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Methicillin-resistant staphylococci: implications for our food supply?

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Abstract

Food-borne intoxication, caused by heat-stable enterotoxins produced by *Staphylococcus aureus*, causes over 240,000 cases of food-borne illness in the United States annually. Other staphylococci commonly associated with animals may also produce these enterotoxins. Foods may be contaminated by infected food handlers during slaughter and processing of livestock or by cross-contamination during food preparation. *S. aureus* also causes a variety of mild to severe skin and soft tissue infections in humans and other animals. Antibiotic resistance is common in staphylococci. Hospital-associated (HA) *S. aureus* are resistant to numerous antibiotics, with methicillin-resistant *S. aureus* (MRSA) presenting significant challenges in health care facilities for over 40 years. During the mid-1990s new human MRSA strains developed outside of hospitals and were termed community-associated (CA). A few years later, MRSA was isolated from horses and methicillin resistance was detected in *Staphylococcus intermedius/pseudintermedius* from dogs and cats. In 2003, a livestock-associated (LA) MRSA strain was first detected in swine. These methicillin-resistant staphylococci pose additional food safety and occupational health concerns. MRSA has been detected in a small percentage of retail meat and raw milk samples indicating a potential risk for food-borne transmission of MRSA. Persons working with animals or handling meat products may be at increased risk for antibiotic-resistant infections. This review discusses the scope of the problem of methicillin-resistant staphylococci and some strategies for control of these bacteria and prevention of illness.

Keywords: MRSA, MRSIG, methicillin-resistance, foodborne, epidemiology, swine, cattle, poultry, cattle, horses, dogs, cats

Staphylococci and foodborne illness

Staphylococcus aureus is a food-borne pathogen that produces heat-stable enterotoxins during growth in a variety of foods including meat and poultry products, eggs, cream-filled pastries, potatoes, and some salads. Staphylococcal enterotoxins are estimated to cause food-borne illness in about 241,000 persons in the U.S. annually. Staphylococcal food poisoning is believed to be greatly underreported and underdiagnosed because of the short duration of illness and infrequent complications (Scallan *et al.*, 2011). Data from Centers for Disease Control and Prevention (CDC) for 1998 to 2010 indicate that the annual number of reported outbreaks peaked at 82 in 2002 and declined to 11 in 2010. Nearly half of the 573 reported outbreaks occurring in 1998–2010 were associated with some type of meat (Table 1). Seafood, potatoes/rice/noodles, vegetables/salads, combination foods, and dairy products were also cited as food vehicles. Approximately 51% of reported outbreaks involved only 2–4 cases while only 7.3% of outbreaks involved more than 50 cases. Table 2 lists some large outbreaks occurring during this period in the U.S. and other countries.

Data from Alberta, Canada indicate that 10.5% of food samples collected from food-borne disease outbreaks in

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Table 1. Reported food vehicles for 573 outbreaks of staphylococcal food poisoning reported by CDC for 1998–2010 (http://wwwn.cdc.gov/foodborneoutbreaks/)

Food vehicle	No. of outbreaks (%)*
Meat (total)	276 (48.2)
Beef	54 (9.4)
Chicken	87 (15.2)
Ham	39 (6.8)
Pork	48 (8.4)
Turkey	19 (3.3)
Meat, cured, except ham	11 (1.9)
Meat, other (alligator, rabbit, deli meat, unspecified)	19 (3.3)
Seafood	37 (6.5)
Vegetables/salad	36 (6.3)
Potatoes	30 (5.2)
Rice/noodles	28 (4.9)
Dairy Products	12 (2.1)
Sauces/dressings	7 (1.2)
Eggs	8 (1.4)
Combination foods	42 (7.3)
Multiple Foods	13 (2.3)
Unknown	104 (18.1)

*Total of outbreak numbers is >573 (and total of percentages is >100) because more than one food was implicated in some outbreaks.

2007–2010 contained *S. aureus*. Of the positive samples, about 65% were meat or prepared foods containing meat, 15% were prepared foods not containing meat, about 14% were dairy products and 4% were produce items (Crago *et al.*, 2012).

S. aureus has been a food safety concern for meat producers and food processors for decades because it is widespread in the environment and often detected in air, dust, water, raw milk, other foods, and on environmental surfaces. Three recent surveys of retail meat in the U.S detected *S. aureus* in a significant number of samples of pork, chicken, turkey, and beef, ranging from 12% to 65% of packages. Cell numbers per gram of meat were low, but can increase rapidly if meats are not kept refrigerated (Bhargava *et al.*, 2011; Kelman *et al.*, 2011; O'Brien *et al.*, 2012). *S. aureus* cells survive desiccation and tolerate high levels of salt. Although these bacteria are destroyed by heat, enterotoxins produced in a food will survive many preservation processes, including pasteurization and approved doses of irradiation (Rose *et al.*, 1988; Genigeorgis, 1989).

Numerous staphylococcal enterotoxins have been described and it is ingestion of these enterotoxins and not of *S. aureus* cells that causes onset of nausea and vomiting within 1–6 h. Less than 200 ng of toxin is sufficient to cause symptoms (Evenson *et al.*, 1988). Although symptoms may be severe, they usually resolve within a day and serious complications, hospitalization, and death are rare, afflicting primarily the very young, the elderly, the chronically ill, and those who have consumed a large amount of contaminated food.

S. aureus has also been a problem for caterers and others involved in food preparation. According to several studies, *S. aureus* is present in nasal passages or skin of about 50% of people and in intestines of about 20% of people in the general population (Acton *et al.*, 2009; Frank *et al.*, 2010). Thus, asymptomatic food handlers may harbor *S. aureus* and can contaminate food during preparation (Todd *et al.*, 2008). If contaminated foods, for example salads or some desserts at a picnic, are left at ambient temperature for extended periods, *S. aureus* may multiply and produce enterotoxins.

In some circumstances, ingestion of staphylococcal cells causes enteritis. Occasionally infants, immunocompromised adults and others treated with large doses of antibiotics develop staphylococcal enterocolitis. When the normal human intestinal microbiota is depleted or absent, ingested *S. aureus* cells may grow in the intestines and produce enterotoxins that cause profuse diarrhea (Lin *et al.*, 2010).

Although *S. aureus* is the most common cause of food-borne staphylococcal intoxication, other species of staphylococci carry genes for enterotoxin production and can potentially cause food-borne illness (Becker *et al.*, 2001). One food-borne outbreak in southwestern U.S. in 1991 affecting over 265 people was traced to *Staphylococcus intermedius* producing type A enterotoxin in a butter blend (Khambaty *et al.*, 1994).

Non-food-borne illness

Staphylococcus aureus

S. aureus causes a variety of mild to severe skin and soft tissue infections and numerous serious infections including endocarditis, endophthalmitis, osteomyelitis, meningitis, bacteremia, pneumonia, and toxic shock syndrome in humans. These bacteria produce many virulence factors (besides enterotoxins) such as exfoliative toxins, toxic shock syndrome toxin, and immunomodulators. The Panton–Valentine leukocidin (PVL) is a toxin often associated with abscess formation and severe necrotizing pneumonia (Watkins *et al.*, 2012). Asymptomatic carriage of *S. aureus* by healthy individuals is a risk factor for developing serious staphylococcal infections (Safdar and Bradley, 2008).

