Original articles

ß-LACTAMASES IN SALMONELLA ENTERICA ISOLATED IN AUSTRALIA

Gino Micalizzi

Abstract

Understanding the antibiotic susceptibility of Salmonella enterica is important both from a clinical treatment and a public health perspective. The emergence of extended spectrum ß-lactamases (ESBLs) and AmpC ß-lactamases in S. enterica is important, as this will limit treatment options and could provide a strain with a significant selective advantage. The aim of the study was to screen isolates of S. enterica, including isolates that had previously shown antibiotic resistance, to gauge the extent of ß-lactamase activity in S. enterica in Australia. Phenotypic detection involved screening in accordance with Clinical and Laboratory Standards Institute double disk synergy test guidelines and assessing susceptibility to cefoxitin. Presumptive positives were then screened using a MAST® AmpC & ESBL detection set.

S. enterica isolates that were consecutively received in the laboratory (n=624), or had previously exhibited some antibiotic resistance (n=351), were screened for ß-lactamase activity. None of the isolates in the second group were included in the first. ß-lactamase activity was detected in nine of the consecutively received isolates; one with demonstrated ESBL activity and eight others with demonstrated AmpC ß-lactamase activity. None of the isolates in the second group were included in the first. ß-lactamase activity was detected in nine of the consecutively received isolates; one with demonstrated ESBL activity and eight others with demonstrated AmpC ß-lactamase activity. None of the isolates in the second group were included in the first. ß-lactamase activity was detected in nine of the consecutively received isolates; one with demonstrated ESBL activity and eight others with demonstrated AmpC ß-lactamase activity.

ß-lactamase activity was detected in 16 of the isolates that had previously demonstrated some antibiotic resistance; three with demonstrated ESBL activity and 13 others with demonstrated AmpC ß-lactamase activity. S. enterica serovar Stanley is a serovar that is frequently acquired overseas and this serovar had the highest proportion of isolates that demonstrated ß-Lactamase activity in consecutively sampled isolates (4.95%), reflecting the emergence of an epidemic clone within South East Asia. While antibiotic resistance is being detected in Salmonella isolates, the data indicates that there is limited awareness of, or screening for, ß-lactamases in S. enterica. This study will help to overcome these deficiencies and provide some baseline surveillance data against which future trends can be measured. Commun Dis Intell 2013;37(1):47–51.

Keywords: extended spectrum ß-lactamase, AmpC ß-lactamase, Salmonella enterica, Queensland, Australia, antibiotic, resistance, surveillance

Introduction

Salmonella enterica belongs to the family Enterobacteriaceae and is one of the main causes of foodborne illness worldwide. The most common form of disease caused by non-typhoidal Salmonella is gastroenteritis or salmonellosis. Extra-intestinal infection resulting in bacteraemia occurs in approximately 5 per cent of cases. Other invasive diseases such as meningitis, osteomyelitis and pneumonia are more likely in high risk groups, such as the very young or the immuno-compromised.1,2

To date, over 2,500 different S. enterica serovars have been identified.3 In Australia, approximately 150 of these are responsible for the majority of human infections, caused mainly by the consumption of contaminated food or drink.2,3 In Australia, 9,249 laboratory-confirmed human cases were notified to the National Enteric Pathogens Surveillance Scheme in 2009, at a rate of 42.1 cases per 100,000 population, with 11 per cent of these infections known to have been acquired overseas.2 Internationally, Salmonella serotyping provides the primary epidemiological information and data required for the surveillance and control of salmonellosis. The global distribution of serovars varies greatly and information about whether a certain serovar or strain exhibits antibiotic resistance is of public health importance to other countries.3 This epidemiological information is critical for developing appropriately targeted interventions to control the emergence and dissemination of a resistant strain.

