July 2013

An HSI Report: Industrial Farm Animal Production and Livestock Associated MRSA (Methicillin Resistant *Staphylococcus aureus*)

Abstract

Staphylococcus aureus is a leading cause of bacterial infection and is increasingly found to be resistant to antibiotic therapy. A newly described type of Methicillin Resistant *Staphylococcus aureus* carried by farm animals, Livestock Associated MRSA (LA-MRSA), is now causing infections in humans with and without direct livestock contact. A reduction in the non-therapeutic use of antibiotics in feed would likely reduce the capacity of industrial animal agriculture to continue to create, disseminate, and perpetuate a large reservoir of LA-MRSA on a global scale, but more fundamental changes in the way animals are raised for food may be necessary forestall a "post-antibiotic age."

HA-MRSA, CA-MRSA, and LA-MRSA

The development of antibiotics revolutionized the treatment of human bacterial infections, dramatically reducing death and disease. Globally, human medicine has come to rely heavily on antibiotics. There is growing concern, however, about the emergence of antimicrobial resistance to these drugs, including *Staphylococcus aureus*, a common cause of foodborne illness, skin, and respiratory infections. Misuse of antibiotics in the health care industry[a] as well as routine[b] use of antibiotics in industrial farm animal operations are credited with driving the rate and extent of antibiotic resistance worldwide.[1,2,3,4]

Because of the adaptive nature of bacteria, antibiotics eventually lose their effectiveness. Antimicrobials must continually innovate in order to remain effective. In the past, pharmaceutical advances had been able to keep ahead of the evolution of resistant *Staphylococcus aureus*,[c] considered "one of the most adaptable and virulent pathogens in modern times."[5] But this is no longer the case. Presently, there is a market failure in the pharmaceutical development and production of human antibiotics, since people generally only need them for acute sporadic interventions. Compared to the profit value of drugs for chronic diseases that may be prescribed for life, antibiotic development has relatively less financial incentive.[6,7] Thus, there is a dearth of new antibiotics coming onto the market.[8,9]

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[a] Misuse of human antibiotics is one reason for the escalating problem of antibiotic resistance in the community and in health care settings. Health providers in the United States have long been aware of the need for prudent dispensation of antibiotics (Colgan R and Powers J. 2001. Appropriate Antimicrobial Prescribing: Approaches that Limit Antibiotic Resistance. American Family Physician 64(6):999-1005).

[b] In 2010, roughly 13.4 million kg of ntimicrobials were sold for food animal production (Food and Drug Administration Animal Drug User Fee Act 2010).

[c] It has been noted that "*Staphylococcus aureus* has the characteristic ability to rapidly develop resistance to virtually any antibiotic drug coming into clinical use" (Pantosti A.2012. Methicillin-Resistant *Staphylococcus aureus* associated with animals and its relevance to human health. Frontiers in Microbiology 3(127):1-12).

The global rise of resistant bacteria coupled with a lack of medical innovation has creates a global health crisis and the danger of entering a "post-antibiotic-era," in which particularly vulnerable populations, including children, the elderly, and the immunocompromised may start dying from common infections.[10,11]

In 2005, Methicillin-Resistant *Staphylococcus aureus* (MRSA)[d] was responsible for an estimated 94,000 infections in the United States, with more than 18,000 deaths reported.[12] *Staphylococcus aureus* is a leading cause of bacterial infections, and the number one cause of hospital infections.[13] Increasingly, those infections are resistant to antibiotic therapy. Historically, most MRSA deaths were Hospital Associated Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA), occurring within healthcare settings in patients with compromised immune systems. HA-MRSA has spread globally in health care settings since the 1960s and has emerged to become endemic in most industrialized nations.[14]

Then MRSA deaths began rising in people who have had no hospital contact. Since 2000, the majority of MRSA infections in most countries are acquired in the community among people with no health care contact.[15,16] These infections are distinguished epidemiologically by the fact that they occur in the community at large and are referred to as Community-Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA). CA-MRSA is especially worrisome because it occurs among otherwise healthy people with no known risk-factors.

More recently, another type of MRSA carried by pigs was discovered causing human infections in Europe.[17] It is now called Livestock Associated Methicillin Resistant *Staphylococcus aureus* (LA-MRSA). Sequence Type 398 (ST398), the founder[18] strain[e] of LA-MRSA, appears to have arisen independently in the pig population, distinct from HA-MRSA and CA-MRSA.[19] LA-MRSA belongs to unique lineages that don't spread well in hospitals.[20] It has however spread rapidly among pigs and between farms and is now found in pig herds, humans, and many other animals globally.[21,22]

Industrial Farm Animal Production and LA-MRSA

There is much about LA MRSA that is unknown. The scientific understanding of LA-MRSA's epidemiology and public health implications has been termed "embryonic."[23] The nomenclature is non-uniform and problematic.[24,25] Data is often difficult to interpret as collection methods are not standardized and few countries have systems in place to monitor the use of antibiotics and antibiotic resistance in animals.[26,27] There is concern that some LA-MRSA strains currently remain undetected.[28,29]

It is clear, though, that industrial farm animal production is widely recognized as being responsible for the development, dissemination, and persistence of an enormous reservoir of the LA-MRSA and its resistance genes on a global scale.[30,31] The resulting problem is now so widespread that it threatens the future effective use of anti-staphylococcal therapy.[32]

[d] Methicillin-Resistant *Staphylococcus aureus* is typically defined as being resistant to a large spectrum of antibacterial agents, including beta-lactamase-resistant penicillins such as methicillin.

[e] LA-MRSA comprises multiple lineages but ST398 is the predominant one in North America and Europe. In Asia, the predominant strain is ST9 (Smith T, and Pearson N. 2011.The Emergence of *Staphylococcus aureus* ST398. Vector-Borne and Zoonotic Diseases 11(4):327-339; Graveland H, Duim B, van Duijkeren E, Heederik D, and Wagenaar JA, 2011. Livestock associated methicillin resistant *Staphylococcus aureus* in animals and humans. International Journal of Medical Microbiology 301:630-634; Larsen J, Imanishi M, Hinjoy S, et al. 2012. Methicillin-Resistant *Staphylococcus aureus* ST9 in Pigs in Thailand. PLoS ONE7(2):1-3). A 2013 study finds that the range of LA-MRSA types is larger than previously understood (Frana TS, Beahm AR, Hanson BM, et al. 2013. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Pork Farms and Visiting Veterinary Students. PLoS ONE 8(1):1-10).