S. aureus has been isolated from many domestic and wild mammals and from wild and domestic birds and some reptiles (Weese and Van Duijkeren, 2010; Ho *et al.*, 2012; Wardyn *et al.*, 2012). Animals may be asymptomatic carriers or may suffer respiratory, gastrointestinal, or skin and soft tissue infections. *S. aureus* is a significant cause of mastitis in cows and small ruminants (Vanderhaeghen *et al.*, 2010b; Foster, 2012). Whether animals can be persistent carriers of *S. aureus* has yet to be determined. However, healthy animals can intermittently carry *S. aureus*. A recent study found that 10% of dogs visiting

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Table 2. Large outbreaks of staphylococcal food intoxication (1998–2008)

No. of cases	Year	Location	Food vehicle*
>13,000	2000	Community (Japan)	Milk, low fat (Asao *et al*., 2003)
~4000	1998	Ordination dinner (Brazil)	Multiple foods, food handlers (Do Carmo *et al*., 2004)
>600	2005	Military base (Greece)	Cheese, grated (Jelastopulu *et al*., 2006)
400	2007	Community (Paraguay)	Milk, ultrapasteurized (Weiler, 2011)
272	2002	Celebration (Australia)	Lamb, potatoes, rice (OzFoodNet Working Group, 2003)
225	1998	Multiple locations (TX)	Ham salad
218	1998	Multiple locations (TX)	Turkey salad
180	1998	Brazil	Salad, chicken, food handlers (Colombari *et al*,. 2007)
166	2007	Schools (Austria)	Milk, pasteurized (Schmid *et al*., 2009)
147	2006	Festival (Argentina)	Cake (López *et al*., 2008)
145	1998	Restaurant, home (HI)	Bento sandwiches
142	2008	Restaurant (KY)	Gravy
138	2005	Workplace (KS)	Sausage
132	2004	Restaurant, Home (OH)	Ice cream
125	2000	Fair (GA)	Pork BBQ
113	2006	Boarding school (Austria)	Rice, boiled; food handler (Schmid *et al*., 2007)
112	2001	Picnic, Fair (OH)	Pork, roasted; ham
101	1998	Prison (IN)	Macaroni salad
100	1999	School (GA)	Pork, BBQ
100	2000	School (TN)	Turkey, stuffing
100	2006	Wedding reception (VA)	Chicken BBQ; ham; potato salad
100	2010	Multiple locations (IL, WI)	Pastries (U.S. Food and Drug Administration, 2010)

U.S. outbreak information from CDC: http://wwwn.cdc.gov/foodborneoutbreaks/

a clinic for regular vaccinations harbored *S. aureus* (Rubin and Chirino-Trejo, 2010). Molecular analyses of isolates from different animals have revealed that there are some strains that appear to be host-adapted to a particular animal species and other strains can colonize many species of animals (Cuny *et al*., 2010). *S. aureus* can be transferred in both directions between humans and animals and frequently infections in companion animals can be traced back to their human caretakers (Rutland *et al*., 2009). Farmers also often harbor some *S. aureus* strains that are similar to those detected in their livestock (Khanna *et al*., 2008).

Other pathogenic staphylococci

Coagulase-positive staphylococci, other than *S. aureus*, also cause infections in humans and animals. Some animal isolates of coagulase-positive staphylococci are classified in the *S. intermedius* group (SIG). *S. intermedius* was originally described in 1976 and is part of the normal microflora of the skin and mucosal membranes of dogs and cats. It has also been detected in a variety of other animals, including horses, mink, goats, foxes, raccoons, and pigeons, but is not commonly present in humans. Recent molecular analyses demonstrated that isolates of *S. intermedius* detected in a large number of various animals living in several geographic locations have some significant differences and the species can best be reclassified into three clusters: *S. intermedius, Staphylococcus pseudintermedius*, and *Staphylococcus delphini* A and B. These three species constitute the SIG (Sasaki *et al*., 2007; Sledge *et al*., 2010). Extensive differences were observed in transposons and other accessory genetic elements among different SIG species that may reflect adaptations to different hosts (Ben Zakour *et al*., 2012).

S. pseudintermedius is the most frequently encountered pathogen in the SIG and its taxonomy, epidemiology and pathogenicity were recently reviewed (Bannoehr and Guardabassi, 2012). This species was first identified as distinct from other SIG in 2005 by examination of rRNA gene sequences in clinical staphylococcal isolates from several animals (Devriese *et al*., 2005). The majority of isolates from dogs are now classified as *S. pseudintermedius* and some surveys report that *S. pseudintermedius* is present in the nares, pharynx and/or rectum of more than 80% of healthy dogs (Rubin and Chirino-Trejo, 2011). *S. delphini* was originally isolated from a dolphin and has been detected in horses. All SIG isolates from 118 mink, 6 badgers, and 1 ferret were recently identified as *S. delphini*, whereas all except one SIG isolate from 33 foxes were *S. pseudintermedius* (Guardabassi *et al*., 2012).

SIG pathogens produce a number of virulence factors (coagulase, hemolysins and exfoliative toxin) similar to those associated with *S. aureus* (Fitzgerald, 2009). When animals are injured, sick, or otherwise weakened, these bacteria may cause skin, ear, and wound infections (Weese and Van Duijkeren, 2010). *S. pseudintermedius* has been isolated from pet owners and veterinarians (Morris *et al*., 2010) and occasionally causes soft tissue or respiratory infections in humans exposed to dogs carrying these bacteria (Chuang *et al*., 2010). Invasive infections have occurred in a few persons, including some who were bitten by dogs (Hatch *et al*., 2012).

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Methicillin resistance in staphylococci

Emergence and spread of methicillin resistance

Staphylococci are notorious for rapidly becoming resistant to many antibiotics. Penicillins and other β-lactam antibiotics kill bacterial cells by interfering with cell wall synthesis. Not long after penicillin was first used to treat human infections, *S. aureus* strains producing penicillinase were detected and it is estimated that now >80% of *S. aureus* cells produce penicillinase. Methicillin (meticillin), a β-lactam antibiotic that is not inactivated by penicillinase, was introduced in the late 1950s. But by 1961, there were reports of methicillin-resistant staphylococci in a hospital in the United Kingdom (Jevons, 1961). Methicillin-resistant *S. aureus* (MRSA) are now resistant to all currently available β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and their derivatives. MRSA have spread worldwide and are currently the most commonly identified antibiotic resistant bacteria in hospitals in Europe, the Americas, North Africa, and the Middle- and Far-East (European Centre for Disease Prevention and Control, 2011). Approximately 478,000 hospitalizations in the U.S. in 2005 were associated with *S. aureus* infections and 58% of those were caused by MRSA (Klein *et al.*, 2007). In 2010, it was estimated that MRSA caused illness in more than 150,000 persons annually in health care facilities in the European Union (Köck *et al.*, 2010). Recent statistics indicate that severe, invasive, health-care associated MRSA infections have declined, but MRSA infections acquired in the community are still increasing (Kallen *et al.*, 2010; Hadler *et al.*, 2012).

