Characterization of Antibiotic Resistance Among Veterinary Bacterial Pathogens

David G. White
Department of Veterinary and Microbiological Sciences
North Dakota State University, Fargo, ND 58105

Since the introduction of antimicrobials into veterinary medicine some 45 years ago, animal health and productivity has improved significantly.[1,4,10,12] Despite considerable use, and some misuse, many antimicrobials continue to remain effective today. However, loss of efficacy through emergence of bacterial antibiotic resistance is always an ever present risk.[1,2,3,6,14,15] Antibiotic resistant bacterial pathogens in animals not only pose a problem with respect to animal health but are a growing concern regarding possible transmission to humans as foodborne pathogens.[7,9,15,18] The dilemma of antibiotic resistance is worsened by the growing number of bacterial pathogens resistant to multiple, structurally unrelated drugs, and to the fact that few veterinary antimicrobials are likely to be available before the end of the decade.[4,8,12] Accordingly, more attention is now being paid to the ease at which resistance can develop to both single and multiple antimicrobials among bacterial pathogens. **If current trends continue, we may encounter bacterial pathogens which are resistant to all known antimicrobials. This situation is being addressed by both FDA and USDA which are currently implementing strategies to head off this potential threat.**

Gram negative bacteria, in particular *E. coli*, have been slowly accumulating multiple antibiotic resistance phenotypes, to a point where in the near future, therapeutic choices could become very limited.[5,8,12,14,16] In the past few years, strains of *Escherichia coli* (animal and human origin) have become increasingly resistant to most frontline antibiotics, including sulfa drugs, aminoglycosides, third generation cephalosporins, and even fluoroquinolones.[1,2,3,6,9,10,17] Infections caused by drug-resistant bacteria are a critical and costly animal health problem; these infections prolong illness and if not treated in time with more costly, alternative antimicrobial agents, can lead to increased morbidity and mortality.

Wild-type *E. coli*, unexposed to antibiotic selective pressures tend to be sensitive to the majority of antimicrobials, but exposure to such agents favors resistance development.[16] This resistance development in *E. coli* has been observed since the initial introduction of antimicrobial agents in both the human and veterinary realms. Prolonged use of antimicrobial agents supports resistant bacterial strains by eliminating more susceptible competitors. If these resistant strains also happen to be more virulent than others, than more pathogenic bacteria could become established at the expense of favorable commensal microorganisms.[12,16]

The development of antimicrobial resistance in *E. coli* creates obstacles due to their increased tendency to distribute multiple antimicrobial resistance genes. Bacterial resistance to antibiotics usually develops by means of chromosomal mutations, or by the acquisition of large, transferable, extrachromosomal DNA elements, called plasmids, on which may be other DNA mobile elements, termed transposons and integrons.[11,17] These DNA mobile elements have been shown to possess genetic determinants for several different mechanisms of resistance to multiple antimicrobial agents.[2,11,16,17] Bacterial antibiotic resistance generally develops through one of four mechanisms: reduced cellular uptake and or increased efflux of the antimicrobial agent; antibiotic inactivation; alteration of target enzyme; and alteration of target binding site. The majority of antibiotics used in veterinary medicine can be inactivated or rendered ineffectual by one or more of these mechanisms. For instance, fluoroquinolone resistance has been linked to chromosomal mutations mediating changes in the A subunit of bacterial DNA gyrase (*gyrA*), or to decreased levels of drug accumulation, or both,[6,10,14] whereas ß-lactam antibiotics can be inactivated by the presence of bacterial enzymes called ß-lactamases which cleave the ß-lactam ring.[5]

The term "antimicrobial resistance" can be interpreted many different ways by many different disciplines. For instance, there is the microbiological definition versus the clinical interpretation of antimicrobial resistance; intrinsic resistance versus acquired;

and chromosomal versus extrachromosomal antimicrobial resistance. All of these aspects should be included when one describes the antimicrobial resistance situation facing us today.

The focus of my research at NDSU is to collect data concerning the prevalence of multiple antibiotic resistance among *E. coli* strains incriminated in bovine calf scours, and identify the antimicrobial resistance mechanisms at work. Over 300 *E. coli* isolates were obtained from clinical calf scours cases submitted to the North Dakota State University Veterinary Diagnostic Laboratory in 1997. Bacterial antibiotic sensitivities were carried out using standard antibiotic disk diffusion assays and micro dilution methods.[13] The resulting antibiograms (antibiotic sensitivities) are already yielding interesting data concerning patterns of resistance. These strains were also screened for several virulence factors that have recently been identified among both human and animal pathogenic *E. coli* strains .[2,9] For *E. coli* strains incriminated in calf scours, antimicrobial resistance percentages ranged from 93% for tetracycline to less than 1% for amikacin. Seventy-seven percent of strains were resistant to ampicillin whereas only 23% were resistant to gentamicin. Eleven percent of strains were resistant to ceftiofur and 5% were resistant to enrofloxacin, even though this drug is prohibited for large animal use. Why are certain *E. coli* strains found to be 100% resistant to certain antimicrobials? One must remember that these isolates are the "worst of the worst" since they are submitted to the NDSU Veterinary Diagnostic Laboratory after other treatments have probably failed.