Emergence

World health governmental bodies agree that non-therapeutic antibiotic use in animal production creates reservoirs of resistant bacteria and is a direct threat to human health.[33,34,35,36] Although antimicrobials have been used in animal production for decades[37] the massive increase of the amounts used, attributable to the growth of industrial farm animal production (IFAP), is relatively recent.[38]

MRSA in livestock developed as a direct result of the routine and widespread use of antibiotics,[f] including Animal Growth Promoters (AGPs) in livestock production.[39,40] AGPs may play a role in maximizing profit, by potentially shortening the time that an animal achieves market weight (i.e., is ready for slaughter), thereby potentially conferring an economic advantage with their use.[41] AGPs are also used as a prophylaxis to address the host of health problems associated with intensive, confined animal production.

The constant exposure to non-therapeutic low-dose antibiotics routinely given in feed or water exerts a selection pressure for the survival of antibiotic resistant pathogens and genes.[42] Every single animal, in every IFAP operation that consumes the low levels of antibiotics contained in AGPs becomes its own unique "factory" for the production and dissemination of both pathogens resistant to those antibiotics as well as resistance genes.[43,g] And the more animals in a confined environment, the more opportunity there is to exchange bacteria and resistance genes.[44,45]

It is believed that LA-MRSA was originally a human adapted Methicillin Susceptible *Staphylococcus aureus* (MSSA). It is thought to have adapted to intensively raised pigs and acquired resistance genes due to the AGPs routinely fed to them.[46,h]

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[f] More than 29 million pounds of antimicrobials were sold for use in animal production in 2010 (Food and Drug Administration 2010 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. Center for Veterinary Medicine). This raw sales data is a record of manufacturer sales to distributors as opposed to end-use and does not lend itself to in-depth analysis. For instance, the veterinary data provided to FDA are not distinguishable by animal species or by usage, prophylactic or therapeutic (Department of Health and Human Services Undersecretary Meister April 2011 letter to Rep. Slaughter; Apley MD, Bush EJ, Morrison RB, Singer RS, and Snelson H. 2012. Use Estimates of In-Feed Antimicrobials in Swine Production in the United States. Foodborne Pathogens and Disease 9(3):1-8). Regardless, the amount of antibiotics used in farm animal production clearly dwarfs that which are used in human clinical medicine.

[g] Antibiotics may stimulate gene transfer (Aminov RI, Mackie RI. 2007. Evolution and ecology of antibiotic resistance genes. Federation of European Microbiological Societies 271:147-161), especially in sites that are highly concentrated in microbes, such as the porcine gut (Amador MP, Fernandes RM, Duarte IM, Brito ML, Barreto MP, and Prudencio MC. 2012. Assessment of Antibiotic Resistance in Water Systems http://cigr.ageng2012.org/comunicaciones-online/htdocs/principal.php?seccion=posters&idcomunicacion=13367&tipo=4). Transfer of resistance genes in LA-MRSA is frequent and greater transference may lead to more virulent MRSA (McCarthy AJ, Witney AA, and Gould KA et al. 2011. The distribution of Mobile Genetic Elements (MGEs) in MRSA CC398 is associated with both host and country. Genome Biology and Evolution 3:1164-1174). AGPs have accelerated the process by which antibiotic resistance is disseminated, through the horizontal gene transfer of mobile genetic elements (McCarthy AJ, Lindsay JA, Loeffler A. 2012. Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. Veterinary Dermatology 23:267-275). Not all AGPs have the same effect in the animal gut, however. Certain antibiotics are far more likely to be involved in genetic exchange than others (Carlson S. 2009. Effects of Subtherapeutic Antibiotics on Antibiotic Resistance and Virulence Gene Transfer in Swine Intestinal Bacteria. Research Report. National Pork Board NPB#08-002).

[h] Antibiotic use on farms is a risk factor for the prevalence and spread of MRSA in animal production (Graveland H, Wagenaar JA, Heesterbeek H, et al. 2010. Methicillin-resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5(6); Cuny C, Friedrich AW, Witte W. 2012. Absence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* Clonal Complex CC398 as a Nasal Colonizer of Pigs Raised in an Alternative System. Applied and Environmental Microbiology 78(4):1296-1297). However, other factors are implicated in MRSA prevalence on farms, including farm size (Alt K, Fetsch A, Schroeter A, et

Dissemination

LA-MRSA spread rapidly among pigs, between farms, and is now widespread globally in countries with important pig production industry.[i] In 2009 U.S. researchers found 49% colonization with LA-MRSA ST398 in a sample of 299 pigs from two industrial pig operations encompassing roughly 87,000 live animals.[47] In the European Union prevalence is estimated at 26.9% in pig production holdings overall, but, vary from 0% to 51.2% among European Union Member States.[48] Prevalence is also growing in South America and many Asian countries.[49,50,51,52] In Asia, ST9 appears to be the dominant LA-MRSA strain,[53,54,j] while in North[55] and South America[56] and Europe, the dominant strain is ST398.[k] Conditions inherent to IFAP appear to have contributed to global increase in LA-MRSA.