Methicillin resistance in canine *S. intermedius* isolates was first reported in the mid-late 1990s (Gortel *et al.*, 1999). Early isolates of methicillin-resistant *S. intermedius* from companion animals were probably strains of methicillin-resistant *S. pseudintermedius* (MRSP) based on the recent changes to SIG taxonomy (Sasaki *et al.*, 2007). For several years, these strains appeared to constitute a small proportion of *S. intermedius* isolates from animals and, although they exhibited some resistance to other drugs, there were other antibiotics effective against them (Van Duijkeren *et al.*, 2011a). Starting in 2006, there were more frequent reports of MRSP and methicillin-resistant SIG (MRSIG) strains that were resistant to multiple classes of antibiotics, in addition to the β-lactam group (Loeffler *et al.*, 2007; Van Duijkeren *et al.*, 2011a; Proietti *et al.*, 2012). Colonization of dogs with MRSP may persist after symptoms of pyoderma have resolved (Beck *et al.*, 2012).

Methicillin resistance has also been detected in other staphylococcal species, including several species isolated from pigs in Belgium (Vanderhaeghen *et al.*, 2012), *Staphylococcus lentus* in chickens (Koller *et al.*, 2011), and several coagulase negative species isolated from livestock, chickens, raw milk and meat in Switzerland (Huber *et al.*, 2011).

Genetic basis of resistance

Resistance to methicillin is mediated by the *mec*A gene that encodes an altered penicillin-binding protein that is located in the cell wall and that has a low affinity for β-lactam antibiotics. Since β-lactam antibiotics interfere with bacterial cell wall synthesis, this decreased antibiotic binding causes cells to be resistant to the effects of methicillin. The *mec*A gene resides on a large heterogeneous mobile genetic element called the staphylococcal cassette chromosome (SCC*mec*) (Ito *et al.*, 1999; Katayama *et al.*, 2000).

To date, 11 major SCC*mec* variations have been described but types I–V are the most common. SCC*mec* types I–III are relatively large and are typically found in strains associated with healthcare facilities. SCC*mec* types IV and V are smaller and are usually found in MRSA associated with community-acquired infections. Molecular analyses of numerous MRSA strains indicate that resistance genes have been transferred to various methicillin-susceptible *S. aureus* (MSSA) strains on multiple occasions (Robinson and Enright, 2004). These resistance genes have also been transferred to other staphylococcal species. Many MRSA strains, particularly hospital-associated (HA) strains, are also resistant to other classes of antibiotics, which makes it a challenge to treat serious infections. MRSA continues to evolve and present new challenges for treatment and identification (Sabat *et al.*, 2012).

Genes coding for resistance to antibiotics may be present on mobile genetic elements (such as transposons), located on bacterial chromosomes, or on extrachromosomal plasmid DNA. In some species, plasmid gene transfer can occur directly from cell to cell by conjugation. In other cases, genes on mobile elements can be transferred by transduction to other bacterial cells of the same or related species with the aid of viruses. Efficient transduction of resistance genes has been demonstrated in MRSA (Varga *et al.*, 2012).

Characterization of MRSA and MRSIG strains

Terms used to designate different MRSA strains are sometimes inconsistent or confusing. Many isolates and clones were originally named according to the geographical areas of origin. In 2002, a proposal was made to identify isolates according to sequence type (ST), antibiotic resistance, and SCC*mec* type. ST is determined by multilocus sequence typing (MLST) of seven housekeeping genes in an isolate and comparing these with known sequences published on the MLST website (http://saureus.mlst.net). As of October 2012, this site contained data on 4588 isolates, representing 2315 STs. Antibiotic resistance is designated as MRSA or MSSA and the SCC*mec* type as I to VII. For example the

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New York/Japan clone is ST5-MRSA-II and USA300 is ST8-MRSA-IV. However, many publications continue to refer to well-known strains by their old names. STs that differ in only a few of the genetic loci tested are grouped into clonal complexes (CCs) using BURST (based on related STs) analysis. The ST number of the isolate considered to be closest to the ancestral sequence is assigned as the CC number for that group of highly related STs. Five major CCs originated in hospitals (Robinson and Enright, 2004). Other CCs developed from *S. aureus* strains circulating in the community, outside of healthcare facilities (David and Daum, 2010). CC398 is a CC that appeared to originate in swine (Leonard and Markey, 2008; Cuny *et al.*, 2010).

S. pseudintermedius ST71 has become established as the most common multidrug resistant strain in Europe, whereas ST68 is most commonly detected in North America. Another clonal group, ST106, was identified as the most important MRSP in Norway (Osland *et al.*, 2012). Nearly all strains are resistant to nine classes of important veterinary antimicrobials. Over 70% of MRSP isolates contain the SCC*mec* elements II or III, and the remaining strains have SCCmec elements III, IV, V, or VII (Perreten *et al.*, 2010; Ruscher *et al.*, 2010; Black *et al.*, 2011). The genetic basis of antimicrobial resistance in *S. pseudintermedius* and other SIG was recently reviewed (Kadlec and Schwarz, 2012).

Epidemiology of MRSA and MRSIG

MRSA carriage and infection in humans

Approximately 50% of people in the general population are carriers of *S. aureus* (Acton *et al.*, 2009; Frank *et al.*, 2010), but only about 1.5% of the population are carriers of MRSA according to CDC. However, certain population subgroups have a significantly higher prevalence of MRSA than others, including those older than 60 years (Enoch *et al.*, 2010; Reilly *et al.*, 2010). Persons with occupational exposure to animals carrying MRSA are also more likely to carry related animal MRSA strains than the general population (Van Loo *et al.*, 2007a, b; Van Cleef *et al.*, 2010). Several studies have demonstrated that carriers of MRSA are at greater risk for developing serious infections compared to people who are not carriers.

MRSA, like MSSA, can cause a range of illness from relatively mild skin disorders to life threatening, invasive infections. MRSA has been a chronic problem in hospitals and long-term care facilities for over 40 years, particularly in surgical wards and intensive care units. Community-acquired infections typically affect skin and soft tissues, causing mild to moderate symptoms, often in healthy, younger people without the usual risk factors for healthcare-acquired MRSA. Severe, invasive community acquired MRSA infections also occur and there is evidence that these more severe infections are increasing as a more virulent strain, USA300, spreads (David and Daum, 2010). At the same time, there is evidence that MRSA infections in hospitals have leveled off or decreased in the past 5 years (Hadler *et al.*, 2012).

One troubling aspect of MRSA infections and colonizations is that they often appear to be chronic and bacteria can be detected in human tissues for extended periods. Persistence of MRSA in 403 German patients had a half-life of 549 days with the duration dependent on the site(s) colonized or infected (Mattner *et al.*, 2010).

HA-MRSA

MRSA was first detected in a UK hospital in 1961 and a few years later was detected in U.S. hospitals and other health care facilities where the widespread use of antibiotics selected for drug resistant bacteria. Until the 1990s, MRSA continued to be almost exclusively an issue in hospitals and long-term care facilities. Some MRSA infections occurred in non-hospitalized persons, but these were usually close contacts of persons who had been hospitalized. Due to the high rate of antibiotic usage in health care facilities, HA-MRSA are often resistant to many classes of antibiotics in addition to the β-lactams. Five major lineages or CCs (CC5, CC8, CC22, CC30 and CC45) originated in hospitals and have spread globally. Most of them possess one of the larger SCC*mec* types I–III, which also carry genes for resistance to other antibiotics. Type II is most common among U.S. HA-MRSA isolates, whereas type III is found more often in other countries (David and Daum, 2010; McCarthy *et al.*, 2010). Two major genotypes of HA-MRSA are circulating in Asia: CC5 which is dominant in Korea and Japan and CC239 which is dominant in China, India, and several countries in southeast Asia (Ko *et al.*, 2005; Li *et al.*, 2012).

