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Salmonella enterica isolated from infections in Australian livestock remain susceptible to critical antimicrobials

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ABSTRACT

Salmonella enterica is a zoonotic pathogen causing a variety of diseases in humans and animals. Many countries are reporting an increase in the prevalence of multidrug-resistant (MDR) *S. enterica* in food animals. The aim of this study was to determine whether *S. enterica* isolated from livestock in New South Wales, Australia, have similar resistance traits to those reported internationally. *Salmonella enterica* ($n = 165$) from clinical infections in food animals between 2007 and 2011 were serotyped and tested for susceptibility to 18 antimicrobials. Also, 22 antimicrobial resistance genes (ARGs), 3 integrons and 18 plasmid replicon types were screened for using PCR. Most isolates (66.1%) remained susceptible to all antimicrobials; 8.5% of the isolates were resistant to four or more antimicrobials. Antimicrobials with the highest prevalence of resistance were sulfafurazole (28.5%), ampicillin (17.0%), tetracycline (15.8%) and trimethoprim (8.5%). There was no resistance to fluoroquinolones or third-generation cephalosporins. The most common ARGs were bla_{TEM} (15.2%), *sul2* (10.3%), *tetB* (9.1%), *tetA* (5.5%), *aphA1* (4.8%) and *dhfrV* (4.8%). Class 1 integrons (7.9%) and IncFIIA (69.7%) were the most commonly detected integron and plasmid replicon types, respectively. Class 1 integrons were positively associated with MDR phenotypes and ARG carriage ($P \leq 0.001$). Internationally prominent MDR serovars associated with severe disease in humans (e.g. AmpC-positive *Salmonella* Newport) were not detected. Overall, the comparatively favourable resistance status of *S. enterica* in Australian livestock represents minimal public health risk associated with MDR strains and supports a conservative approach to the registration of antimicrobial drug classes in food-producing animals.

1. Introduction

Salmonella enterica is a zoonotic pathogen causing a variety of diseases in humans and animals [1]. The emergence of multidrug-resistant (MDR) forms of *S. enterica* in food-producing animals is a substantial threat to human and animal health [2], especially when it involves serovars with a propensity to cause severe clinical disease in humans [3]. Examples of MDR *S. enterica* serovars with elevated virulence for humans that have recently emerged in animals include serovar Newport in the USA [4,5], serovar Heidelberg in Canada [6] and serovar Kentucky in Egypt and Africa [7,8]. Possession of AmpC and/or extended-spectrum β-lactamase-producing genes on MDR plasmids by some of these serovars eliminates many options for treatment of invasive salmonellosis in humans [2,9], especially since concurrent fluoroquinolone resistance has been observed in some strains [10]. These near pan-resistant *Salmonella* isolates could become more widely disseminated in much the same way that penta-resistant *S. enterica* serovar Typhimurium DT104 established a near-global distribution in the 1990s [11].

A number of factors present in Australia may influence the epidemiology of *Salmonella* in food animals, favouring selection of susceptible over MDR strains. First, Australia's unique geography, quarantine restrictions and predominance of extensive livestock systems act to prevent the entry of exotic MDR serovars and subsequent colonisation of food animals, as has been suggested for DT104 [12,13]. In addition, Australia is the only country never to have permitted the use of fluoroquinolones and gentamicin in food-producing animals [14]. However, there are currently no published studies comprehensively describing the resistance determinants in *Salmonella* isolated from food animals in Australia [13,15]. There

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is also a pressing need to define the extent of multidrug resistance in serovars linked to more clinically virulent forms of human salmonellosis. We therefore investigated the occurrence of phenotypic resistance traits as well as the rate of carriage of specific antimicrobial resistance genes (ARGs), integrons and plasmid replicon types among a fully serotyped collection of *S. enterica* serovars isolated from clinical infections in food animals over a 5-year period from New South Wales (NSW), Australia.

2. Materials and methods

2.1. Bacterial strains

A collection of 165 *S. enterica* sequential isolates from confirmed cases of salmonellosis in livestock (dairy cattle, $n = 85$; beef cattle, $n = 21$; sheep, $n = 32$; pigs, $n = 21$; poultry, $n = 4$; and goats, $n = 2$) was obtained from diagnostic submissions by the NSW State Veterinary Diagnostic Laboratory (this laboratory processes the vast majority of food animal submissions in NSW). The collection represented all isolates obtained from food-producing animals between 2007 and 2011. Isolates were serotyped by the Institute for Medical and Veterinary Science (Adelaide, Australia). DNA extraction from the isolates was performed as previously described [16].