IFAP operations are anthropogenic ecosystems which constrain the natural mobility and interactions of animals and displace natural selection through breeding and genetic modification.[57] Because profits depend on predictability and uniform product, pig herds are bred for homogeneity,[58] which contributes to bacterial evolution toward antibiotic resistance and virulence.[59] As such, these systems produce unique public health dangers beyond the traditional risks which have always been associated with animal production. The

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al. 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BioMed Central Veterinary Research 7(69):1-8; Smith TC, and Pearson N. 2011. The Emergence of *Staphylococcus aureus* ST398. Vector-Borne Zoonotic Diseases 11(4):327-339). Two of many key factors likely to influence pathogen transmission in pig production are herd size and animal density (Davies, PR. 2010. Pork Safety: Achievement and Challenges. Zoonoses and Public Health 57(supp.1):1-5). Lack of proper hygiene, which is often assumed to be a marker for antibiotic use, may be related to herd size or number of animals at a facility (Broens EM, Graat EAM, Van Der Wolf PJ, Van De Giessen AW, De Jong MCM. 2011. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Preventive Veterinary Medicine 102:41-49). Studies examining risk factors for human colonization of LA-MRSA have found unexpected results regarding hygiene, including a inverse correlation between good hygiene practices and MRSA colonization (Smith TC, and Pearson N. 2011. The Emergence of *Staphylococcus aureus* ST398. Vector-Borne Zoonotic Diseases 11(4):327-339, citing Denis O, Suetens C, Hallin M, et al. 2009. Methicillin resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. Emerging Infectious Diseases 15:1098-1101). For example, MRSA-colonized farmers in a 2012 study were the only ones to wear protective overalls, in accordance with good hygiene practice (Osadebe LU, Hanson B, Smith TC, and Heimer R. 2012. Prevalence and Characteristics of *Staphylococcus aureus* in Connecticut Swine and Swine Farmers. Zooneses and Public Health doi: 10.1111/j.1863-2378.2012.01527.x).

[i] ST398 is not limited to pigs. It is present in a range of animals, including sheep, birds, and cows (Lo YP, Wan MT, Chen MM, Su HY, Lauderdale TL, and Chou CC. 2012. Molecular characterization and clonal genetic diversity of methicillin-resistant *Staphylococcus aureus* of pig origin in Taiwan. Comparative Immunology, Microbiology and Infectious Diseases 35(6):513-521). It has been found in horses as well as pets (Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, and Witte W. 2009. Nasal Colonization of Humans with Methicillin-Resistant *Staphylococcus aureus* (MRSA) CC398 with and without Exposure to Pigs. PLoS One 4(8):1-6). It has even been found in rats on pig farms (van de Giessen AW, van Santen-Verheuvel MG, Hengeveld PD, Bosch T, Broens EM, and Reusken CB. 2009. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Preventive Veterinary Medicine 91(204):270-273). And it has been identified on a farm with both pigs and cows in both animals, contributing to mounting evidence that it spreads easily between species (Tavakol M, Riekerink R, Sampimon O, Van Wamel W, Van Belkum A, and Lam T. 2012. Bovine-associated MRSA ST398 in the Netherlands. Acta Veterinaria Scandinavica 54:28).

[j] The first detection of LA-MRSA ST9 in animals in Korea has been reported. A wide sampling of pigs and farms were found to harbor not only LA-MRSA, but also human MRSA (Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, and Kwak HS. 2012. The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. Veterinary Microbiology 155:88-92).

[k] Genes normally associated with ST398 have now been found in MRSA CC30, another common pig *S. aureus* lineage in Denmark. This suggests the spread of resistance genes normally associated with ST398 into another *S. aureus* lineage in Danish pigs. Further, LA-MRSA ST398 was found in Danish beef cattle for the first time, which suggests a spread between animal hosts (Agerso Y, Hasman H, Cavaco LM, Pedersen K, and Aarestrup FM. 2012. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Veterinary Microbiology 157: 246-250).

environmental strains resulting from tens of thousands of animals produced on strictly limited space have long been recognized as producing public health and environmental hazards for surrounding ecosystems.[60]

The number of animals produced in IFAP is increasing dramatically worldwide. The United States has dramatically intensified pig production during the past thirty years. While the number of U.S. pig farms fell by more than 70% between 1992 and 2004, the number of pigs produced stayed the same. The average operation grew from 945 animals in 1992 to 4,646 animals in 2004. The percentage of IFAP operations with 2,000 or more pigs increased from fewer than 30% to 80%. And those with 5,000 or more pigs held more than 50% of the total number of pigs in 2004.[61] From 2002 to 2007, U.S. sales of hogs and pigs increased 46 percent to $18.1 billion.[62] More than 50 percent of the value of U.S. sales of hogs and pigs comes from just three states: Iowa, North Carolina, and Minnesota. Production in the United States is geographically very concentrated, as 53% of all IFAP operations are within one mile of one another.[63]

IFAP produces far more waste than the surrounding environment can absorb. There are well-known hazards from human proximity to waste lagoons, including respiratory diseases and quality of life issues such as limited ability to be outdoors, due to foul air and insect infestations.[64,65] Less obvious consequences include the collateral effects from massive antimicrobial use in intensive animal production.[l]

CAFO animals shed an enormous amount of resistant pathogens and resistance genes directly amongst themselves and to humans, and indirectly through their waste into the environment via soil, air, and water contamination.[66,m] Pigs typically produces 1.5 tonnes of fresh manure before slaughter[67] and, in mammals in general, bacteria account for about 50% of the feces.[68]

Land application of swine manure is a significant route of environmental contamination.[69,70,71] Livestock manure is known to promote horizontal gene transfer (HGT) of antibiotic resistance genes in soil.[72,73,74] Swine feedlot wastewater has also been found to spread antibiotic resistance genes to adjacent fields through waste amendment and irrigation.[75]

Resistance genes can remain and perpetuate their reservoir in indigenous soil bacteria[76] which can be further disseminated to other environments, like groundwater and surface water.[77] As it disseminates, these commensal bacteria can also share resistance genes through HGT[n] with pathogenic bacteria.[78,79,o]

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[l] The pressure exerted by the use of AGPs in creating resistant microbes is straightforward. In contrast, the known and unknown collateral effects of AGPs fed to pigs are complex and numerous (Looft T, Allen HK. 2012. Collateral effects of antibiotics on mammalian gut microbiomes. Gut Microbes 3(5):1-5). For instance, AGPs are known to stimulate gene transfer, including virulence genes, and significantly alter bacterial community structures in the animal gut (Looft T, Johnson TA, Allen HK, et al. 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proceedings of the National Academy of Scientists of the United States of America 109(5):1691-1696; Allen HK, Looft T, Bayles DO, et al. 2011.Antibiotics in Feed Induce Prophages in Swine Fecal Microbiomes. mBio 2(6):1-9). Direct selection force exerted by AGPs is only one factor which contributes to the dissemination and persistence of resistance genes in bacterial populations (Aminov RI and Mackie RI. 2007. Evolution and ecology of antibiotic resistance genes. Federation of European Microbiological Societies 271:147-161).

