ORIGINAL ARTICLES

Multiresistant clones of *Salmonella enterica*: the importance of dissemination

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Increasing anitmicrobial resistance among foodborne pathogens has prompted calls for the reduction of anitmicrobial use in livestock to prevent future emergence or resistance. In the case of *Salmonella enterica*, clonal dissemination may play a more critical role in regional changes in antimicrobial resistance in *Salmonellae* than antimicrobial selection pressure. Multi-resistant *Salmonella* Typhimurium definitive type 104 (mr-DT104) emerged from an unknown location and was disseminated globally during the 1980s and 1990s. Other clones of *Salmonella* Typhimurium and non-Typhimurium *Salmonellae* have demonstrated an ability to disseminate widely. The clonal epidemiology of mr-DT104 is in contrast with that of *Campylobacter jejuni*, in which antimicrobial resistance is polyclonal and seems to develop in response to local antimicrobial pressures. The epidemiology of mr-DT104 is more similar to that of methicillin resistant *Staphylococcus aureus*, which is also characterized by international transmission of a few clonal subtypes. Control measures for multiresistant disseminated clones of *Salmonellae* must focus on the interruption of dissemination in order to be effective. (J Lab Clin Med 2002;140:135-41)

Abbreviations: ACSSuT = serovars resistant to the antimicrobials ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides, and tetracycline

The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades.[1-6] This increase has been judged to be primarily the result of selection pressure exerted by antimicrobials in the feeds of food-producing animals.[2,3,7-9] While this may be true for other foodborne pathogens like *Campylobacter jejuni*, we have presented evidence that clonal dissemination plays a critical role for both human and animal infections with Salmonella, and that this is not driven only by, or even primarily by, antimicrobial selection pressure.[4,10,11]

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SALMONELLA ENTERICA SEROVAR TYPHIMURIUM DEFINITIVE TYPE 104

Nontyphoidal salmonellosis continues to be a major public health burden, with 45,000 cases and 400 to 600 deaths reported annually to the Centers for Disease Control and Prevention.[12,13] In the United States, Enteritidis and Typhimurium are the two most frequently isolated serovars of *Salmonella* in humans with salmonellosis.[12] The majority of these cases are a self-limited gastroenteritis and do not require antimicrobial therapy; however, some infections can be invasive, and even fatal, particularly for patients who are immune-compromised. In these cases, antimicrobial therapy is necessary.[13] Therefore, the increase of a penta-resistant subtype of Typhimurium was of great concern when this

was observed in the United Kingdom, Europe, and North America in the 1990s.[10] This subtype was primarily identified by two phenotypic markers: 1) resistance to at least the five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline [ACSSuT+]) and 2) the definitive phage type 104 (DT104).

Molecular genetic studies conducted in Great Britain,[14] France,[15] Ireland,[16] Germany,[17,18] Denmark,[19] and the United States[20] have contributed to the consensus that mr-DT104 represents a globally disseminated clone. For the most part, these studies used pulsed-field gel electrophoresis (PFGE) in combination with other genotyping methods, including polymerase chain reaction (PCR)-based fingerprinting,[20] hybridizations with IS200 probes,[17] or hybridizations with probes targeting integrase genes and resistance genes.[14,15] The studies in France,[14] the United Kingdom,[15] and Denmark[19] compared isolates from different countries, including the United States, the United Kingdom, and Europe, and concluded that they were clonally related. Another study from Greece, which compared 14 mr-DT104 isolates using PFGE and PCR to detect integrase and resistance genes, found that 5 of 14 were identical, and the other 9 were diverse.[21] PFGE is a very discriminating method in which minor genetic changes can produce major band pattern differences,[22] and findings of local genetic diversity in mr-DT104 populations do not necessarily contradict the idea that mr-DT104 has an essentially clonal population structure.

Mr-DT104 emerged from an unknown location and has spread globally very rapidly, as it was first detected in Europe, Asia, and North America[10] almost simultaneously. In at least one region (the Pacific Northwest), the major changes in antimicrobial resistance among *Salmonella* Typhimurium isolates from humans and cattle between 1989 and 1997 were attributable to the rise in mr-DT104.[4] ACSSuT phage type 104 *Salmonella* Typhimurium were isolated from humans in Asia before 1982,[23] although the clonal relationship of these early isolates with the mr-DT104 clone is unknown. The earliest isolations of mr-DT104 were from humans and birds in the United Kingdom in 1984,[24,25] and from a human in the United States in 1984 and 1985.[3] The first isolates from agricultural animals were observed in Britain in 1988[24] and in the United States in 1990.[10] These observations fail to support the hypothesis that this clone originally emerged in cattle;[26] in fact, the species and location in which the mr-DT104 clone originated are unknown.