Community-associated MRSA (CA-MRSA)

Cases of MRSA that genuinely originated in the community were first reported from Australia in the early 1990s. MRSA isolates from these cases were not resistant to multiple antibiotics and genetic analyses revealed that they were different from other MRSA in Australia (Udo *et al.*, 1993; Coombs *et al.*, 2010). More frequent reports of CA-MRSA emerged in other countries in the late 1990s, primarily among healthy persons with skin and soft tissue infections. These CA-MRSA isolates were susceptible to more classes of antibiotics than HA-MRSA and they generally carried smaller, more mobile SCC*mec* elements, usually types IV or V (David and Daum, 2010). Many CA-MRSA strains produce a toxin, PVL, that attacks white blood cells and is not commonly present in HA-MRSA (Li *et al.*, 2010). More extensive information on the evolution, virulence, and epidemiology of CA-MRSA can be found in a recent comprehensive review article (David and Daum, 2010).

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Several CA-MRSA clones originated in Europe (ST80), North America (ST1 and ST8), and Australia (ST30), and subsequently spread worldwide with reported cases in countries as diverse as South Africa, Nepal, Argentina, Saudi Arabia, Japan, Malaysia, and most countries in Europe (Tristan *et al.*, 2007). A great deal of genetic diversity exists in currently circulating CA-MRSA in Europe (Rolo *et al.*, 2012). A highly virulent CA-MRSA clone, ST93 (Queensland CA-MRSA), has emerged in Australia and has now spread to become the dominant CA-MRSA there (Otto, 2010; Coombs *et al.*, 2012).

A particularly virulent clone, USA300 (ST8), first reported in a prison outbreak in 2000 (Culpepper *et al.*, 2001), now causes nearly all CA-MRSA cases in the U.S. USA300 infections have also been increasing rapidly in Canada (Kim *et al.*, 2010) and this clone is now a major international epidemic strain having spread to numerous countries on five continents (Nimmo, 2012). USA300 contains SCC*mec* type IV and a gene encoding PVL appears to be more capable of colonizing human epithelial surfaces and causing skin and soft tissue infections than other CA-MRSA clones (Thurlow *et al.*, 2012). In the past 5 years, USA300 has acquired a number of additional antibiotic resistance genes, apparently from USA100, a common HA-MRSA strain (Huang *et al.*, 2007; Kennedy *et al.*, 2008; McDougal *et al.*, 2010; David and Daum, 2010).

Human HA-MRSA and CA-MRSA infections

In the past, categorizing MRSA as HA or CA, depending on where patients acquired infections, was useful. HA strains were more likely than CA strains to be resistant to multiple classes of antibiotics and therefore treatment for the two would differ. However, the emergence of CA-MRSA in healthcare settings and the appearance of HA-MRSA in the community, along with changes in virulence and the scope of antibiotic resistance have blurred distinctions between HA-MRSA and CA-MRSA.

CA-MRSA are now causing an increasing proportion of MRSA infections, including invasive infections, in hospitalized patients (Van De Griend *et al.*, 2009; Valsesia *et al.*, 2010; Zhanel *et al.*, 2010; Tenover *et al.*, 2012; Farr *et al.*, 2012). An analysis of discharge data on 616,375 pediatric cases of skin and soft tissue infections occurring in the U.S. during a 10-year period revealed that hospitalizations for infections caused by CA-MRSA increased dramatically from <1 case/100,000 in 1996 to 25.5 cases/100,000 in 2006 (Frei *et al.*, 2010). It has been predicted that CA-MRSA strains may eventually displace conventional strains of HA-MRSA. Therefore, it may now be more appropriate to define MRSA strains based on genetic differences revealed by SCC*mec* typing and MLST analysis in addition to noting whether MRSA was acquired in the hospital or the community and whether disease symptoms first appeared in the hospital or community (Otter and French, 2011).

MRSA carriage and infection in animals

MRSA colonizes and infects a variety of animals, including livestock, companion animals and some wild animals. Epidemiology of MRSA and predominant strains differ in different animals; human-associated MRSA strains are often isolated from pets while some horse- and livestock-adapted strains are more commonly present in other animals (McCarthy *et al.*, 2012; Petinaki and Spiliopoulou, 2012). The earliest published report of MRSA in farm animals described the detection of MRSA, in 1972, in dairy cows with mastitis in Belgium (Devriese and Hommez, 1975). Although current methods for typing MRSA strains were not available then, it is believed that these cases resulted from human to animal transmission of HA-MRSA. Later reports documented HA-MRSA in horses, dogs, and other animals at veterinary clinics and hospitals (Hartmann *et al.*, 1997; Seguin *et al.*, 1999). Some later reports described dogs, horses and cats at veterinary hospitals with CA-MRSA infections (Middleton *et al.*, 2005).

A new MRSA strain, ST398, was first detected in 2003 in swine and swine farmers in the Netherlands and was designated LA (livestock associated) MRSA (Van Loo *et al.*, 2007a, b; Fluit, 2012). ST398 has also been detected in pigs and pig farmers in other European countries (Denis *et al.*, 2009; Köck *et al.*, 2009; Pomba *et al.*, 2010), the U.S. (Smith *et al.*, 2009), and Canada (Khanna *et al.*, 2008; Golding *et al.*, 2010). ST398 has also been isolated from humans and other animals living on pig farms (Van De Giessen *et al.*, 2009; Cuny *et al.*, 2010; Hasman *et al.*, 2010). Surveys indicate that pigs, veal calves, and broilers are the main reservoirs for ST398 (ECDC 2009), but this strain has also been detected in dairy cattle and milk (Kreausukon *et al.*, 2012; Tavakol *et al.*, 2012). A different swine-associated MRSA strain, CC9, is circulating among pigs and pig farmers in China (Cui *et al.*, 2009; Ho *et al.*, 2012).

It appears, from genome analysis of numerous human and animal strains belonging to the CC 398, that the LA-MRSA ST398 was derived from a human MSSA ST398 strain that was transferred to pigs. When this occurred, some genes coding for immune modulators specific to humans were lost and the ST398 in pigs acquired resistance to tetracycline and methicillin (Price *et al.*, 2012; Jamrozy *et al.*, 2012). The loss of genes specific for human infections appears to limit transmission of ST398 among people (Bootsma *et al.*, 2011). Over 70% of 54 LA-MRSA strains tested were resistant to three or more classes of antibiotics. All of the strains tested were PVL negative and only four strains had genes coding for enterotoxins (Kadlec *et al.*, 2009, 2012).

Certain horse-adapted MRSA strains have been described and have apparently been transmitted to humans (Catry *et al.*, 2010). A majority of horse isolates in Canada belong to a subtype of the Canadian epidemic strain, MRSA-5, which has a type IV SCC*mec*. This strain is also present in horses in other countries and has been reported in numerous people working with horses (Weese *et al.*, 2005; Catry *et al.*, 2010).