[m] In addition to disseminating pathogens and resistance genes, CAFOs also disseminate a massive amount of whole antibiotics into the environment. Roughly 75% of the antibiotics given to animals are not absorbed by them and are excreted in waste, largely unmetabolized (Chee-Sanford JC, Mackie RI, Koike S, et al. 2009. Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genes following Land Application of Manure Waste. Journal of Environmental Quality 38:1086-1108). As a result, antibiotics can be acquired by food crops. The contaminated "grey water" used to irrigate vegetable crops for human consumption is considered a potential public health risk (Salyers A, and Shoemaker NB. 2006. Reservoirs of Antibiotic Resistance Genes. Animal Biotechnology 17(2):137-146).

[n] Bacteria such as *S. aureus* exchange genetic material with other various bacteria, crossing species boundaries (Levy. 1997. Antibiotic Resistance: Origins, Evolution, Selection and Spread. Wiley Chichester (Ciba Foundation Symposium 207):1-14); Wang Y, Zhang W, Wang J et al. 2012. Distribution of the Multidrug Resistance Gene *cfr* in *Staphylococcus*

Wind is another contamination route. A 2012 study detected LA-MRSA in the majority of soil surfaces sampled as a result of it having blown downwind from pig barns.[80] The researchers reason that because human MRSA strains are tenacious with a high survival rate for weeks on hard surfaces, the LA-MRSA blown onto soil surfaces would be able to accumulate provided they are not washed away by rainfall. The researchers identify the concern of perpetual contamination or recontamination via humans, animals, or machines that pick up contaminated soil and enter a disinfected barn. Subsequent studies on turkey, broiler chicken, and laying hen operations found similar downwind contamination.[81],[82] MRSA rates in chickens have ranged up to 71% tested.[83]

Persistence

IFAP enables the persistence of antibiotic resistant genes and pathogens through the use of antibiotics, but can also enable persistence in the absence of direct antibiotic pressure. Simply put, resistance is an "easy to get, hard to lose phenomenon."[84]

One way for resistance genes to persist in the absence of direct antibiotic selection pressure is through co-selection. Co-selection occurs when resistance elements are physically linked on the same MGE, for example.[85,86,87] This means, the removal of any particular antimicrobial from an animal's regime does not necessarily lead to a corresponding loss of resistance to that particular antimicrobial, since it could be tied to another gene for which active selection pressures continue.[88,89]

While the direct or indirect selection pressure exerted by an antibiotic can expedite the proliferation of antibiotic resistant bacteria, it is not necessary for the dissemination of antibiotic resistance genes among bacteria. One way for antibiotic resistance genes to persist in the absence of *any* antibiotic selection pressure happens when heavy-metal resistance genes and antibiotic resistance genes are physically linked on the same stretch of DNA. Co-selection for antibiotic resistance frequently occurs in heavy-metal polluted environments,[90] so antibiotic resistance may be transferred and maintained even in environments with no antibiotic selection pressure.[91]

Heavy-metals have been used extensively as feed additives in intensively produced animals, especially copper, zinc, and arsenic, and are seen as alternatives to antibiotic growth promoters.[92,93,94] Yet any additive which affects an animal's microbial flora has the potential to select for resistance.[95] The use of heavy-metals in feed might be tantamount to opening a "Pandora's Box,"[96] because of the metals' antibiotic resistance co-selection properties.[97,98] Researchers suspect that the use of zinc in pig feed as a growth promoter may have contributed to the emergence of LA-MRSA.[99]

Species Isolates from Swine Farms in China. Antimicrobial Agents and Chemotherapy 56(3):1485-1490). The main way resistance genes and virulence factors move among bacteria, even bacteria that are not closely related, is via Horizontal Gene Transmission or Transfer (HGT). HGT of MGEs in CC398 is frequent (McCarthy AJ, Witney AA, and Gould KA. 2011. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. Genome Biology and Evolution 3:1164-1174). A 2012 study concludes that staphylococci on pig farms act as a reservoir of resistance genes and inter-species HGT (Tulinski P, Fluit AC, Wagenaar JA, Mevius D, van de Vijver L, and Duim B. 2012. Methicillin-Resistant Coagulase-Negative Staphylococci on Pig Farms as a Reservoir of Heterogeneous Staphylococcal Cassette Chromosome mec Elements. Applied and Environmental Microbiology 78(2):299-304).

[o] Resistance among commensals (ubiquitous non-pathogenic bacteria) is a major avenue for development of resistance in bacterial pathogens since resistance often increases first in commensals and then transfers to pathogens (Chee-Sanford JC, Mackie RI, Koike S, et al. 2009. Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genes following Land Application of Manure Waste. Journal of Environmental Quality 38:1086-1108; Federation of Animal Science Societies. Webinar I: Antibiotics in Animals and People Webinar. 2010. fass.org).