In south Asia, opportunities for sustained *Salmonella* transmission to occur in situations with intense antimicrobial selection pressure have been extensively documented, particularly for nosocomial outbreaks of salmonellosis.[10] The rapid dissemination of mr-DT104 is more consistent with human travel than with bovine or animal movements: humans travel rapidly—and often globally—by air with great frequency, and very little intercontinental movement of agricultural animals occurs. Consistent with this hypothesis, transcontinental dissemination of a bovine host-specific bovine *Salmonella* serovar, Dublin, occurred at a far slower pace.[27,28] In contrast, multi-resistant strains of the human host-specific *Salmonella* Typhi have disseminated rapidly and globally, much like mr-DT104.[10]

The importance of human travel as a vehicle for global dissemination of *Salmonella* strains may have been underestimated. Foreign travel is a well-established risk factor for salmonellosis in developed countries.[29-33] Imported *Salmonella* infections are more likely to be multi-resistant than those domestically acquired in developed countries.[32,34] The first observed isolates resistant to quinolones[35] and ceftriaxone[8] in the United States were from imported cases.

Although the frequency with which human fecal pathogens are ingested by food-producing animals is likely to be limited in most developed countries, reported examples of sewage transmission of *Salmonella* from humans to livestock suggest that this phenomenon occurs more frequently than is generally imagined. In 1980, the first appearance of *Salmonella* serovar Zanzibar occurred in Scotland after a man returned from Malaysia; *S.* Zanzibar subsequently was isolated from a bulk milk sample from a nearby dairy.[36] In 1994, the first appearance of *Salmonella* Enteritidis phage type 4 in a poultry flock in the United States prompted an environmental investigation, which found evidence that sewage effluent upstream from the farm was the likely source of the infection.[37]

The relatively high rate of *Taenia saginata* cysticercosis in feedlot cattle in the northwestern United States provides evidence that cattle in this region frequently ingest feed contaminated with human feces.[38,39] Humans are the only definitive host for the parasite, and cattle are the only intermediate host. The encysted larvae are detected by visual inspection of the muscle of cattle at the time of slaughter. Since slaughter inspection for cystic infection is insensitive, the reported frequency probably underestimates the frequency of human fecal contamination of cattle feed, at least in the northwest United States.[10]

Even if human travel is largely responsible for intercontinental dissemination of Salmonella strains, it may be expected that local dissemination would be promoted by use of antimicrobials in food animals. However, in the case of mr-DT104, there is evidence of its local dissemination in the absence of selective antimicrobial pressure. In the Pacific Northwest of the United

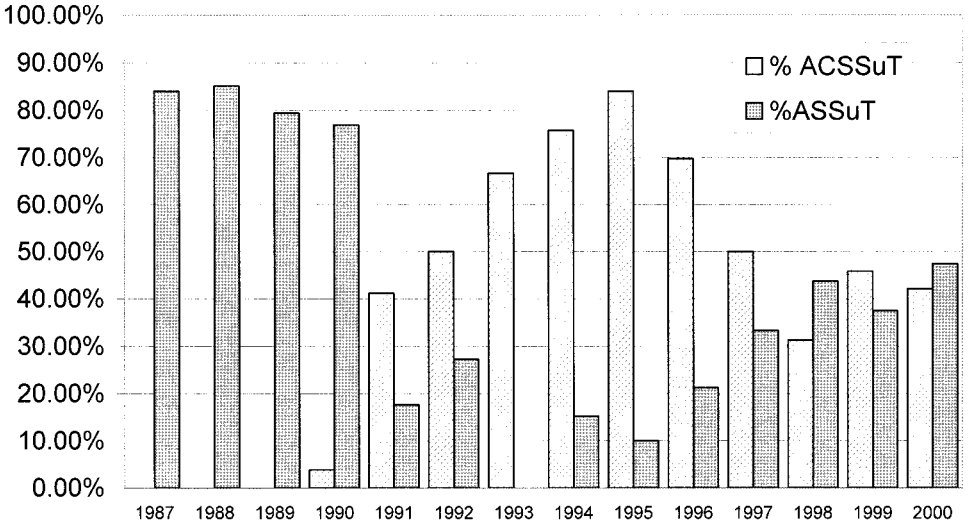

Fig 1. Percent of clinical bovine *Salmonella* Typhiurium isolates from the Washington Animal Disease Diagnostic Laboratory with resistance pattern. Chloramphenicol was banned for use in cattle in 1983, and florfenicol was not licensed for use until 1996. *ACSSuT*, resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline plus or minus other resistance; *ASSuT*, resistant to ampicillin, streptomyicin, sulfonamides, and tetracycline plus or minus other resistance but not resistant to chloramphenicol.