Swine

Of all livestock, swine appear to most commonly harbor MRSA. In most cases, MRSA does not appear to seriously affect the health of pigs, but there have been reports of MRSA in pathological lesions in pigs (Atyah *et al.*, 2010; Meemken *et al.*, 2010; Vanderhaeghen *et al.*, 2010b). Data from the EU on MRSA in 4597 swine holdings (breeding and production) in 26 countries revealed that overall 14% of breeding and 27% of production herds tested positive for MRSA. However, in some countries, no herds tested positive, whereas in others, up to 51% of holdings contained MRSA. Highest prevalence of MRSA was recorded in Spain, Germany, Belgium, and Italy. LA-MRSA (ST398) accounted for 92.5% of isolates tested (European Food Safety Authority, 2009).

Other recent surveys report the presence of MRSA in:

- 70% of pigs sampled at one U.S. production facility and none of the pigs at another facility. Isolates were ST398 (Smith *et al.*, 2009).
- 2.9% of pigs tested on 10 Ohio farms (Molla *et al.*, 2012).
- 58–79% in pigs in Spain and Hungary sampled in 2010 (European Food Safety Authority and European Centre for Disease Prevention and Control, 2012).
- 4.6% of pigs on 45 Canadian farms. The predominant strain was ST398 (Weese *et al.*, 2011).
- 70% of German pig farms. All were ST398 (Köck *et al.*, 2009).
- 71% of Dutch finishing pig herds and 67% of breeding herds (Broens *et al.*, 2011).
- 44% of pigs 50 Belgian farms (Crombée *et al.*, 2012).
- 0.9% of swine nasal samples in Japan. No ST398 was detected (Baba *et al.*, 2010).

Factors positively associated with prevalence of MRSA in swine include larger herd sizes and greater numbers of imported pigs (European Food Safety Authority, 2010; Broens *et al.*, 2011), 'open' versus 'closed' farms (Crombé *et al.*, 2012), conventional rearing of pigs as compared to organic rearing (Meemken and Blaha, 2009) and poorer hygiene practices (Nathaus *et al.*, 2010). Trading of MRSA colonized pigs between farms was documented as a mode of transmission to new farms as molecular typing confirmed that MRSA isolates at the receiving farm were indistinguishable from those at the supplying farm (Espinosa-Gongora *et al.*, 2012). LA-MRSA, ST398, readily spread from pigs nasally inoculated with a low dose to other pigs in the same pen. MRSA was not detected in feces, but was present in lymph nodes (Jouy *et al.*, 2012).

Cattle

S. aureus is a significant cause of mastitis in cows and small ruminants (Vanderhaeghen *et al.*, 2010b). However, the prevalence of methicillin-resistant strains in European cows appears to be low, although there is some intercountry variation (Hendriksen *et al.*, 2008; Alves *et al.*, 2009). CA-, HA-, and LA-MRSA were all recently detected in bulk tank milk from cows in Minnesota (Haran *et al.*, 2010). Human-associated MRSA strains have also been detected in mastitic cows in Hungary (Juhász-Kaszanyitzky *et al.*, 2007), Korea (Nam *et al.*, 2011), and Turkey (Turutoglu *et al.*, 2009), and recent studies have demonstrated that ST398 is present in German (Fessler *et al.*, 2010; Spohr *et al.*, 2011), Dutch (Tavakol *et al.*, 2012), and Belgian (Vanderhaeghen *et al.*, 2010a) cows. Some healthy dairy cows in Denmark carry a CC130 strain that harbors a new mecC gene and these MRSA strains can be transferred between cattle and humans (Petersen *et al.*, 2012).

MRSA has been detected in retail beef, but nasal and fecal sampling of nearly 500 Canadian feedlot cattle, shortly before slaughter, detected no MRSA (Weese *et al.*, 2012b). MRSA was detected in 18–31% of veal calves on Dutch farms. Prevalence was strongly correlated with the use of antimicrobials and was also related to management and hygiene practices on different farms (Bos *et al.*, 2012). In 2010, the European Union reported that 20% of veal calves in Germany carried MRSA (European Food Safety Authority and European Centre for Disease Prevention and Control, 2012).

Poultry

Methicillin resistance was observed in *S. aureus* isolates from chickens in Korea in 2001–2003 (Lee, 2003). MRSA was later detected in broiler chickens in Belgium (Nemati *et al.*, 2008; Persoons *et al.*, 2009) and in broilers, but not in breeder chickens in the Netherlands (Mulders *et al.*, 2010). These isolates were identified primarily as the livestock associated strain, ST398. A survey of the prevalence of MRSA in broilers on three Belgian mixed poultry-pig farms revealed that from 0 to 28% of chickens carried MRSA strain 398 as compared to 82–92% of pigs on the same farms. No MRSA was isolated from barns containing chickens and it may be that the shorter production period for broilers and more frequent disinfection of their quarters explains the lower prevalence of MRSA. Chickens may also be naturally less susceptible to MRSA infection (Pletinckx *et al.*, 2011). A report on MRSA in animals, sampled in 2010, in countries in the

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European Union reported that 20–46% of turkeys in Germany and Hungary carried MRSA. MRSA was also detected in 71% of chickens tested in Hungary, but was not found in 398 flocks of broilers in Switzerland (European Food Safety Authority and European Centre for Disease Prevention and Control, 2012).

Horses

MRSA was first reported in a horse with a surgical wound in 1996 (Hartmann *et al.*, 1997). Transmission of MRSA from humans appeared to cause the early infections in horses in veterinary hospitals and is still a factor (Seguin *et al.*, 1999; Couto *et al.*, 2012). Surveys of horses on farms usually report a low prevalence of MRSA of 0–4.7%. A higher prevalence (up to 12%) has been observed in horses admitted to veterinary hospitals (Tokateloff *et al.*, 2009; Weese and Van Duijkeren, 2010). The most common MRSA strain now identified in horses in Europe, North America, and Australia, Canadian CMRSA-5 or USA500, is a member of the CC8 clone (Abbott *et al.*, 2010b). Although this clone appears to be of human origin, it seldom causes illness in humans and now appears to be horse-adapted (Weese *et al.*, 2006; Axon *et al.*, 2011). Unlike other farm animals that are primarily transported only for slaughter, horses are transported internationally for breeding, racing, and show-jumping, and these movements have contributed to the spread of this clone. Recently, there have been reports of the livestock-associated (LA) strain ST398 in horses (Loeffler and Lloyd, 2010; Van Duijkeren *et al.*, 2010), including veterinary hospital outbreaks in Finland (Salmenlinna *et al.*, 2010) and Sweden (Bergstrom *et al.*, 2012a). Tracking of antibiotic resistant *S. aureus* strains in a Swiss veterinary hospital over a 6-year period revealed that strain ST398 was first detected in an employee in 2006 and then appeared in 10–15% of horses as ST398 rapidly replaced the antibiotic resistant strains of human origin within 6 months (Sieber *et al.*, 2011).

Dogs and cats

A human-associated MRSA was first detected in companion animals in Nigeria in 1972 (Loeffler and Lloyd, 2010). Later, MRSA was detected in dogs with surgical wounds or skin infections (Gortel *et al.*, 1999; Tomlin *et al.*, 1999) and the use of veterinary drugs and IV catheters were identified as risk factors for MRSA infections in dogs (Faires *et al.*, 2010; Magalhães *et al.*, 2010). Generally, cases of MRSA infection in dogs and cats involve lesions in the skin or ears but invasive infections sometimes occur. Healthy dogs and cats can also transiently carry MRSA asymptomatically (Weese, 2005; Morris *et al.*, 2012). Surveys indicate that prevalence of MRSA in companion animals is low (<2%) (Leonard and Markey, 2008). Epidemiology of MRSA in companion animals was recently reviewed (Loeffler and Lloyd, 2010).