Even if there were large-scale decreases in heavy-metal or antibiotic use in animal production, the antibiotic resistance problem is likely to remain for some time.[100,101,102,103,p] In the United States, for example, there is documented persistence of resistant pathogens after antimicrobial use has been discontinued for a significant duration. Fluoroquinolone[q]-resistant pathogens still appear in chicken products sold in the US, even after FDA restrictions in 2005 on fluoroquinolone use in production.[104]

LA-MRSA and other antibiotic resistant pathogens[r] appear in retail meat products from "alternative" production systems which claim not to use antibiotics on the animals.[105] The virulence gene PVL has recently been detected for the first time in the United States in LA-MRSA positive pigs on small, free-range farms, where fewer than 10% of the farms sampled used antibiotics on a regular basis.[106,s] The MRSA positive pigs were carrying HA-MRSA, and CA-MRSA, as well as LA-MRSA[t] showing that even less-intensively raised pigs may be reservoirs for human disease.[u]

Sweden banned all AGPs in the 1980s and subsequently, vancomycin resistant *enterococci* (VRE) was not detected in chickens or pigs through the mid-1990s. However, a study from 1998-2000 confirmed the first case of VRE in livestock and by 2005, 40% of commercial broilers (chickens) studied in a national monitoring program were colonized. The "Swedish Paradox"[107] indicates that eliminating or decreasing antibiotic use in intensive animal production does not necessarily lead to a direct correlation in the elimination or decrease of resistant pathogens such as LA-MRSA. The persistence, spread and increased occurrence of one clone of VRE, apparently without selective pressure within the Swedish broiler production shows how complex and unpredictable the epidemiology of antimicrobial resistance can be once it arises. No matter when and where antimicrobials are used injudiciously, this dangerous practice can have implications on remote resistance both geographically and temporally.[108]

A 2012 publication from McGill University shows a high prevalence of resistant bacteria in the swine complex years after antibiotics in feed had been discontinued.[109] The evidence demonstrates that while a reduction of

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[p] Human intervention studies conducted in various countries during the past decade to measure the effect of removing certain antibiotics for the intended result of reducing resistance have had disappointingly small effects, due in large part to the power of co-selection (Andersson DI and Hughes D. 2011. Persistence of antibiotic resistance in bacterial populations. Federation of European Microbiological Societies 35:901-911).

[q] Fluoroquinolones are considered critically important for human medicine (Collingnon P, Powers JH, Chiller TM, Aidara-Kane A, and Aarestrup FM. 2009. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies for the Use of Antimicrobials in Food Production Animals. Clinical Infectious Diseases 49:132-141).

[r] Researchers describe the persistence of resistant *Campylobacter* on an antimicrobial-free farm (Quintana-Hayashi MP and Thakur S. 2012. Phylogenetic Analysis Reveals Common Antimicrobial Resistant Campylobacter coli Population in Antimicrobial-Free (ABF) and Commercial Swine Systems. PLoS One 7(9):1-6).

[s] Interestingly, most of the farms sampled in this study reported using commercial feed. The researchers of this study discovered that this particular brand of feed used oxytetracycline for its piglet feed. However, the researchers reject any direct antibiotic-resistance link, instead indicating that their study more appropriately supports findings where MRSA-colonized pigs are found on antibiotic-free farms.

[t] A 2012 literature review by Faccioli, et al indicates that livestock are a reservoir for CA-MRSA and HA-MRSA as well as LA-MRSA. Faccioli-Martins PY and de Souza da Cunha LR. 2012. Epidemiology Insights, Dr. Maria De Lourdes Ribeiro De Souza Da Cunha (Ed.), ISBN: 978-953-51-0565-7, InTech, Available from: http://www.intechopen.com/books/epidemiologyinsights/mrsa-epidemiology-in-animals**.**.

[u] However, a recent study of 25 "alternative" pig farms in Germany, i.e. those with far fewer number of pigs (<600 pigs) than conventional farms of 3,000 pigs or more found no MRSA CC398 colonization of pigs or farmers. Alt, *et al* find that the number of pigs on the farm is a major indication for MRSA colonization in the pigs. Alt K, Fetsch A, Schroeter A, et al. 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BioMedCentral Veterinary Research 7(69):1-8.

antimicrobial use on pig farms is probably necessary for the eradication of LA-MRSA on those farms, it is likely insufficient.[110]

Transmission of LA-MRSA

Roughly 75% of the new infectious diseases in humans over the past 10 years originated from animals. They are zoonotic, meaning they can transmit (colonize and infect) directly or indirectly from animals to humans.[111] Colonization of a person or animal occurs when the pathogen is present, but does not cause infection. These people or animals are referred to as "carriers" and may be at increased risk of getting sick and spreading the pathogen because of their carrier status.[112] LA-MRSA ST398 is one such zoonotic pathogen[113] and generally does not cause clinical illness in pigs.[114,115]

Animal-to-Human Transmission

ST398 LA-MRSA colonization in humans has emerged in step with the increase in livestock production[116] and it is generally understood as an occupational hazard.[117] Most published studies of the transmission of LA-MRSA have been performed in Europe. Studies have shown that the proportion of colonization in humans correlates with livestock density in Europe,[118,119] with low prevalence of infection for humans outside farming communities.[120,121] The current risk to human health from animal-to-human transmission of the LA-MRSA depends on the intensity of the contact.[122,123] Higher risk populations include those who regularly work with livestock, including veterinarians, livestock production workers,[v] and slaughterhouse workers, but may also present a concern for those with limited contact with livestock, such as truck drivers[124] and those making regular on-farm visits.[125,126,w] Historically, studies seem to suggest that colonization is transient, and diminishes when the intensity of animal contact diminishes.[127,128,129,130,131]

Contact with raw meat is also a potential vehicle for transmission. Meat is can be contaminated with MRSA and MSSA pathogens[132,133,134] A 2012 study in The Netherlands confirms MRSA presence in 11.9% of a range of raw meat sold for human consumption, the majority of which was associated with ST398.[135] Another 2012 study reported widespread pathogenic contamination of retail meat products in the United States, with 26.9% of the MRSA contaminated meat LA-MRSA.[136] Though no difference was found between meat from conventionally versus alternatively raised animals,[x] a 2013 study in North Carolina only found LA-MRSA in individuals exposed to industrial operations as opposed to those working on farms raising pigs outdoors on pasture, raising concerns about both antibiotic use and confinement.[137] Though singing pig carcasses may reduce surface contagion, fecal recontamination can occur at evisceration.[138] The association between poultry consumption and MRSA carriage may be explained by the abundant presence of MRSA in meat,[139] but overall, foodborne MRSA is currently not believed to be an important source of human infection.[140,141,142,143,144]

[v] IFAP operations potentially create additional biosecurity hazards for their own workers and animals in that the shower facilities which employees use before and after their work may be contaminated with resistant bacteria and serve as a fomite for transmission (Harper AL, Ferguson DD, Larson KR, et al. 2010. An Overview of Livestock-Associated MRSA in Agriculture. Journal of Agromedicine 15(2):101-104).