States, the proportion of Typhimurium isolates that were ACSSuT+ increased (for both human and cattle origin isolates) while the proportion of those that were ASSuT decreased (Figure 1).[4] Use of any one of the antimicrobials ampicillin, streptomycin/spectinomycin, sulfamethoxizole, or tetracycline would select for either ASSuT or ACSSuT, but would not offer the ACSSuT strains any advantage over ASSuT. Thus, the only obvious resistance advantage enjoyed by mr-DT104 over the displaced Typhimuriums was resistance to chloramphenicol. The fitness advantage that allowed mr-DT104 to disseminate successfully compared to Typhimurium strains resistant to ASSuT has not been identified. The gene that confers resistance to chloramphenicol in mr-DT104 also confers cross-resistance to florfenicol.[40] Chloramphenicol was banned for use in food animals in 1983,[41] and florfenicol was not approved until 1996,[42] so the displacement of ASSuT Typhimurium by ACSSuT Typhimuriums among cattle could not have been due to the selective advantage of florfenicol or chloramphenicol resistance. Likewise, the rapid dissemination of mr-DT104 through the United Kingdom occurred well after the pertinent antimicrobials were banned for use as growth promotants, per the recommendations of the Swann Committee in 1969.[43] Of note in this context was the occurrence of an outbreak of fluoroquinolone-resistant DT104 in Denmark associated with swine herds in which fluoroquinolones had not been used for more than a year.[44] Recent Norwegian surveillance of human *Salmonella* infections showed an increase in domestically-acquired mr-DT104[45] in spite of very restricted antimicrobial use in livestock.[46] In Belgium, a decrease from 25% to 0% in enrofloxacin resistance among cattle *Salmonella typhimurium* strains was noted between 1991 and 1998. This decrease could be explained by the displacement of enrofloxacin-resistant phage type 204c clones by enrofloxacin-sensitive DT104, although there was no reduction in the use of enrofloxacin during that time among cattle.[47] The same phenomenon was observed in Germany at about the same time.[17]

Salmonella Typhimurium phage type 10 (PT10) disseminated across Canada between 1970 and 1979, although 90% of the isolates were sensitive to all antimicrobials tested. PT10 was most prevalent among poultry isolates, and most foodborne outbreaks were associated with poultry, but PT10 was prevalent in other food animal sources, as well as humans.[48] In addition to mr-DT104, other phage types of the serovar Typhimurium have demonstrated an ability to disseminate widely, producing marked changes in the prevalence of antimicrobial resistance in a region. In the 1960s, a multiresistant Typhimurium phage type 29 was disseminated in the United Kingdom by means of the sale and distribution of infected calves, and became prevalent among dairy cattle and humans until 1969.[7,49] In the 1970s, phage types 204, 193, and 204c rose to prominence among cattle-origin *Salmonella* Typhimurium isolates.[6] Type 193 was derived from CSSuT-resistant Type 204 by the acquisition of a plasmid that encoded additional resistance to ampicillin and kanamycin.[50] Type 204c differed from Type 204 by an

additional resistance to trimethoprim (and thus it was CSSuTTm). Phage types 204 and 193 also became disseminated internationally by calf traders who sold infected calves to locations throughout the United Kingdom and in Europe.[51,52] Although multiresistant phage type 204c was prevalent among cattle in the UK through the beginning of the 1980s, the proportion of human Typhimuriums that were phage type 204c remained low.[52] The epidemic in calves of DT204c peaked in 1986 and was ending in 1993[53] while mr-DT104 was on the rise.[6]

Clones of non-Typhimurium *Salmonella* serovars have also disseminated regionally and internationally. A human-adapted serovar with multiple resistance, *Salmonella* Wien, disseminated through Europe from Northern Africa. After having first been reported in association with an Algerian pediatric ward in 1969, Wien became the most frequently isolated serovar in France and Italy in the 1970s.[53]

Disseminated multi-resistant clones of *Salmonella enterica*, after a period of increase in proportion of total isolates, typically decline to become relatively minor subtypes. The mechanism of expansion and subsequent replacement of *Salmonella* clones in both human and animal populations is unknown.

COMPARISON TO FLUOROQUINOLONE RESISTANCE IN *CAMPYLOBACTER JEJUNI*

Campylobacteriosis is, like salmonellosis, a primarily foodborne infection associated with the consumption of foods of animal origin. Fluoroquinolone resistance among *Campylobacter* isolates is of particular concern because of the importance of ciprofloxacin for therapy in human patients.[5,56] The epidemiology of fluoroquinolone resistance in *Campylobacter* species contrasts with the epidemiology of mr-DT104 in several important ways. *Campylobacter* exhibits a polyclonal population structure: studies of its molecular epidemiology using PFGE of genomic DNA or restriction fragment analysis of the *flaA* gene among human and poultry isolates have consistently found a wide genetic diversity.[57-59] In contrast, mr-DT104 from diverse sources are genetically homogeneous.[14-20] The frequency of resistance to fluoroquinolones among *Campylobacter* isolates seems consistently responsive to local antimicrobial selection pressure. A temporal relationship between veterinary licensure of fluoroquinolones and increasing resistance among human *Campylobacter* has been observed in Europe, the United Kingdom, and the United States,[5,60,61] whereas the dissemination of mr-DT104 did not correlate temporally or geographically with veterinary antimicrobial use that would confer a selective advantage.