MRSA strains isolated from companion animals are primarily HA-MRSA (Abbott *et al.*, 2010a) and these have been detected in therapy dogs and cats visiting human long-term care facilities (Lefebvre and Weese, 2009; Coughlan *et al.*, 2010). However, both CA-MRSA (Van Duijkeren *et al.*, 2005) and LA-MRSA (Witte *et al.*, 2007; Floras *et al.*, 2010) have caused infections in dogs. Similar MRSA strains have been detected in dogs and their owners but surveys of dogs or humans colonized with MRSA have demonstrated that only a small number of human–dog pairs are infected with the same MRSA strain (Boost *et al.*, 2008; Faires *et al.*, 2009b; Morris *et al.*, 2012). MRSA does not appear to spread easily from dog to dog (Loeffler *et al.*, 2010).

Other animals

In addition to the companion animals and livestock described above, MRSA has been detected in avian pets, including a parrot (Rich and Roberts, 2006; Rodriguez *et al.*, 2007), goats (Alves *et al.*, 2009), sheep (Goñi *et al.*, 2004), farmed fish (Abrahim *et al.*, 2010), wild rats living on a farm (Van De Giessen *et al.*, 2009), a zoo elephant (Janssen *et al.*, 2009), seals, dolphins and walrus from marine parks/sanctuaries (O'Mahony *et al.*, 2005; Faires *et al.*, 2009a), and guinea pig, rabbit, bat, and turtle in a veterinary hospital (Walther *et al.*, 2008). Origins of MRSA were unknown in some cases, but appeared to be from human caretakers for the birds, seal, and elephant calf and from pigs for the farm rats.

MRSA in foods

MRSA has been detected in raw meat (pork, poultry, and beef) and raw milk from countries throughout the world (Table 3). In some cases, the MRSA isolates were identified as HA- or CA-MRSA indicating that meat processors and other food handlers were likely to be the source of the bacteria (Pu *et al.*, 2009; Hata *et al.*, 2010; Türkyilmaz *et al.*, 2010; Weese *et al.*, 2010a). In other surveys, LA-MRSA strains were the primary isolates, indicating an animal source of contamination (De Boer *et al.*, 2008; Benedetti *et al.*, 2010; Vanderhaeghen *et al.*, 2010a; Weese *et al.*, 2010a, b; Fessler *et al.*, 2011; O'Brien *et al.*, 2012). LA-MRSA has been detected in lymph nodes of pigs and if these tissues are included in ground pork, they could be a source of MRSA in meat (Jouy *et al.*, 2012; Szabó *et al.*, 2012).

Reports of MRSA contamination in meats in Europe, North America and Asia have been published in an increasing number of scientific articles. Prevalence of contamination in samples tested ranges from relatively low (1–3%) (Bhargava *et al.*, 2011; Hanson *et al.*, 2011;

Table 3. Reports of MRSA detected in foods

Food	No. of reports	% Positive	Countries	References
Beef	8	1.0–10.6	U.S., Canada, Denmark, Iran, Japan, Korea, and Netherlands	(Pu *et al.*, 2009; De Boer *et al.*, 2009; Rhee & Woo, 2010; Weese *et al.*, 2010; Bhargava *et al.*, 2011; Agersø *et al.*, 2012; Hiroi *et al.*, 2012; Shahraz *et al.*, 2012)
Veal	3	2.2–15.2	Spain and Netherlands	(De Boer *et al.*, 2009; Lozano *et al.*, 2009; de Jonge *et al.*, 2010)
Pork	15	0.04–50	U.S., Canada, Denmark, Germany, Japan, Thailand, Netherlands, Poland, and Spain	(De Boer *et al.*, 2009; Lozano *et al.*, 2009; Pu *et al.*, 2009; Bystron *et al.*, 2010; de Jonge *et al.*, 2010; Weese *et al.*, 2010; Beneke *et al.*, 2011; Hanson *et al.*, 2011; Kelman *et al.*, 2011; Agersø *et al.*, 2012; Hiroi *et al.*, 2012; Molla *et al.*, 2012; O'Brien *et al.*, 2012; Vestergaard *et al.*, 2012)
Boar, wild	1	25	Spain	(Lozano *et al.*, 2009)
Lamb/mutton	1	6.2	Netherlands	(De Boer *et al.*, 2009)
Chicken	12	0.7–43.8	U.S., Canada, China, Germany, Japan, Spain, Netherlands, and Turkey	(De Boer *et al.*, 2009; Lozano *et al.*, 2009; de Jonge *et al.*, 2010; Weese *et al.*, 2010; Bhargava *et al.*, 2011; Citak & Duman, 2011; Cuny *et al.*, 2011; Fessler *et al.*, 2011; Agersø *et al.*, 2012; Hiroi *et al.*, 2012; Ogata *et al.*, 2012; Wang *et al.*, 2013)
Turkey	3	35.3–51	U.S., Germany, and Netherlands	(De Boer *et al.*, 2009; Bhargava *et al.*, 2011; Fessler *et al.*, 2011)
Duck	1	50	Japan	(Ogata *et al.*, 2012)
Rabbit	1	12.5	Spain	(Lozano *et al.*, 2009)
Fish	2	2.5–20	Japan and Korea	(Rhee & Woo, 2010; Hammad *et al.*, 2012)
Milk, cows	5	1.3–17.6	U.S., Belgium, Brazil, India, and Switzerland	(Huber *et al.*, 2010; Vanderhaeghen *et al.*, 2010a; Dias *et al.*, 2011; Haran *et al.*, 2012; Prabhu *et al.*, 2012)
Milk, goats	1	3.5	Iran	(Ebrahimi *et al.*, 2010)
Milk, water buffalo	1	2.5	Turkey	(Pamuk *et al.*, 2012)
Sprouts	1	1.8	Korea	(Seo *et al.*, 2010)

Agersø *et al.*, 2012) to as many as a quarter to a third of the meat samples (Knödl *et al.*, 2010; Fessler *et al.*, 2011). Since sample sizes and sampling and culture methods differed among the studies, results are not strictly comparable. Most of this research was aimed at detecting the presence of MRSA and there was no effort to quantify the bacteria. A recent Canadian study found that most positive meat samples contained <100 cfu g^{-1} (Weese *et al.*, 2010) and a Dutch study reported that most probable numbers (MPN) of MRSA in meat ranged from 0.06 (veal) to >10 (pork) bacteria per gram (de Jonge *et al.*, 2010).

MRSA carried in pigs during lairage, prior to slaughter, can be detected at different stages during processing and in commercial pork products (Molla *et al.*, 2010). A study investigating the fate of MRSA on German pigs at slaughter and at several steps during processing found that 65% of pigs were positive at stunning. However, only 6% of carcasses on the slaughter line, 4.2% of meat samples during processing, and 3% of finished meat products tested positive (Beneke *et al.*, 2011). A Dutch survey of meat handlers found that they were not colonized with MRSA, but LA-MRSA was detected on meat (de Jonge *et al.*, 2010).