[w] Rule *et al* describe resistant bacteria contamination risks to the community at large from the open transport of livestock fed AGPs (Rule AM, Evans SL, and Silbergeld EK. 2008. Food animal transport: A potential source of community exposures to health hazards from industrial farming (CAFOs). Journal of Infection and Public Health 1:33-39).

[x] One reason the researchers give as to why there was no difference between meat labeled as "raised without antibiotics" and conventionally raised meat is that the products carrying these claims on their labels are typically not verified by an independent third-party, unlike products labeled "organic."

Human-to-Human Transmission

Overall, the current risk of serious infection from human-to-human transmission of the resistant pathogen appears to be low. Colonization via farm workers and veterinarians to their household members happens in a small but consistent percentage of transmission.[145] A 2012 study found that of the MRSA positive chicken farms identified, a high percentage of farm residences were also MRSA positive with identical spa types, indicating that humans play an important role in the transmission of the pathogen.[146] Until very recently, though, it has been generally held that LA-MRSA does not spread into the community.[147,148]

The conventional understanding of how the main lineage of LA-MRSA, ST398, spreads seems to be changing rapidly, however.[149] There are documented incidences of human-to-human transmission, including recently reported ST398 LA-MRSA outbreaks in vulnerable populations in geographic areas of intense pig farming.[150,151] While it has been generally held that colonization of farm workers is largely transient[152,153] new evidence suggests that colonization of ST398 in humans has the potential to be far more persistent.[154,155]

A 2012 study indicates that LA-MRSA is likely to spread in areas with a high density of livestock production among those without direct livestock contact.[156,y] This suggests the pathogen may be transmitted via air, water, or land contamination, or human-to-human. Notwithstanding a ban on AGPs in 2006, indices of LA-MRSA as a percentage of MRSA detected in people in some European countries have subsequently increased substantially.[z] In The Netherlands for instance, LA MRSA rose from first detection in 2003 to more than 40% of MRSA identified in that country by 2010.[157,aa] LA-MRSA was responsible for about 12.5% of all MRSA cases in Denmark in 2011, a significant increase in that country from 2010.[158,159]

Methicillin Sensitive *Staphylococcus aureus*

There is a broad consensus among scientists, watch-dog groups, and governments that LA-MRSA poses a significant risk for public health due to the vast reservoirs of antibiotic resistance genes and pathogens produced by IFAP operations. Presently, LA-MRSA ST398 appears to be a relatively poor colonizer of humans. Dutch researchers concluded in a 2011 study that LA-MRSA ST398 is 5.9 times less transmissible than other MRSA in hospitals.[160] Until very recently, ST398 was considered a small percentage of total MRSA prevalence in Europe, with a few important exceptions in dense pig producing areas.[161] Even in areas of high human colonization with ST398, the percentage of human infection appears to be relatively low[162] (although overall prevalence of LA-MRSA may be increasing).[163,bb] The greatest concern involves the potential for genetic exchange between LA-MRSA ST398 and other bacteria, combining resistance and virulence in an easily transmissible human

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[y] Researchers in 2011 concluded that for the MRSA of Unknown Origin that is genotypically defined as CC398, (MUO CC398) investigation into how it spreads is essential "despite the current dogma" of it not spreading outside of high risk populations. It is estimated that at least a quarter of the total Dutch MRSA is MUO, i.e. not from defined risk groups (Lekkerkerk W, van de Sande-Bruinsma N, van der Sande M et al. 2011. Clinical Microbiology and Infection 18:656-661).

[z] For instance, in a prospective longitudinal study, LA-MRSA was found to have increased significantly in Germany from 2004 to 2011 (Schaumburg F, Kock R, Mellman A, et al. 2012. Population dynamics among methicillin resistant *Staphylococcus aureus* in Germany during a 6-year period. Journal of Clinical Microbiology 1-31).

[aa] What portion of these cases represent colonization versus infection is unclear (email correspondence with the author, Beth Feingold 1-12-13). A 2011 study indicates that despite the large increase of ST398 as a proportion of MRSA in *colonized* hospital patients in pig-dense areas in Germany, human ST398 MRSA *infections* are relatively low (Kock R, Siam K, Al-Malat S, et al. 2011. Characteristics of hospital patients colonized with livestock-associated methicillin resistant *Staphylococcus aureus* (MRSA) CC398 versus other MRSA clones. Journal of Hospital Infection 79(4):292-296).

[bb] Increases can to some extent be explained by changes in screening protocol (Wulf M, Verduin CM, van Nes A, Huijsdens X, and Voss A. 2012. Infection and colonization with methicillin resistant *Staphylococcus aureus* ST398 versus other MRSA in an area with a high density of pig farms. European Journal of Clinical Microbiology and Infectious Diseases 31(1):61-5).

pathogen.[164] Several novel resistance genes have already been discovered in ST398 MRSA[165]and "sooner or later" they are likely to transfer to human strains.[166,cc]
The main strain of LA-MRSA, ST398, is increasingly reported globally in Methicillin Sensitive[dd] *Staphylococcus aureus* (MSSA) infection, causing serious illness and death in people who have had no animal contact[167,168,169,170] suggesting it has pandemic potential.[171] How these people acquired ST398 infections remains unclear.