COMPARISON TO METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

The epidemiology of mr-DT104, in which one or a few internationally disseminated, clonal subtypes account for a high percentage of total isolates, is similar to that of methicillin resistant *Staphylococcus aureus* (MRSA).[62-66] Epidemic MRSA clones are different genetically from non-epidemic ones, and selection pressures that favor epidemic clones are not limited to those deriving from antimicrobial usage.[67,68]

Successful control strategies for MRSA require infection control measures in addition to changes in antimicrobial usage.[69-71] The contribution of control strategies that involve changes in antimicrobial use, relative to that of infection control, is subject to debate,[72,73] but infection control practices must play a central role in successful MRSA control programs.[71,73] A recent discussion of the successful Dutch program appears to corroborate the conclusion that infection control has been the decisive element in MRSA control and that this has resulted in a much lower prevalence of MRSA in Dutch hospitals in comparison to those in most other European countries.[69]

CONCLUSION

There is good evidence that the dissemination of multi-resistant clones of *Salmonella* Enterica contribute substantially to changes in *Salmonella* resistance, and in the case of *S.* Typhimurium, clonal dissemination may be the most important factor regionally, nationally, and even globally. The ability of mr-DT104 to disseminate widely and rapidly does not appear to be primarily attributable to its antimicrobial resistance; the traits responsible for the success of mr-DT104 and its predecessors remain unidentified. Reports on the epidemiology of resistant foodborne *Salmonella* Typhimurium, which call for restrictions on antimicrobial use in food animals, often fail to mention the issue of dissemination. Given the global nature of transmission of mr-DT104 and other disseminated clones of *Salmonella*, it is not reasonable to assume that the multiresistance problem can be controlled by preventing genetic emergence of multiresistant subtypes, just as reliance on antimicrobial use restrictions in the absence of infection control could not be expected to reduce the incidence of MRSA.

In the case of multiresistant *Salmonella* clones, control efforts should also be aimed at interrupting dissemination. In the United States, there is no restriction on animal movements for herds with positive *Salmonella* Typhimurium status, although the purchase of infected animals is a known risk factor for dissemination.[10] Surveillance for pathogenic *Salmonella* serotypes in

live animals is sporadic in the United States and dependent on clinical submissions or research projects that, however long-lived, are only temporary. In comparison, routine surveillance, coupled with appropriate interventions, have significantly reduced the incidence of salmonellosis in food-producing animals in Norway and Sweden.[74,75] In those countries, positive Salmonella test results from live animals or carcasses at slaughter prompt traceback and quarantine procedures. In addition, feedstuffs are also routinely monitored for *Salmonella* contamination, with appropriate action following a positive result. A similar program in Denmark detected and contained the spread of mr-DT104 in swine herds.[43] The larger scale of food animal and animal feed operations in the United States does not preclude implementation of biosecurity, which could include protection of feed from rodents and birds, limiting human traffic, disinfection of premises, and separation of incoming animals from the herd.[76] Biosecurity measures have been explored more thoroughly for poultry and swine than for cattle in this country,[77-79] but herd testing and quarantine of incoming animals is feasible and would reduce the risk of introducing mr-DT104 (or a new epidemic clone) into the herd.

Research targeted at evaluating these interventions will be an essential first step towards implementing effective controls. There have been few herd-level observational studies to clarify risk factors for pathogenic *Salmonella* shedding by cattle. The recent purchase of animals was a risk factor for mr-DT104[80] and *S.* Dublin.[81] Warnick et al[82] found an association between rodent and bird access to feed and clinical salmonellosis in cattle herds. Based on current knowledge, control efforts aimed at mr-DT104 (or similar pathogens) would include reduction of wildlife and cat density on a farm, housing sick animals separately from periparturient cows, and improving the microbial quality of cattle feed.[10] Besides more herd-level observational studies designed to evaluate risk factors for presence of multiresistant pathogens on a farm, controlled trials comparing management strategies (for example, enhanced biosecurity) are needed.

Certainly, the reduction of antimicrobial use in veterinary medicine and livestock production would prevent emergence of resistant strains of pathogens, particularly in situations such as calf-raisers and feedlot sick pens where intense antimicrobial use and pathogen transmission co-exist.[10] However, new resistant strains of *Salmonella enterica* are likely to emerge somewhere in the world. Until the issue of dissemination of resistant *Salmonella* clones is addressed by public health and animal health policy makers, such clones will continue to spread unimpeded through food animal populations in the United States, with consequent human foodborne infection.

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