Vitek 2's advanced expert system to detect ESBLs in these organisms has been noted in other studies.[21]

Hyperproduction of K1 β-lactamase in *K. oxytoca*

A small sample of *K. oxytoca* isolates that were hyperproducers of the chromosomally-mediated K1 β-lactamase found in this species was included in the test panel of isolates, as this resistance can be mistaken for ESBL. Isolates hyperproducing K1 β-lactamase have frank resistance to aztreonam, usually ceftriaxone resistance, sometimes reduced cefotaxime susceptibility, but are ceftazidime susceptible. Any resistance to ceftriaxone is usually greater than any resistance to cefotaxime. The enzyme is usually inhibited to some extent by clavulanate.

The test panel included nine K1-positive *K. oxytoca* isolates. In contrast to ESBL producers, multiresistance was not common in K1-positive *K. oxytoca*. In the ESBL screening and confirmatory tests, the K1-positive *K. oxytoca* often tested positive for ESBL production with aztreonam, ceftriaxone, cefotaxime, cefpodoxime and/or cefepime. However, with one

exception, all K1-positive *K. oxytoca* isolates were negative in all tests with ceftazidime. Both Vitek systems mis-identified K1-positive *K. oxytoca* as ESBL producers.

It is recommended that *K. oxytoca* isolates that test positive for ESBLs with cephalosporins other than ceftazidime but negative with ceftazidime, be considered as possible K1 hyperproducers. To confirm K1 hyperproduction, the relative susceptibility to aztreonam, ceftriaxone, cefotaxime and ceftazidime needs to be tested. At ESR, any *K. oxytoca* isolates are tested by double-disc synergy using aztreonam, ceftriaxone, cefotaxime and ceftazidime discs adjacent to a co-amoxiclav disc at a distance of 20 mm (centre-to-centre).

Limitations

This study has several limitations. First, our estimates of the sensitivity of the various ESBL screening and confirmatory tests have an inherent bias. The ESBL-positive *E. coli* and *Klebsiella* included in the test panel had been confirmed at ESR with CLSI confirmatory disc and microbroth tests using cefotaxime and ceftazidime. ESBLs in Enterobacteriaceae other than *E. coli* and *Klebsiella* had been confirmed with the double-disc synergy test using cefpodoxime, cefotaxime, ceftazidime and cefepime. Second, test specificity was not systematically evaluated, as, with the exception of the nine K1-positive *K. oxytoca*, the test panel included only ESBL-positive isolates. Third, the isolates in the test panel were only confirmed as ESBL producers phenotypically and their β-lactamases have not been characterised either biochemically or genetically.

Reporting Practices

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.[23,24] Therefore it is important to identify ESBL producers and report them as resistant to all cephalosporins and monobactams (aztreonam). While it is recommended that the actual susceptibility test results obtained for β-lactam/β-lactamase inhibitor combinations and for cephamycins (eg, cefoxitin) are reported,[8] there is a lack of clinical data to support the use of either inhibitor combinations or cephamycins for the treatment of serious infections due to ESBL-producing organisms.[25]

Most (82.4%) laboratories correctly reported ESBL-producing organisms as resistant to cephalosporins and monobactams, if they reported susceptibility to these agents at all. Most laboratories did not test or report cephamycin susceptibility. However, among those laboratories that did report cephamycin susceptibility, most reported the actual results obtained.

The practices were more mixed with the reporting of susceptibility to β-lactam/β-lactamase inhibitor combinations. About one-third (31.0%) of laboratories reported the actual results obtained. Nearly the same number (27.6%) standardly reported ESBL-producing organisms as resistant to β-lactam/β-lactamase inhibitor combinations, while 10.3% did the reverse and standardly reported ESBL producers as susceptible to these combinations.

Almost all laboratories appropriately reported the isolation of an ESBL-producing organism to their infection control services.

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APPENDIX

Your laboratory's name: _____

Name of person completing the questionnaire: _____

This questionnaire is divided into three main parts: (A) methods used to screen for ESBLs, (B) methods used to confirm ESBLs, and (C) reporting the susceptibility of ESBL-producing organisms, testing and reporting susceptibility to additional antibiotics, and reporting to infection control.