A 2012 study finds ST398 to be a frequent source of MSSA infections in New York City with high transmissibility in households of infected persons, who have had no known animal contact. The researchers conclude that ST398 MSSA efficiently spreads among people independent of animal contact and is well adapted to humans. In gene analysis, the team found little variation in MGEs among their diverse collection of ST398 MSSA human isolates, suggesting recent clonal expansion and dissemination of a human ST398 lineage. Further, in comparing LA-MRSA and MSSA isolates, the scientists found that while most of the core genome was conserved between the isolates, the strains differed substantially in their adhesion abilities. The researchers contend that this accounts for the MSSA's efficient spread among people and that this strain could acquire resistance genes.[172]

In a 2011 study of patients with MSSA ST398 blood stream infections, the researchers suggest that a human-adapted ST398 strain recently evolved from a pig strain by virtue of a particular phage and is able to accept virulence genes giving it the ability to colonize humans. "This would explain the sudden ability of the emerging ST398 strain to infect humans in the absence of livestock or meat." And although the researchers conclude further research is necessary to understand how the human and animal clones emerged and spread, they say ST398 appears to be shifting toward human hosts.[173]

MSSA ST398 infections are increasingly documented among more among vulnerable populations. There are infections in hospitals in China[174] with a 2012 study indicating a high prevalence of the virulence gene PVL in ST398 isolates.[175] The first report of ST398 MSSA in a hospital patient in Greece was published in 2012.[176] The first incidence of an ST398 MSSA infection in Columbia was recently reported.[177] These MSSA ST398 infections have also been identified in among people with no known livestock contact in the Caribbean islands, and the Amazonian forest of French Guiana.[178]

Among otherwise healthy people, case reports describe MSSA ST398 necrotizing pneumonia and invasive bloodstream infections[179] and deaths.[180] However, the MSSA ST398 being found amongst those without livestock contact appears to be of different spa types, specifically t571, than those lineages directly related to contact with livestock. Spa type t571 is uncommon in animal isolates.[181]([ee])

[cc] The issue of novel LA-MRSA lineages is also important. Researchers of a 2012 study discuss the finding of a novel MRSA-ST9 lineage established in the pig population in Thailand, which differs substantially from LA-MRSA lineages found in other areas of the continent. "The emergence of novel LA-MRSA lineages in the animal agriculture setting is worrisome and poses a serious threat to global public health." (Larsen J, Imanishi M, Hinjoy S, et al. 2012. Methicillin-Resistant *Staphylococcus aureus* ST9 in Pigs in Thailand. PLoS ONE7(2):1-3).

[dd] Sensitive strains lack key resistance components which would otherwise classify them as MRSA.

[ee] Although, increasingly, more spa-types are being identified as associated with LA-MRSA **(**van Cleef B, Monnet DL, Voss A, et al. 2011. Livestock associated Methicillin-Resistant *Staphylococcus aureus* in Humans, Europe. Emerging Infectious Diseases 17(3):502-505). For example, a 2012 study documents first time LA-MRSA spa-types found in the U.S. (Osadebe LU, Hanson B, Smith TC, and Heimer R. 2012. Prevalence and Characteristics of *Staphylococcus aureus* in Connecticut Swine and Swine Farmers. Zooneses and Public Health doi: 10.1111/j.1863-2378.2012.01527.x).

Whether these MSSA infections are a direct result of LA-MRSA, or whether they have originated from an ancestral strain[182,183,184,185,186,ff] is unknown. There may be two lineages of ST398: "ancestral" which are human and mostly MSSA (tetracycline-sensitive) and the more recently "livestock-adapted" which are mostly MRSA (tetracycline-resistant). Both appear to be circulating in human populations.[187,188] Some contend that recent research implies that the low-levels of ST398 circulating in human populations are indicative of human ancestral ST398 and do not represent an emergent human clone which originated in livestock. Rather, the "emergent theme" is one of humans as important source of new bacterial strains which cause disease in livestock, and accordingly represent a potential threat to food security.[189]

Staphylococcus aureus is a constantly evolving, dynamic human pathogen.[gg] Some view it not a question of "if" LA-MRSA will acquire novel transmission and virulence mechanisms, but rather "when."[190,hh] The public health community uniformly recognizes that close monitoring of this rapidly evolving zoonotic pathogen is necessary to protect public health.[191,192,193,194,195,196,197,198,199]

Banning Antibiotics for Growth Promotion

While there has been mounting global pressure to ban nontherapeutic uses of antibiotic important to human medicine in animal agriculture,[200,201,202,203,ii] only countries within the European Union have imposed restrictions on the use of growth-promotion antibiotics in livestock production as a means to address antibiotic resistance.[204]

Denmark showed that antibiotic use can be dramatically reduced in major livestock industry.[205] The Danish pork industry, which holds itself as a world leader in this regard, reduced total antimicrobial use in food animals per kilogram of pig produced from 1992 to 2008 by about 50% without serious consequences to industry.[206] Notwithstanding a countrywide ban on AGPs in 2000, the total antimicrobial use in Denmark actually increased 47% from 2002 to 2009 (31 mg/kg meat to 49 mg/kg meat). Following the introduction of a "yellow card" monitoring system in 2010, total use then fell significantly and in 2011 antimicrobial use returned to 2001 levels.[207] Thus, the 2011 data can be seen as a reversal or break in the upward trend of consumption of antimicrobial agents for pigs.[208] The data underscore the admonition that having a legal ban on antimicrobial use is meaningless absent an effective monitor for compliance.[209]

[ff] Potential scenarios associated with transmissibility include a double-host adapted organism: "Adaptation to livestock hosts resulted in deadaptation to the human host. This explains why the livestock-associated ST398 strains were so infrequently transmitted from livestock to humans and from human to human....Nothing excludes the possibility that MRSA-CC398 clones adapted to livestock, and recently sending back migrants to farmers could increase their fitness in the human population without losing their adaptations to the livestock hosts....This is favored by the strong reduction in diversity of farm animals…." (Baquero F. 2012. On the Shifting Balance: the Case of *Staphylococcus aureus* CC398. mBio 3(2):1-2).