2.2. Phenotypic detection of antimicrobial resistance

Phenotypic detection of antimicrobial resistance was performed by disc diffusion using the calibration dichotomous susceptibility (CDS) test [17]. The following antimicrobials were tested: ampicillin (25 μg); amoxicillin/clavulanic acid (AMC) (60 μg); ticarcillin/clavulanic acid (TIM) (85 μg); cefalexin (100 μg); cefoxitin (30 μg); cefotaxime (5 μg); cefepime (10 μg); nalidixic acid (30 μg); ciprofloxacin (2.5 μg); imipenem (10 μg); sulfafurazole (300 μg); trimethoprim (5 μg); tetracycline (10 μg); apramycin (15 μg); neomycin (30 μg); gentamicin (10 μg); azithromycin (15 μg); and chloramphenicol (30 μg). The CDS test method is commonly used both in human and veterinary diagnostic microbiology laboratories within Australia and the breakpoints are calibrated by internationally standardised minimum inhibitory concentration testing for each antimicrobial [17]. *Escherichia coli* NCTC 10418 was used as the control strain [17]. Isolates exhibiting resistance to at least three classes of antimicrobials were classified as MDR.

2.3. Antimicrobial resistance gene profiling

A combination of three multiplex [18] and five uniplex PCR assays were performed to screen for 22 ARGs. The genes tested and the nucleotide sequences are described in Supplementary Table S1.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2013.10.014.

2.4. Detection of integrons

A multiplex PCR assay targeting class 1, 2 and 3 integrons (*int1*, *int2* and *int3*) was performed to investigate the occurrence of integrons in the isolate collection [19].

2.5. Plasmid replicon typing

Plasmid replicon typing was carried out to investigate the presence of 18 replicons using three multiplex PCR assays and one uniplex PCR assay. These assays were performed using previously described primers [20], multiplex panels and reaction conditions [21].

2.6. Statistical analysis

Comparisons of prevalence and co-association of traits were analysed by Fisher's exact test using IBM SPSS Statistics for Windows v.20 (IBM Corp., Armonk, NY).

3. Results

3.1. Phenotypic detection of antimicrobial resistance

The most frequently detected serotypes among the 165 *S. enterica* isolates were Typhimurium (46.1%), Dublin (20.6%) and Bovismorbificans (13.3%). The majority of isolates (66.1%) remained susceptible to all tested antimicrobials, and a high proportion (17.0%) showed resistance to only one antimicrobial. Few isolates were resistant to two (1.8%), three (6.7%) or four or more antimicrobials (8.5%). No isolates were resistant to AMC, cefalexin, cefoxitin, cefotaxime, cefepime, nalidixic acid, ciprofloxacin, imipenem or azithromycin. Resistance was most frequently detected to sulfafurazole (28.5%), followed by ampicillin (17.0%), tetracycline (15.8%), trimethoprim (8.5%), neomycin (4.2%), apramycin (3.0%), chloramphenicol (2.4%), gentamicin (1.2%) and TIM (0.6%). Specific MDR phenotypes were not serovar- or host-animal-associated (Table 1). For the 5 years of isolate collection, there was no suggestion of a temporal increase in either the mean count of phenotypic resistance traits per isolate or in the percent of isolates identified as MDR (Fig. 1).

3.2. Detection of antimicrobial resistance genes

Antimicrobial resistance genes were detected in 17.6% of the 165 isolates. Most common were bla_{TEM} (15.2%), *sul2* (10.3%), *tetB* (9.1%), *tetA* (5.5%), *aphA1* (4.8%), *dhfrV* (4.8%), *sul1* (2.4%), *aadA* (2.4%), *aac(3)-IV* (2.4%), *dhfrI* (1.8%), *tetC* (1.2%) and *cmlA* (1.2%). None of the following genes were detected in the collection: $bla_{SHV;}$ *cat1*; *floR*; bla_{OXA}; bla_{CMY-2}; *ereA*; bla_{MOX} or bla_{CMY}; bla_{DHA}; bla_{MIR-1} or $bla_{ACT-1;}$ and $bla_{FOX1–5}$.

3.3. Detection of integrons

Only class 1 integrons (*int1*) were detected (in 7.9% of isolates). All *int1*-positive isolates except one were resistant to at least four antimicrobials (Table 1). There was a strong association between carriage of *int1* and expression of a MDR phenotype (resistance to at least three classes of antimicrobials) as well as possession of at least four ARGs ($P \leq 0.001$) (Tables 1 and 2).

3.4. Detection of plasmid replicon types

Plasmid replicon typing revealed that 79.4% of the 165 isolates carried at least one plasmid. Replicon IncFIIA (69.7%; 115/165) was the most frequently detected plasmid among the *Salmonella* isolates. Less commonly, IncI1 (8.5%), IncN (5.5%), IncFIB (3.6%), IncH12 (1.8%), IncL/M (1.8%), IncA/C (1.2%) and IncFIC (0.6%) were also detected. The IncFrep that amplifies FII, FIII, FIV and FV variants of IncF plasmids was also detected at a low frequency (4%). Plasmid types IncB/O, P, T, K/B, W, FIA, Y, X and HI1 were not detected.

4. Discussion

These data from NSW, Australia, are remarkable for the high proportion of *Salmonella* isolates that were fully susceptible to all of the antimicrobials tested as well as the absence of resistance to critical antimicrobials in human medicine (fluoroquinolones and third- and fourth-generation cephalosporins). This contrasts with