Original articles

ß-lactamases in *Salmonella enterica* isolated in Australia

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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 (ESßLs) 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 & ESßL 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 ESßL activity and eight others with demonstrated AmpC ß-lactamase. ß-lactamase activity was detected in 16 of the isolates that had previously demonstrated some antibiotic resistance; three with demonstrated ESßL 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.[4] 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 ß-lactamases that confer resistance to 3rd generation cephalosporins that are being reported in *S. enterica*.[5] The first group are extended-spectrum beta-lactamases (ESßLs), which belong to the Molecular Class A ß-lactamase enzymes.[6,7] First described in *Klebsiella sp* (SHV) and *Escherichia coli* (TEM), an evolving group of over 600 different ESßLs have been reported, mainly arising from point mutations of the original SHV and TEM genes with many emerging in *S. enterica*.[6,7,8] More recently, a new rapidly proliferating group of ESßL enzymes called CTX-M that are preferentially active against cefotaxime have arisen, found mainly in strains of *S.* Typhimurium and *E. coli*.[6,8] The second group are AmpC ß-lactamases, which were first reported in 1988 and belong to molecular class C ß-lactamase enzymes and 50 variants have since been described.[7,9] 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*.[7,9]

Routine antimicrobial susceptibility testing (AST) may not detect the presence of an ESßL or AmpC ß-lactamase as the high breakpoints used in AST and the differing levels of activity against various cephalosporins can make detection difficult.[10] The inappropriate use of cephalosporins to treat invasive infections caused by ESßL or AmpC ß-lactamase producing *Enterobacteriaceae* such as *S. enterica* have been associated with increased rates of morbidity and mortality.[11] This is a particular problem in health care environments where antibiotic selective pressure is conducive to the acquisition of these resistant genes.[10] This risk extends beyond patients. Infection through exposure with carriage of ESßL producing *Salmonella* sp has been reported among health care workers.[12]

In the last decade, the emergence of ESßLs and AmpC ß-lactamases in *S. enterica* populations have been reported from many countries.[5,10,13–18] 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 ESßLs or AmpC ß-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 ESßL or AmpC ß-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.* Weltevreden (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 ß-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 ESßLs in *Klebsiella pneumoniae, Klebsiella oxytoca, E. coli* and *Proteusmirabilis* (M100-S19).[19,20] The screen is based on demonstrating synergy with cephalosporin (ceftazidime, 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).[19,20] Cefoxitin 30 µg (FOX 30 µg) was included in the screen as AmpC ß-lactamase can also demonstrate activity against cephamycins.[16,20,21] In organisms that harbour both an ESßL and AmpC ß-lactamase, the induction of AmpC ß-lactamases by clavulanic acid can potentially mask the positive synergistic reaction that confirms ESßL activity.[10,20] This concern is particularly pertinent as ESßLs and AmpC ß-lactamases have reportedly been found in conjunction in *S. enterica*.[22] 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 ESßL detection and reporting in the presence of AmpC ß-lactamase.[19,20]

MAST® (D68C) has developed an antibiotic detection set incorporating four discs that contain a combination of cefpodoxime with either and both an AmpC ß-lactamase and ESßL 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 ESßL positive control and *E. cloacae* NCTC 13406 as the AmpC ß-lactamase positive control.

Results

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

Table 1: ESßL and AmpC ß-lactamase phenotypes in isolates

Serovar	Number of isolates	Number of ESβLs	Number of AmpC	Total
S. Typhimurium	100	0	0	0
S. Virchow	111	0	1	1
S. Aberdeen	97	1	0	1
S. Enteritidis	114	0	2	2
S. Weltevreden	101	0	0	0
S. Stanley	101	0	5	5
Total	624	1	8	9

Table 2: Resistance profiles of ß-lactamase positive *Salmonella* Typhimurium and *S.* Virchow isolates that had previously demonstrated antibiotic resistance

Number of positive / total	ß-lactamase class	Antibiotic resistance profiles
S. Typhimurium	ESβL	Amp, S, T, C, Su, Tm, K, Sp, G, Cp, Cf
n=11 / 298 (3.7%)	ESβL	Amp, Tm, G, Cf
	AmpC	Amp, Cf
	AmpC	Amp, S, Su, Cf
	AmpC	Amp, Cf
	AmpC	Amp, T, C, Tm, Na, Sp, Cp, Cf
	AmpC	Amp, S, T, Cf
	AmpC	Amp, Cf
	AmpC	Amp, Cf
	AmpC	Amp, Cf
	AmpC	Amp, Cf
S. Virchow	ESβL	Amp, G, Cf
n= 5 / 53 (9.4%)	AmpC	Amp, Cf
	AmpC	Amp, Cf
	AmpC	Amp, Cf
	AmpC	Amp, Cf

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 ß-lactamase positive, with two demonstrating ESßL activity and nine possessing AmpC ß-lactamase. (Table 2). Five of the 53 *S*. Virchow isolates that had previously demonstrated antibiotic resistance demonstrated ß-lactamase activity. One of these isolates demonstrated ESßL activity and four AmpC ß-lactamase activity (Table 2).