[gg] (MRSA) Bacteria are said to have "interminable adaptive qualities" which have enabled them to achieve "superbug" status (Aminov R. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in Microbiology 1(134):1-7). Schmidt declares that S. aureus is "one of the most adaptable and virulent pathogens in modern times." (Schmidt T, Zundorf J, Gruger T, et al. 2012. Phenotyping of Staphylococcus aureus reveals a new virulent ST398 Lineage. Clinical Microbiology and Infection: 1-7).

[hh] McCarthy *et al* state that genetic pressures are restraining plasmid-driven virulence among S. *aureus*, delaying fully virulent and resistant strains (McCarthy AJ and Lindsay JA. 2012. The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. BioMed Central 12(104):1-8). For more discussion of the Restriction Modification systems which are thought to play a role in pathogenicity and novel strains, see Bloemendaal ALA, Brouwer EC, and Fluit AC. 2010. Methicillin resistance transfer from *Staphylococcus epidermidis* to Methicillin-Susceptible *Staphylococcus aureus* in a patient during antibiotic therapy. PLoS ONE 5(7):1-5.

[ii] Formal recommendations to reduce or eliminate AGPs in the US are decades old and include: IOM, 1980, 1989; Council for Ag Science and Tech Report 1981, Committee on Drug Use in Food Animals Report 1998. Dibner JJ and Richards JD. 2005. Antibiotic Growth Promoters in Agriculture: History and Mode of Action. Poultry Science 84:634-643.

Even if antibiotic use for growth promotion is banned, the crowding, stress, and unhygienic nature of IFAP may necessitate significant amounts of antibiotics be used for therapeutic purposes.[210,211,212,213] Denmark, for all of its leadership in protecting the efficacy of antibiotics, saw the use of third-generation cephalosporins, a class of antibiotics critically important to human medicine, increase to 0.8% of all pig antimicrobial consumption in 2008. While this total amount is low, it is an increase of 200% from 2002 to 2008. Further, this 2012 study estimates that between 15 and 30% of all piglets produced in 2007 were treated with this critically important drug, despite the relatively small amount used. This implies broad exposure.[214]

LA-MRSA remains widespread in many European pig herds, despite the ban on AGPs that went into effect for the EU in 2006. In the Netherlands, for instance, overall total antibiotic use has remained stable despite a decrease in the total number of livestock animals. This is due to an increase in the average size of farms and to increased therapeutic use of antibiotics resulting from a ban on AGPs.[215] LA-MRSA is endemic in the Dutch pig industry,[216] and the reservoir of ST398 remains pervasive among livestock.[jj] Danmap 2011 indicates a dramatic increase in MRSA prevalence at slaughter in Danish pigs between 2010 and 2011,[217] showing that resistant microbes are still prevalent in Danish and EU meat products[218] years after the ban on AGPs.

In 2012 the U.S. Food and Drug Administration (FDA) announced that it considers the growth promotion use of clinically important antibiotics "injudicious" use and that therapeutic use should require veterinary oversight.[219] Even if this were more than just recommendation, it is not clear that those directly involved in IFAP are able to discern the role of growth promoting drugs versus therapeutic drugs.[kk] Industry experts have called the growth promotion/therapeutic distinction a "mythical" one.[220] FDA Draft Guidance #213 for animal pharmaceutical companies is aimed at getting the drug companies to change their labeling claims from "growth-promotion" claims to "disease prevention" claims.[221] The value of changing labels appears more political than practical.

Conclusion

Ultimately, the human impact of antibiotic resistance from IFAP may be far more significant than the burden of in-hospital transmission given the extent of agricultural contamination of the environment with resistance genes. A large population with low exposure to resistant bacteria may result in a great public health threat than a small number of hospital patients with a high risk of transmission.[222]

Whatever its epidemiology, LA-MRSA is able to acquire and stably maintain resistance genes from other bacteria and can accept foreign genetic material including virulence genes, rendering it a threat to human health.[223,ll] Medical microbiologist A.C. Fluit wrote in *Clinical Microbiology and Infection*:

[jj] There are many examples which disprove the idea that once a selective pressure (such as an antibiotic) is removed, the targeted resistance genes will cease from a bacterial population. This idea is a holdover of vertical inheritance of single resistance mutation studies done in highly controlled laboratory settings (Summers AO. 2006. Genetic Linkage and Horizontal Gene Transfer, the Roots of the Antibiotic Multi-Resistance Problem. Animal Biotechnology 17(2):125-135). In contrast, the messy real-world realities include the amelioration of fitness costs, integration of resistance genes into a genotype, co-selection by other factors such as heavy metals, and novel re-combinations of resistance genes via ubiquitous commensals. (Aminov RI. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in Microbiology 1(134):1-7).

[kk] Sometimes, medicated feed is broadly labeled to be used for growth promotion as well as to treat animal with active disease, or simply disease prevention. When this feed is given to the animals "free choice," there is no way to know how much of the drugs any particular animal, well or sick, actually consumes (Love DC, Davis MF, Bassett A, Gunther A, and Nachman KE. 2011. Dose Imprecision and Resistance: Free-Choice Medicated Feeds in Industrial Food Animal Production in the United States. Environmental Health Perspectives 119(3):279-283).

[ll] LA-MRSA CC398 has a wide variety of resistance genes (Tulinski P, Fluit AC, Wagenaar JA, Mevius D, van de Vijver L, and Duim B. 2012. Methicillin-resistant coagulase-negative Staphylococci on pig farms as a reservoir of heterogeneous

> The most important danger is when host adapted strains acquire virulence factors that enable them to colonize and infect new hosts. The biggest threat in this respect is formed by further adaptation of ST398 to humans because of its pandemic nature and the huge reservoir of livestock animals.[224]

While a reduction of antibiotic use is probably necessary for the eradication of LA-MRSA in animal production, it is likely insufficient. The critical question is whether the AGP bans in some countries are enough to eliminate or reduce the global reservoirs of resistant pathogens and resistance genes. This may require more fundamental shifts in the way animals are raised to decrease disease susceptibility, so as to lower the use of antibiotics for all purposes in animal agriculture.[mm]

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