Whilst intestinal infections are usually self-limiting, antimicrobial treatment of non-typhoidal Salmonella is required with invasive disease or in high risk patients.3 The highest age-specific incidence of Salmonella infection in 2009 was 300 cases per 100,000 population in children aged 0–1 year.1 Consequently, treatment is often required in these higher risk patients. Third generation cephalosporins are in many cases the drugs of choice for treatment, particularly in infants to whom fluoroquinolones should not be administered due to concerns regarding toxicity.5 Therefore, the emergence of resistance in S. enterica to this group of antibiotics will severely restrict treatment options.
There are two main classes of β-lactamases that confer resistance to 3rd generation cephalosporins that are being reported in *S. enterica*. The first group are extended-spectrum β-lactamases (ESβLs), which belong to the Molecular Class A β-lactamase enzymes. First described in *Klebsiella sp* (SHV) and *Escherichia coli* (TEM), an evolving group of over 600 different ESβLs have been reported, mainly arising from point mutations of the original SHV and TEM genes with many emerging in *S. enterica*. More recently, a new rapidly proliferating group of ESβL enzymes called CTX-M that are preferentially active against cefotaxime have arisen, found mainly in strains of *S. Typhimurium* and *E. coli*. The second group are AmpC β-lactamases, which were first reported in 1988 and belong to molecular class C β-lactamase enzymes and 50 variants have since been described. These enzymes are derived from chromosomal genes in organisms such as *Enterobacter cloacae* and *Citrobacter sp* that can be transferred via mobile genetic elements to other *Enterobacteriaceae* that do not possess these genes, such as *S. enterica*.

Routine antimicrobial susceptibility testing (AST) may not detect the presence of an ESβL or AmpC β-lactamase as the high breakpoints used in AST and the differing levels of activity against various cephalosporins can make detection difficult. The inappropriate use of cephalosporins to treat invasive infections caused by ESβL or AmpC β-lactamase producing *Enterobacteriaceae* such as *S. enterica* have been associated with increased rates of morbidity and mortality. This is a particular problem in health care environments where antibiotic selective pressure is conducive to the acquisition of these resistant genes. This risk extends beyond patients. Infection through exposure with carriage of ESβL producing *Salmonella* sp has been reported among health care workers.

In the last decade, the emergence of ESβLs and AmpC β-lactamases in *S. enterica* populations have been reported from many countries. Whilst AST is carried out when required for individual patient treatment and by reference laboratories as an epidemiological tool, little is known of the prevalence of ESβLs or AmpC β-lactamases within *S. enterica* isolated in Australia.

The main aim of this study was to determine if *S. enterica* isolated in Australia had phenotypically detectable ESβL or AmpC β-lactamase resistance and whether there were differences among serovars, thereby providing the initial baseline data against which to measure future trends.

**Methods**

**Isolates**

The study included a selection of isolates from two separate groups, all of which were human clinical isolates received by the *Salmonella* Reference Laboratory in Queensland from diagnostic laboratories between 2000 and 2009. Isolates were serologically classified according to the White-Kauffmann-LeMinor scheme, as recommended by the World Health Organization Collaborating Centre for Reference and Research on *Salmonella*.

The first group included isolates from six different serovars consecutively received by the laboratory (n=624). These included *S. enterica* serovar Typhimurium (n=100), *S. Virchow* (n=111), *S. Aberdeen* (n=97), *S. Enteritidis* (n=114), *S. Weltervreden* (n=101) and *S. Stanley* (n=101).

The second group consisted of *S. Typhimurium* (n=298) and *S. Virchow* (n=53) isolates that had exhibited resistance to at least one of the following antibiotics (μg/ml): ampicillin 32; streptomycin 5; tetracycline 20; chloramphenicol 10; sulphathiazole 550; trimethoprim 50; kanamycin 10; nalidixic acid 50; spectinomycin 50; gentamicin 2.5; ciprofloxacin 0.06 or cefotaxime 1.0 as part of epidemiological typing. The screening of these isolates for this study was thought to improve the likelihood of detecting β-lactamase resistance, which was expected to be very low. None of the isolates from the second group were included in the first group.

