Expanded-Spectrum Cephalosporin Resistance and Multi-Drug Resistance in *Salmonella* Isolates from Swine.

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**Abstract:** We examined a group of cephalosporin resistant isolates from various swine sources submitted to the National Antimicrobial Resistance Monitoring System (NARMS). A total of 50 isolates were obtained between the years 1997 and 1999. Each of the isolates had MIC’s to Ceftriaxone, Ceftiofur, and Cephalothin of ≥32, ≥64 and ≥32, respectively. A total of 24 serotypes were identified with no one serotype being predominant. The most common serotypes were Agona (8), Derby (6), typhimurium-copenhagen (4), and Heidelberg (4). Isolates were obtained from diagnostic laboratories (29) federally inspected slaughter and processing plants (18) as well as on farm swine surveys (1). Interestingly, the majority of these isolates were found to be resistant to multiple antimicrobials. Of the 50 isolates 64% were found to be resistant to 8 or more of 17 antimicrobials and 44% of the isolates were resistant to 10 or more antimicrobials as compared to 0.8% of the general population of *Salmonella* isolates tested (2391) having resistance to 10 or more antimicrobials in 1997. Seventy four percent (37) of the isolates were resistant to amoxicillin-clavulanic acid. The most common non beta-lactam resistances were streptomycin, kanamycin, sulfamethoxasole, chloramphenicol and gentamicin. A subset of isolates was examined for cephalosporin resistance mechanisms; 19 of 22 isolates were found to have an AmpC like beta lactamase and tested positive for the *cmy-2* gene by PCR and two of the isolates had a putative ESBL. Resistance of *Salmonella* strains to 3rd generation cephalosporins is a concern and these analyses indicate the predilection of these strains to acquire multiple antimicrobial resistances.

**Keywords:** Salmonella, Antimicrobial resistance, Cephalosporin

**Introduction:** *Salmonella* infections are commonly treated with Antimicrobial agents. In human patients with severe diarrhea or a systemic infection antimicrobial treatment may be lifesaving. Flouroquinolones are a common drug of choice for adults with *Salmonella* however these drugs are not available for use in children. Children with invasive *Salmonella* infections are typically treated with
expanded-spectrum cephalosporins (eg, ceftriaxone) because of the low occurrence of side effects and favorable pharmacology (Bryan 1992, Cherubin 1986, Stutman 1994). Ceftriaxone-resistant *Salmonella* have been observed in North and South Africa, Asia, Europe and the United Sates. The NARMS survey in 1995 found 3 of 4003 isolates resistant to ceftriaxone, all of these infections were acquired outside the United States (Fiorentino 2000, Dunne 2000, Fey 2000). A recent report reviewed the national surveillance data to determine the magnitude and molecular mechanisms of this problem (Dunne et al, 2000).

**Materials and Methods**

**Bacterial strains**

Isolates were obtained from the general stocks of the NARMS system. All cultures were initiated by swabbing the surface of the frozen stock with a sterile cotton-tipped applicator, and then inoculated onto the appropriate media. *Salmonella typhimurium* strains were grouped according to their antimicrobial resistance pattern and phage-type.

**Determination of serotype and phage-type**

All serotyping and phage-typing was performed by the National Veterinary Services Laboratory (NVSL), Ames, IA.

**Determination of antimicrobial susceptibility**

Antimicrobial susceptibility testing was performed at USDA’s Agricultural Research Service facility in Athens, GA. Susceptibility testing was performed using as semi-automated system as per manufacturers’ instructions (Sensititre™, TREKT™ Diagnostics, Inc., Westlake Ohio). National Committee for Clinical Laboratory Standards (NCCLS) guidelines were followed throughout the testing procedure.

**DNA extraction and PCR analysis**

*Salmonella typhimurium* chromosomal DNA was prepared by boiling bacteria in distilled water for 5 minutes. All PCR assays contained a total volume of 25 μl using standard thermocycling parameters. Template was added to a mixture containing 10X buffer with 15 mmol l⁻¹ MgCl₂, 40 μmol l⁻¹ oligonucleotides, 50 pmol of each primer, and 1.25 U *taq* polymerase.

**Results:**

*Multiple Drug Resistance*
- 64% resistant to 8 or antimicrobials
- 44% resistant to 10 or more antimicrobials
- 0.8% of the general population resistant to 10 or more antimicrobials

*Phage types*
- no DT104 isolates
* Majority of isolates cmy-2 positive and have AmpC like beta-lactamase
* Two isolates likely have an ESBL
* Genetic Analysis
  - Riboprinting and PFGE indicate isolates are not clonal in nature
  - Plasmid profiles variable with at least one large plasmid
  - Cephalosporin Resistance is Transferable

**Conclusions:**
* 50 Isolates detected between 1997-1999
* Resistance to multiple 3rd generation cephalosporins is uniform
* Mechanism of Resistance – similar (AmpC B-Lactamase)
* Isolates have a predilection to acquire and carry multiple antimicrobial resistances
  - Serotypes from humans are not the same serotypes but have the same resistance mechanisms
    - *S. newport* 1997-2000

**References:**