Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 119 (2007) 221–230
veterinary microbiology
www.elsevier.com/locate/vetmic

Antimicrobial resistance in *Salmonella enterica* serovar Dublin isolates from beef and dairy sources

Margaret A. Davis [a,*], Dale D. Hancock [b], Thomas E. Besser [a], Joshua B. Daniels [a], Katherine N.K. Baker [b], Douglas R. Call [a]

[a] *Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164, United States*
[b] *Field Disease Investigation Unit, Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164, United States*

Received 19 June 2006; received in revised form 24 August 2006; accepted 28 August 2006

Abstract

Salmonella enterica serovar Dublin (*S.* Dublin) is a cattle-adapted *Salmonella* serovar, so if antimicrobial resistance in *S.* Dublin arises as a result of antimicrobial use this most likely occurs within the cattle reservoir without impact from antimicrobial use in humans. We tested the antimicrobial resistance of bovine-origin *S.* Dublin isolates from 1986 through 2004 using a standard disk diffusion method. High proportions of isolates throughout the time period were resistant to one or more antimicrobials, and a marked increase in resistance to ceftazidime occurred between 2000 and 2004. Dairy-origin isolates were more likely to be resistant to several antibiotics than were isolates from beef operations where exposure to antimicrobials is likely to be less frequent. Plasmid analysis of a subset of isolates also supported the hypothesis that antimicrobial resistance traits in the cattle-adapted serovar Dublin were acquired within the bovine host environment.
Published by Elsevier B.V.

Keywords: *Salmonella enterica* serovar Dublin; Antimicrobial drug resistance; Ceftazidime; Cattle

1. Introduction

Zoonotic salmonellosis epidemiology often exhibits a pattern of emergence and clonal expansion of specific *Salmonella enterica* strains, which can be multi-drug resistant (Hancock et al., 2000; Davis et al., 2002; Velge et al., 2005). We hypothesized that ''selection foci'' play a key role in the development of epidemic clones with stable resistance traits. The two key elements of a selection focus are sustained transmission of bacterial pathogens and continual exposure to antimicrobial drugs (Hancock et al., 2000). Both of these elements are frequently found in dairy calf-raising operations in the United States. Intensive therapeutic and prophylactic use of antimicrobial drugs is common on dairy calf raising operations (USDA, 2006) while biosecurity measures are often incomplete (USDA, 2002). This is in contrast to beef cow–calf operations where calves are

* Corresponding author. Tel.: +1 509 335 5119; fax: +1 509 335 8529.
E-mail address: madavis@vetmed.wsu.edu (M.A. Davis).

0378-1135/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetmic.2006.08.028

raised at lower density (USDA, 1998) and with comparatively little antimicrobial use (McEwen and Fedorka-Cray, 2002).

S. Dublin is a cattle-adapted serovar (Olsen and Skov, 1994; Liebana et al., 2002) that is very uncommon in other species. It is therefore almost certain that a selection focus giving rise to resistant *S.* Dublin strains would be within the cattle reservoir (in contrast to *S. enterica* serovar Typhimurium, whose selection focus could be within any of the many host species that this serovar infects). *S.* Dublin is uniquely suitable for examination of this question because human infections with *S.* Dublin are relatively rare, representing less than seven percent of all reported serotypes (Vugia et al., 2004; CDC, 2005). Thus, antimicrobial resistance in *S.* Dublin is expected to be the result of selection within the cattle reservoir, unaffected by use of antimicrobials in human populations.

We hypothesized that dairy calf raising operations are selection foci and that *S.* Dublin isolates from dairy breed calves should exhibit greater resistance to antimicrobial drugs compared to those from beef breed calves. To test this hypothesis we compared the antimicrobial resistance profiles for *S.* Dublin strains from dairy and beef sources over a 19-year period in the Pacific Northwest.

2. Materials and methods

2.1. Bacterial isolates

The Washington Animal Disease Diagnostic Laboratory (WADDL) serves as a veterinary reference laboratory for the Pacific Northwest region of the United States. All *S.* Dublin isolates from clinical bovine samples submitted by veterinarians to WADDL are transferred to the Field Disease Investigation Unit (FDIU) (College of Veterinary Medicine, Pullman, WA) and banked in BHI broth containing 25–30% buffered glycerol at −80 °C. Isolates are serotyped at the National Veterinary Services Laboratory, Ames, Iowa (NVSL). A small number of non-clinical *S.* Dublin isolates are acquired by the FDIU during herd sampling for other research efforts. All of the non-clinical isolates in this study originated from dairies. Between 1986 and 2004 a total of 442 *S.* Dublin isolates from cattle were obtained by the FDIU, of which 376 were submitted through WADDL and the remainder were collected during research efforts. If multiple *S.* Dublin isolates from the same herd and year shared identical resistance profiles, only the first was retained for analysis. After eliminating those with duplicate resistance profiles from the same herd and year, there were 337 bovine *S.* Dublin isolates for analysis. Among these 337, 273 were from dairy breeds, 19 were from beef breeds, and 45 were from unknown breeds. All beef-origin isolates derived from clinical submissions and 253 of 273 dairy-origin isolates derived from clinical submissions.

2.2. Antimicrobial susceptibility testing

Susceptibility testing was done by a disk diffusion method (Bauer et al., 1966) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (NCCLS, 2003a,b). Isolates collected between 1986 and 2000 were tested for susceptibility to a panel of antimicrobials, which included ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nitrofurantoin (300 μg), streptomycin (10 μg), tetracycline (30 μg), triple-sulfa (a combination of sulfadiazine, sulfamethazine, and sulfamerazine) (250 μg), and trimethoprim-sulfamethoxizole (1.25–23.75 μg) (BD Diagnostics, Sparks, MD, USA). In the year 2000, ciprofloxacin (5 μg) was added to the panel and in 2001 ceftazidime (30 μg) was added so isolates collected from 2001 through 2004 ($n = 49$) were tested with a panel of 11 antimicrobials. All available pre-2001 beef-origin isolates ($n = 10$) were also tested for resistance to ceftazidime. A randomly selected (PROC SURVEYSELECT, SAS, Cary, NC) set of dairy-origin isolates from the same years ($n = 15$) were also tested for ceftazidime resistance. The same beef- and dairy-origin isolates that were tested for ceftazidime resistance were also tested for resistance to neomycin (30 μg) (Table 1).

2.3. Pulsed-field gel electrophoresis and plasmid profiles

A subset of *S.* Dublin isolates was chosen for pulsed-field gel electrophoresis (PFGE) analysis to maximize the diversity of source location, resistance phenotype and operation type. *Xba*I restriction enzyme profiles were compared using a standard