**Phenotypic method**

Isolates were screened as per Clinical and Laboratory Standards Institute (CLSI) recommendations for screening and confirmatory tests for ESβLs in *Klebsiella pneumoniae, Klebsiella oxytoca, E. coli* and *Proteus mirabilis* (M100-S19). The screen is based on demonstrating synergy with cephalosporin (cefazidime, CAZ, 30 μg and cefotaxime, CTX, 30 μg) and with cephalosporin in combination with clavulanic acid (CAZ/CLA 30/10 μg and CTX/CLA 30/10 μg). Cefoxitin 30 μg (FOX 30 μg) was included in the screen as AmpC β-lactamase can also demonstrate activity against cephemycins. In organisms that harbour both an ESβL and AmpC β-lactamase by clavulanic acid can potentially mask the positive synergistic reaction that confirms ESβL activity. This concern is particularly pertinent as ESβLs and AmpC β-lactamases have reportedly been found in conjunction in *S. enterica*. To overcome this problem isolates that exhibited resistance to CAZ, CTX or
FOX were further tested to differentiate and distinguish between the two mechanisms of resistance. Currently, no recommendations exist for ESβL detection and reporting in the presence of AmpC β-lactamase.\(^{19,20}\)

MAST\(^\text{®}\) (D68C) has developed an antibiotic detection set incorporating four discs that contain a combination of cefpodoxime with either and both an AmpC β-lactamase and ESβL inhibitor to enable the differentiation of both mechanisms of resistance.

Controls included were *E. coli* ATCC 25922 as the negative control, *K. pneumoniae* ATCC 700603 as the ESβL positive control and *E. cloacae* NCTC 13406 as the AmpC β-lactamase positive control.

### Results

Nine of the 624 consecutively isolated serovars screened demonstrated β-lactamase activity. Phenotypic ESβL activity was detected in one of these isolates and phenotypic AmpC β-lactamase activity was detected in eight others (Table 1).

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Number of isolates</th>
<th>Number of ESβLs</th>
<th>Number of AmpC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. Virchow</td>
<td>111</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. Aberdeen</td>
<td>97</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>114</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. Weltevreden</td>
<td>101</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. Stanley</td>
<td>101</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>624</strong></td>
<td>1</td>
<td><strong>8</strong></td>
<td><strong>9</strong></td>
</tr>
</tbody>
</table>

**Table 1**: ESβL and AmpC β-lactamase phenotypes in isolates

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Number of isolates</th>
<th>ESβL</th>
<th>AmpC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>n=11 / 298 (3.7%)</td>
<td>ESβL</td>
<td>Amp, S, T, C, Su, Tm, K, Sp, G, Cp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ESβL</td>
<td>Amp, Tm, G, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, S, Su, Cf</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, T, C, Tm, Na, Sp, Cp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, S, T, Cf</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
</tr>
<tr>
<td>S. Virchow</td>
<td>n= 5 / 53 (9.4%)</td>
<td>ESβL</td>
<td>Amp, G, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
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<td>Amp, Cf</td>
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<td>Amp, Cf</td>
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<tr>
<td></td>
<td></td>
<td>AmpC</td>
<td>Amp, Cf</td>
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</tr>
</tbody>
</table>

Amp ampicillin (32 μg/ml); S streptomycin (25 μg/ml); T tetracycline (20 μg/ml); C chloramphenicol (10 μg/ml); Su sulphathiazole (550 μg/ml); Tm trimethoprim (50 μg/ml); K kanamycin (10 μg/ml); Na nalidixic acid (50 μg/ml); Sp spectinomycin (50 μg/ml); G gentamicin (2.5 μg/ml); Cp ciprofloxacin (2.0 μg/ml); Cf cefotaxime (1)
Eleven of the 298 S. Typhimurium isolates that had previously demonstrated antibiotic resistance were β-lactamase positive, with two demonstrating ESβL activity and nine possessing AmpC β-lactamase. (Table 2). Five of the 53 S. Virchow isolates that had previously demonstrated antibiotic resistance demonstrated β-lactamase activity. One of these isolates demonstrated ESβL activity and four AmpC β-lactamase activity (Table 2).

The antibiograms for each of the β-lactamase positive S. Typhimurium and S. Virchow isolates are shown in Table 2.