S. aureus is a known cause of mastitis in ruminants and several studies reported MRSA in raw milk from cows in the U.S. and Europe (Benedetti *et al.*, 2010; Vanderhaeghen *et al.*, 2010a; Haran *et al.*, 2012), from milk in South Africa, India, and Brazil (Prabhu *et al.*, 2012), and from goats (Ebrahimi *et al.*, 2010) and water buffalo (Ateba *et al.*, 2010; Dias *et al.*, 2011; Pamuk *et al.*, 2012). Some of these strains also produced enterotoxins. MRSA has also been detected in other foods, including lamb and mutton, rabbit, duck, wild boar meat, minimally processed vegetables, and fresh fish. Although *S. aureus* is not part of the normal flora of fish, MRSA and some other methicillin-resistant staphylococci have been detected on raw fish in Japan. These contaminants may be deposited on fish by workers with poor personal hygiene (Hammad *et al.*, 2012).

MRSIG infection in animals and humans

Published reports generally indicate a low prevalence of MRSP in dogs and cats, but the reported prevalence of MRSP appears to be increasing in some areas (Jones *et al.*, 2007; Weese and Van Duijkeren, 2010; Nienhoff *et al.*,

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2011a, b). Recent hospitalization and antibiotic use are positively correlated with the presence of MRSP in dogs (Nienhoff *et al.*, 2011a; Weese *et al.*, 2012a). MRSP can persist in dogs for many months after clinical infections. Treatment of MRSP-infected dogs with systemic antibiotics to which the MRSP strains were resistant appeared to prolong carriage of these strains (Windahl *et al.*, 2012).

Dogs with clinical infections can contaminate their environment and bacteria can remain after ordinary cleaning and disinfection. Infected dogs can readily transmit MRSP to cats and other dogs. However, transmission to humans in the household and to veterinarians occurs at a lower rate than infection of other animals (Laarhoven *et al.*, 2011; Van Duijkeren *et al.*, 2011b).

Methicillin resistance has been detected in other staphylococci including *Staphylococcus schleiferi* and *Staphylococcus epidermidis* from dogs (Kania *et al.*, 2004; Kawakami *et al.*, 2010), and several staphylococcal species on freshwater fish in Greece (Abrahim *et al.*, 2010).

MRSP/MRSIG strains are seldom isolated from human food, but there is one report of MRSIG in camel meat in Jordan (Al-Tarazi *et al.*, 2009). Methicillin-resistant coagulase-negative staphylococci have been detected in bulk tank milk and minced meat in Switzerland. These bacteria are not normally pathogenic, but could transfer their antibiotic resistance to *S. aureus* or other bacteria (Huber *et al.*, 2011).

Routes of infection

Staphylococci are spread among humans and animals and between species by direct physical contact or indirectly through clothing, towels, equipment, food, air, or surfaces contaminated by infected or colonized persons or animals.

Person to person

Hospital outbreaks of MRSA have been traced to lax hygiene practices among health care workers. MRSA transmission in a UK hospital was audited by swabbing patients' skin and their environment and the hands of healthcare workers. MRSA was transmitted from a source, most commonly a patient's skin, to other patient skin areas, furniture, or note pads by the hands of healthcare workers in 22 of 24 cases. In one case, a doctor entering an intensive care unit with MRSA on his/her hands contaminated a notes trolley near a patient (Ludlam *et al.*, 2010). Another study found that the frequency of transfer of MRSA from the skin of a colonized patient to a gloved hand was 40% (Stiefel *et al.*, 2011). A study in four Danish hospitals found that the transmission rate of HA-MRSA from an introduced non-isolated patient to others in the hospital was about nine times greater than the transmission rate of CA-MRSA (Hetem *et al.*, 2012). A mathematical model has been developed to estimate the importance of numerous factors influencing the persistence and spread of MRSA in nursing homes. This model may aid in determining the most effective ways of controlling this pathogen in such communities (Chamchod and Ruan, 2012).

MRSA outbreaks in the community often occur in groups of people living in close quarters where they may transmit MRSA through direct physical contact or through commonly used items. Athletes participating in team sports are a recognized at-risk group. MRSA may be transmitted during direct physical contact during practices and games, and may also be present on towels or other equipment (Buss *et al.*, 2009; Redziniak *et al.*, 2009; Creech *et al.*, 2010). Several outbreaks of HA-MRSA have occurred in prisons where inmates share soap, shower infrequently, and are not well informed about prevention of staphylococcal infections. Other risk factors are related to pre-incarceration factors such as a low educational level and little contact with the health care system (Maree *et al.*, 2010; Malcolm, 2011).

A cluster of CA-MRSA cases (strain USA300) in the Netherlands occurred in a beautician, her customers, family members, and contacts. Skin treatments (waxing) performed by the beautician were identified as the likely mode of transmission (Huijsdens *et al.*, 2008).

Airborne transmission

MRSA is present in the nose and on the skin, and is shed into the environment by infected or colonized people and animals, indicating that airborne transmission is a possible route for infection. MRSA strains, identical to clinical isolates from patients, were detected in the air of hospital rooms (Gehanno *et al.*, 2009). LA-MRSA was detected in the air inside commercial pig barns in Germany at concentrations ranging from 6 to 3619 CFU m^{-3} and at lower concentrations (11–14 CFU m^{-3}) in air samples taken 150 m downwind from the barns. LA-MRSA was also present on soil surfaces 300 m downwind from the barns. Airborne transmission and deposition does occur around barns containing MRSA-positive pigs and this is likely affected by wind speed and direction as well as temperature and rainfall (Friese *et al.*, 2012; Schulz *et al.*, 2012).

Animal contact

LA-MRSA ST398 was first described in pigs in the Netherlands in 2003 (Voss *et al.*, 2005; De Neeling *et al.*, 2007). Subsequent studies reported detection of this strain among farmers and a survey indicated that human carriers of ST398 were 12.2–19.7 times more likely to be pig or

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cattle farmers than to be workers at other jobs (Van Loo *et al.*, 2007a, b). While the overall number of MRSA infections in the Netherlands appears to have stabilized, an increasing percentage of MRSA infections in the country are caused by this LA strain, even among people without known exposure to pigs or veal calves. Total MRSA isolates submitted to the national laboratory in the Netherlands in 2008 numbered 2693. Of these, 42% were identified as the LA-MRSA ST398 strain as compared to 30% in 2007 and 14% in 2006. Only 29% of people surveyed indicated contact with live pigs or veal calves (Haenen *et al.*, 2010). A recent study in Germany found that 86% of farmers and 45% of veterinarians exposed to pigs with ST398 also carried this strain. However, it was not readily transmitted from the workers to others as only 4–9% of family members and other close contacts tested positive for ST398 (Cuny *et al.*, 2009). LA-MRSA ST398 was reported in 1.4% of Danish livestock veterinarians and in 7.5% of Belgian veterinarians (Garcia-Graells *et al.*, 2012). An increased risk for MRSA ST398 colonization was observed in persons who visited farms to purchase milk or eggs (Bisdorff *et al.*, 2012). A survey of 51 veal calf farms in the Netherlands indicated that an average of 38% of farmers and 16% of family members were colonized with LA-MRSA. However, carrier status decreased dramatically when farmers took a break from direct animal care duties. Farm hygiene and antibiotic administration to calves were related to MRSA carriage in calves (Graveland *et al.*, 2010, 2011). Another study of pig farmers on holiday found that 59% did not clear carriage of MRSA while on vacation (Köck *et al.*, 2012). LA-MRSA can be acquired by humans with short-term occupational exposure to infected/colonized animals, but in most cases the strain is lost after a day (Van Cleef *et al.*, 2011). Studies of carriage of ST398 in workers at Dutch pig slaughterhouses found that 11 of 341 workers tested positive and that those working in lairage and the scalding and dehairing areas were most likely to be colonized (Gilbert *et al.*, 2012).