A Screening for ESBLs, that is, initial screening of clinical specimens for possible ESBL-producing organisms and initial screening of isolates for ESBL production

A1 Do you attempt to detect or screen for ESBLs? Yes / No
(circle correct choice)

If no, go to section D of this questionnaire.

A2 Do you use selective media to screen clinical samples (for example, faecal specimens)? Yes / No

If yes, what media and what specimens is it used for:

_____

_____

A3 Do you use an ESBL antimicrobial susceptibility screening test, such as the NCCLS ESBL initial screen disc or MIC breakpoint tests, to screen isolates? Yes / No

A3.1 If yes, and NCCLS initial screen tests used, please complete this table:

Organism	**NCCLS ESBL initial screen disc test**	**NCCLS ESBL initial screen MIC breakpoint test**
	Please list antibiotics used	**Please list antibiotics used**
E. coli		
Klebsiella spp		
Other Enterobacteriaceae		
Other organisms (please specify)		

A3.2 If yes, and an ESBL antimicrobial susceptibility screening test other than the NCCLS tests used, please detail the method, including what organisms it is used for and what antibiotics are used:

_____

_____

_____

_____

A4 Do you use an isolate's resistance profile, as obtained in routine antimicrobial susceptibility tests (ie, as opposed to the results obtained in the specific ESBL antimicrobial susceptibility screening tests asked about in section A3), to detect possible ESBLs? Yes / No

If yes, please elaborate on the profiles, including what organisms they are used for:

_____

_____

_____

_____

A5 Do you use some other screening test(s)? Yes / No

If yes, please describe and include a reference if applicable.

_____

_____

_____

_____

A6 If the protocols you have described above are only used selectively, please specify when they are used, for example, for specimens or isolates from particular high-risk patients or for periodic screening rather than on an ongoing basis:

_____

_____

_____

_____

B Confirmation of EBSL production

B1 If you use either the double-disc synergy (Jarlier) test, Etest ESBL strips, or the NCCLS ESBL disc confirmatory test to confirm ESBL production, please complete this table by placing a tick in the appropriate boxes:

Organism	Method			Antibiotics						
	Double-disc synergy*	**E-test ESBL strip**	**NCCLS disc**	**Cefotaxime**	**Ceftazidime**	**Ceftriaxone**	**Cefpodoxime**	**Cefepime**	**Aztreonam**	**Other antibiotics** (please specify)
E. coli	□	□	□	□	□	□	□	□	□	
Klebsiella spp	□	□	□	□	□	□	□	□	□	
Other Enterobacteriaceae	□	□	□	□	□	□	□	□	□	
Others (please specify)	□	□	□	□	□	□	□	□	□	

* a disc containing clavulanate (eg, co-amoxiclav) placed adjacent to third/fourth-generation cephalosporin discs

B2 If you use the double-disc synergy (Jarlier) test, please specify the distance(s) between the placement of the clavulanate-containing disc and the cephalosporin discs:

_____

_____

_____

B3 Do you use a confirmatory test other than the double-disc synergy (Jarlier) test, Etest ESBL strips, or NCCLS ESBL disc confirmatory test? Yes / No

If yes, please describe and include a reference if applicable. Please include which cephalosporins are used and which organisms you use the test for:

_____

_____

_____

B4 Do you refer the isolate to another lab, for example ESR, for confirmation? Please answer 'no' to this question if you refer ESBL-producing isolates to ESR primarily for ESR's surveillance programme rather than confirmation per se. Yes / No

C Reporting and further testing the susceptibility of ESBL-producing organisms

C1 Do you override the susceptibility test results of all cephalosporins (including fourth-generation cephalosporins) and aztreonam, and report as resistant? Yes / No

If no, how do you report cephalosporin and aztreonam susceptibility:

_____

_____

_____

C2 How do you report cephamycin susceptibility:

_____

_____

_____

C3 How do you report the susceptibility of ß-lactam/ß-lactamase inhibitor combinations (eg, co-amoxiclav and piperacillin/tazobactam):

_____

_____

_____

C4 If an ESBL-producing organism is multiresistant, do you test or report susceptibility to additional antibiotics? Yes / No

If yes, which antibiotics:

_____

_____

_____

C5 Do you notify the infection control service when an ESBL is confirmed in a hospital inpatient or resident of a residential-care facility? Yes / No

D Please add any further comments you wish to make about this survey:

_____

_____

_____

_____