**Discussion**

This study demonstrates the presence of β-lactamase activity in some isolates of *S. enterica* in Queensland and therefore antibiotic treatment failure is a distinct possibility. This may not apply to the rest of Australia as geographical distribution of salmonellosis and serovars varies greatly between states and territories.1,2 Specifically, of the isolates that had previously demonstrated some antibiotic resistance, 21 produced AmpC β-lactamase and four were ESβL producers. AmpC β-lactamase was observed more frequently than ESβL in *S. enterica* indicating that resistance to 3rd generation cephalosporins is more likely to be a result of an AmpC β-lactamase rather than an ESβL. In this study, there was no evidence of the coexistence of both an ESβL and AmpC β-lactamase in the same *S. enterica* isolate, which has been found elsewhere.22

The results of this study suggest an epidemiological association between certain *Salmonella* serovars and β-lactamase activity. The serovars with the highest proportions of isolates demonstrating AmpC β-lactamase activity were *S. Enteritidis* (1.75%) and *S. Stanley* (4.95%). These serovars are predominately associated with overseas-acquired infections particularly in Indonesia and Thailand.23 The high proportion of *S. Stanley* isolates demonstrating AmpC β-lactamase activity reflects the recently emerged epidemic clone seen in South East Asia, particularly Thailand and Taiwan. Not surprisingly, 3 cases reported overseas travel specifically to Thailand prior to onset of illness.25

Laboratories should remain vigilant regarding the possibility of β-lactamase activity when overseas travel has been reported, particularly if the isolate demonstrates antibiotic resistance to any β-lactam antibiotics. It is also imperative from a clinical perspective that the case’s travel history be reported in clinical notes. From a public health perspective, the introduction of these exotic serovars into Australia is a concern, as the exchange of genetic material occurs readily across species of the same genera. This could provide a native strain with a significant selective advantage, resulting in the rapid dissemination of the strain into environmental niches as seen with *S. Newport* multidrug-resistant-AmpC overseas.3,18

Among isolates that had previously demonstrated resistance as part of epidemiological typing, β-lactamase activity contributed to 3.7% of the resistance observed in *S. Typhimurium* isolates and a much higher 9.4% in *S. Virchow* isolates. This is of concern because *S. Virchow* continually demonstrates a higher rate of invasiveness (8.7%) compared with other serovars.2 Overall, multiple antibiotic resistance was more evident with ESβLs than with AmpC β-lactamases, as all 3 positive ESβL isolates demonstrated resistance not only to β-lactams but to a wide range of antibiotics. By contrast, only three (3/13) of the positive AmpC β-lactamase isolates demonstrated resistance to antibiotics other than β-lactams. This would suggest that the coexistence of multiple resistance mechanisms is more likely to occur with strains that demonstrate ESβL activity than with AmpC β-lactamase. Both of these antibiograms should alert laboratories to the potential existence of β-lactamase activity in *S. enterica* and should be further investigated.

Laboratory capacity to detect β-lactamase activity in *S. enterica* will also be of public health benefit as antibiotic resistance profiling has in the past proven to be a valuable tool in linking cases in outbreak investigations.2 Whilst none of the β-lactamase-producing isolates in this study can be directly associated with a defined outbreak, outbreaks caused by β-lactamase-producing *S. enterica* have occurred overseas.10,12 The probability of such an event occurring in Australia will increase as the dissemination of β-lactamase resistance in *S. enterica* continues.

It is apparent from the results of this study that many laboratories still face significant challenges when detecting β-lactamases, as only three (3/25) of the isolates in which β-lactamase activity was detected in this study submitted were reported as exhibiting ESβL and or AmpC β-lactamase activity. Whilst the CLSI guidelines have been evaluated as applying quite well to *S. enterica*, the low incidence of resistance observed at the time of validation concluded that the inclusion of *S. enterica* was not warranted and so the guidelines specifically cover only *K. pneumoniae*, *K. oxytoca*, *E. coli* and *P. mirabilis*.10 The evidence presented in this study would support the suitability of this detection methodology for *S. enterica* and warrant its inclusion in the guidelines. The need to improve the awareness of β-lactamase activity in *S. enterica* worldwide has been recognised by the World Health Organization Global Foodborne Infections Network. It now incorporates guidelines for β-lactamase confirmation requirements as part of their Salm-Surv External Quality Assurance System annual proficiency test.
In conclusion, whilst surveillance in itself does not control resistance, accurate surveillance data informs the clinical use of antimicrobials and provides a powerful tool to prevent the spread of resistance. The results of this study provide the first surveillance data of β-lactamase activity in *S. enterica* in Australia. This will provide some baseline surveillance data against which future trends can be measured, and against which to gauge the dissemination of β-lactamases in *S. enterica* within Australia in the coming years.

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