In conclusion, bacterial antibiotic resistance has emerged with surprising rapidity among *E. coli* strains incriminated in bovine calf scours following the extensive use of antimicrobials in veterinary medicine. Many of these also possess several virulence factors that enable these strains to produce disease in both animals and humans. This association of increased virulence coupled with multi-drug resistance is an increasing threat to successful treatment of *E. coli* related animal diseases and possibly to human health. As is the case for particular bacterial related human infections, therapeutic options for treatment of diseases in animals is lessening. **Because multiple drug resistance is such a growing dilemma, it is conceivable that in the near future, veterinarians may confront bacterial infections for which there is no effective therapy.**

References

1.Adesiyun, A.A., and J.S. Kaminjolo. 1992. Susceptibility to antibiotics of *Escherichia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad. *Rev. Elev. Med. Vet. Pays. Trop.* 45:260-262 2.Blanco, J., D. Cid, J.E. Blanco, M. Blanco, J.A. Ruiz Santa Quiteira, and R. de la Fuente. 1996. Serogroups, toxins, and antibiotic resistance of *Escherichia coli* strains isolated from diarroeic lambs in Spain. *Vet. Microbiol.* 49:209-217. 3.Blanco, J.E., M. Blanco, A. Mora, and J. Blanco. 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *J. Clin. Microbiol.* 35:2184-2185. 4.Brown, S.A. 1996. Fluoroquinolones in animal health. *J. Vet. Pharmacol. Ther.* 19:1-14. 5.Bush, K., G.A. Jacoby, and A.A. Medeiros. 1995. A functional classification scheme for ß-lactamases and its correlation with its molecular structure. Antimicrob. *Agents Chemother.* 39:1211-1233. 6.Everett, M.J., Y.F. Jin, V. Ricci, and L.J.V. Piddock. 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. Antimicrob. *Agents Chemother.* 40:2380-2386. 7.Farina, C., A. Goglio, G. Conedera, F. Minelli, and A. Caprioli. 1996. Antimicrobial susceptibility of *Escherichia coli* O157 and other enterohaemorrhagic *Escherichia coli* isolated in Italy. Eur. J. Clin. Microbio. Infect. Dis. 15:351-353. 8.Gold, H.S., and R.C. Moellering, Jr. 1996. Antimicrobial drug resistance. *N. Eng. J. Med.* 335:1445-1453. 9.Gonzalez, E.A. and J. Blanco. 1989. Serotypes and antibiotic resistance of verotoxigenic (VTEC) and necrotizing (NTEC) *Escherichia coli* strains isolated from calves with diarrhoea. *FEMS Microbiol. Lett.* 51:31-36. 10.Griggs, D.J., M.C. Hall, Y.F. Jin, and L.J.V. Piddock. 1994. Quinolone resistance in veterinary isolates of salmonella. *J. Antimicrob. Chemother.* 33:1173-1189. 11.Kruse, H., and H. Sorum. 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. Appl. Environ. *Microbiol.* 60:4015-4021. 12.Levy, S.B. 1992. The Antibiotic paradox. Plenum Publishing. 13.National Committee for Clinical Laboratory Standards. 1997. (NCCLS Document M-31 Proposed Standard). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. National Committee for Clinical Laboratory Standards, Villanova, PA. 14.Piddock, L.J.V. 1995. Mechanisms of resistance to fluoroquinolones: state of the art 1992-1994. Drugs. 49 *Suppl.* 2:29-35. 15.Piddock, L.J.V. 1996. Does the use of antimicrobial agents in veterinary medicine andanimal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy. *J. Antimicrob. Chemother.* 38:1-3. 16.Salyers, A.A., and C.F. Amiable-Cuevas. 1997. Why are antibiotic resistance genes so resistant to elimination? *Antimicrob. Agents Chemother.* 41:2321-2325. 17.Sandvang, D., F.M. Aarestrup, and L.B. Jensen. 1997. Characterisation of integrons and antibiotic resistance genes in danish multiresistant *Salmonella enterica typhimurium* DT104. *FEMS Microbiol. Lett.* 157:177-181. 18.Singh, M., M.A. Chaudhry, J.N. Yadava, and S.C. Sanyal. 1992. The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* form 1984-1986 in northern India. *J. Antimicrob. Chemother.* 29:159-168.