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

Discussion

This study demonstrates the presence of ß-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 ß-lactamase and four were ESßL producers. AmpC ß-lactamase was observed more frequently than ESßL in *S. enterica* indicating that resistance to 3rd generation cephalosporins is more likely to be a result of an AmpC ß-lactamase rather than an ESßL. In this study, there was no evidence of the coexistence of both an ESßL and AmpC ß-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 ß-lactamase activity. The serovars with the highest proportions of isolates demonstrating AmpC ß-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.[2,4] The high proportion of *S*. Stanley isolates demonstrating AmpC ß-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.[15]

Laboratories should remain vigilant regarding the possibility of ß-lactamase activity when overseas travel has been reported, particularly if the isolate demonstrates antibiotic resistance to any ß-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.[1,18]

Among isolates that had previously demonstrated resistance as part of epidemiological typing, ß-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 ESßLs than with AmpC ß-lactamases, as all 3 positive ESßL isolates demonstrated resistance not only to ß-lactams but to a wide range of antibiotics. By contrast, only three (3/13) of the positive AmpC ß-lactamase isolates demonstrated resistance to antibiotics other than ß-lactams. This would suggest that the coexistence of multiple resistance mechanisms is more likely to occur with strains that demonstrate ESßL activity than with AmpC ß-lactamase. Both of these antibiograms should alert laboratories to the potential existence of ß-lactamase activity in *S. enterica* and should be further investigated.

Laboratory capacity to detect ß-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 ß-lactamase-producing isolates in this study can be directly associated with a defined outbreak, outbreaks caused by ß-lactamase-producing *S. enterica* have occurred overseas.[10,12] The probability of such an event occurring in Australia will increase as the dissemination of ß-lactamase resistance in *S. enterica* continues.

It is apparent from the results of this study that many laboratories still face significant challenges when detecting ß-lactamases, as only three (3/25) of the isolates in which ß-lactamase activity was detected in this study submitted were reported as exhibiting ESßL and or AmpC ß-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 ß-lactamase activity in *S. enterica* worldwide has been recognised by the World Health Organization Global Foodborne Infections Network. It now incorporates guidelines for ß-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 ß-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 ß-lactamases in *S. enterica* within Australia in the coming years.

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References

1. Davos D. A Compendium of Laboratory Diagnostic Methods for Common and Unusual Enteric Pathogens—an Australian persective. Chapter 10, *Salmonella*. 2005:119–135.
2. Powling J. National Enteric Pathogens Surveillance Scheme: Human Annual Report 2009.
3. Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, et al. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog Dis* 2011;8(8):887–900.
4. Bright A. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFoodNet Network, 2009. *Commun Dis Intell* 2010;34(No. 4):375–483.
5. Su L-H, Chiu C-H, Chu C, Ou JT. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: A global challenge. *Clin Infect Dis* 2004;39(4):546–551.
6. Bush K, Jacoby GA. Updated functional classification of ß-lactamases. *Antimicrob Agents Chemother* 2010;54(3):969–976.
7. Bush K. New ß-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 2001;32(7):1085–1089.
8. Bradford PA. Extended-spectrum ß-lactamases in the 21st Century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001;14(4):933–951.
9. Thomson KS. Controversies about extended-spectrum and AmpC beta-lactamases. *Emerg Infect Dis* 2001;7(2):333–336.
10. Paterson DL, Bonomo RA. Extended-spectrum ß-lactamases: a clinical update. *Clin Microbiol Rev* 2005;18(4):657–686.
11. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 2002;8(5):490–495.
12. Hammami A, Arlet G, Ben Redjeb S, Grimont F, Ben Hassen A, Rekik A, et al. Nosocomial outbreak of acute gastroenteritis in a neonatal intensive care unit in Tunisia caused by multiply drug resistant *Salmonella* wien producing SHV-2 beta-lactamase. *Eur J Clin Microbiol Infect Dis* 1991;10(8):641–646.
13. Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD, Davies RH, et al. blaCTX-M genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. *Antimicrob Agents Chemother* 2005;49(4):1319–1322.
14. Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008;13(47).
15. Huang KY, Hong YW, Wang MH, Chu C, Su LH, Chiou CS, et al. Molecular epidemiology and antimicrobial susceptibility of *Salmonella enterica* serotype Stanley isolates in Taiwan. *J Microbiol Immunol Infect* 2007;40(5):411–418.
16. Kruger T, Szabo D, Keddy KH, Deeley K, Marsh JW, Hujer AM, et al. Infections with Nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother* 2004;48(11):4263–4270.
17. Tassios PT, Gazouli M, Tzelepi E, Milch H, Kozlova N, Sidorenko S, et al. Spread of a *Salmonella* Typhimurium clone resistant to expanded-spectrum cephalosporins in three European Countries. *J Clin Microbiol* 1999;37(11):3774–3777.
18. Whichard JM, Gay K, Stevenson JE, Joyce KJ, Cooper KL, Omondi M, et al. Human *Salmonella* and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. *Emerg Infect Dis* 2007;13(11):1681–1688.
19. Clinical and Laboratory Standards Institute. Performance Standard for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute. P.A.Wayne. 2009.
20. Clinical and Laboratory Standards Institute. Performance Standard for Antimicrobial Disc Susceptibility Tests; Approved Standards – Tenth Edition, CLSI document M02-10. Clinical and Laboratory Standards Institute. P.A.Wayne. 2009.
21. Livermore DM, Brown DFJ. Detection of ß-lactamase-mediated resistance. *J Antimicrob Chemother* 2001;48(Suppl 1):59–64.
22. Hanson ND, Moland ES, Hossain A, Neville SA, Gosbell IB, Thomson KS. Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 beta-lactamases. *J Antimicrob Chemother* 2002;49(6):1011–1014.