Children and others interacting with animals at fairs and petting zoos are known to be exposed to zoonotic bacteria and viruses and some *Escherichia coli* and *Salmonella* outbreaks have been traced to these locales. A survey of 157 pigs at two state fairs detected *S. aureus* in 25 animals and MRSA in two pigs (Dressler *et al.*, 2012). Although MRSA infections have not been traced to fairs, this is a potential site for transmission.

Horses may be colonized or infected with an uncommon horse-adapted MRSA strain, CMRSA-5, and several studies have reported this strain in horse caretakers and veterinarians (Weese *et al.*, 2005; Abbott *et al.*, 2010b). Australian veterinarians whose major emphasis was treatment of horses were found to be more likely than other veterinarians to be carriers of MRSA (Jordan *et al.*, 2011). Suspected transmission of MRSA ST398 from a horse to a human has also been reported (Van Duijkeren *et al.*, 2011).

Companion animals may also be carriers of MRSA and MRSP. MRSA was first detected in a companion animal in a ward cat in a geriatric rehabilitation unit in England. The cat was apparently infected by a resident and then served as a reservoir spreading the infection to human residents (Scott *et al.*, 1988). HA-MRSA strains have been detected in therapy dogs and cats visiting human long-term care facilities and may be a source of infection to residents (Lefebvre and Weese, 2009; Coughlan *et al.*, 2010).

Similar MRSA and MRSP strains have been detected in dogs and their owners, but surveys of dogs or humans colonized with MRSA have demonstrated that only a small number of human–dog pairs are infected with the same MRSA strain. Evidence indicates that pets can acquire MRSA from humans and dog owners and veterinarians may also acquire MRSP from dogs (Boost *et al.*, 2008; Faires *et al.*, 2009b; Paul *et al.*, 2011; Soedarmanto *et al.*, 2011; Walther *et al.*, 2012).

Contaminated equipment and surfaces

Surfaces and equipment in healthcare facilities may be contaminated with MRSA for extended periods. CA-MRSA (USA300) inoculated on to various fomites (towels, sheets, ceramic, wood, vinyl, and plastic) survived for extended periods and were transmissible for up to 8 weeks. Transmissibility decreased more rapidly from porous surfaces. HA-MRSA tested were not as readily transmitted from fomites (Desai *et al.*, 2011). MRSA was detected on about 10% of 183 mobile phones belonging to nurses, laboratory workers and other health care staff (Ustun and Cihangiroglu, 2012). Investigations at a veterinary hospital found 12% of environmental samples harbored MRSA (usually USA100) and that surfaces frequently touched by people, such as doors, or by animals, for example carts, were most likely to be contaminated with MRSA (Scott *et al.*, 2008; Hoet *et al.*, 2011). Transmission of MRSA in healthcare facilities can occur by touching contaminated surfaces; gloved hands can pick up MRSA from bedrails, call buttons, tables, and phones at a frequency of 45% (Stiefel *et al.*, 2011).

Patients with end stage renal disease (ESRD) are particularly vulnerable to invasive *S. aureus* infections because their blood must be treated using dialysis machines at least three times per week. These patients are frequently hospitalized, receive long courses of antibiotic treatment, and 14% die annually as a result of infections. Incidence of invasive MRSA was estimated at 45.2 cases/1000 population among dialysis patients, the highest for any patient population and about 100 times greater than the incidence in the general population (Collins *et al.*, 2007). An increasing proportion of MRSA infections in ESRD patients is due to CA-MRSA strains (Johnson *et al.*, 2006).

MRSA has also been detected on a variety of surfaces outside of healthcare facilities. Contamination

of household surfaces is a source of infection for people and pets (Bocher *et al.*, 2010; Davis *et al.*, 2012). A swab survey of 400 ATM machines in Hong Kong found that over 15% had detectable levels of *S. aureus*, whereas MRSA was present on only two machines. Many of the antibiotic-resistant *S. aureus* isolates also carried genes encoding resistance to antiseptics (Zhang *et al.*, 2012). CA-MRSA strains on contaminated needles have been transmitted among illicit drug users (Kreisel *et al.*, 2010) and non-sterile equipment used in tattooing was cited as the cause of CA-MRSA infections in tattoo recipients in several states (Long *et al.*, 2006).

Numerous reports have detailed outbreaks in high school and collegiate athletes where MRSA was detected on equipment and surfaces in athletic facilities and on towels and clothing (Bowers *et al.*, 2008; Redziniak *et al.*, 2009). Experiments testing survival of CA-MRSA on artificial turf indicated that these bacteria could survive for at least a week in large numbers and for a month at lower concentrations, depending on environmental conditions (Waninger *et al.*, 2011). MRSA has been detected at relatively low levels in beach sand and water in California (Goodwin *et al.*, 2012).

Contaminated food

MRSA strains have been detected in meat and milk and may also be present in a variety of other foods (Table 3). The origin of these contaminants has been traced to infected/colonized food handlers in some outbreaks (Kluytmans *et al.*, 1995; Jones *et al.*, 2002). Studies have demonstrated that meat can also become contaminated during slaughter and processing of animals carrying MRSA (Molla *et al.*, 2010; Beneke *et al.*, 2011). In some surveys, MRSA detected on meat was identified as the LA strain, ST398 (de Jonge *et al.*, 2010).

Significance of MRSA contamination of foods remains uncertain. If meat and other foods are cooked properly, MRSA cells will be killed. However, as with enterotoxigenic MSSA strains, under conditions of temperature abuse, MRSA cells could grow in foods, produce heat-stable enterotoxins, and cause food-borne intoxication. In some individuals, whose normal flora has been depleted by antibiotic treatment, MRSA cells on ready-to-eat foods, including processed meats, cheeses, and fresh produce, could cause staphylococcal enterocolitis. Finally, MRSA present on foods could potentially cause skin infections in food handlers. To date, there have been only two reported outbreaks associated with MRSA-contaminated food. A community outbreak of food-borne illness caused by CA-MRSA occurred in Tennessee in 2000 (Jones *et al.*, 2002). Identical MRSA isolates were recovered from three ill persons, the coleslaw they purchased from a convenience store deli, and the nose of a food handler at the convenience store. This strain produced enterotoxin C. The second reported outbreak of MRSA occurred in a Dutch hospital and affected 27 patients and 14 health care workers from 1992 to 1993, resulting in five deaths. Epidemiological investigations indicated that a colonized food handler apparently contaminated food (a peeled banana tested positive for MRSA) served to hospital patients and some nurses may have inadvertently spread the bacteria to different wards (Kluytmans *et al.*, 1995).

MRSA does not appear to be transferred readily from meat to meat handlers. It was not detected on hands or in noses of 89 persons working in cold meat processing facilities or institutional kitchens in the Netherlands even though 14% of samples of meat (veal, pork, and chicken) that they worked with did contain MRSA. Most of the MRSA isolates were identified as ST398, LA MRSA (de Jonge *et al.*, 2010). However, MRSA swabbed on pork loins was transferred at low levels to knives and cutting boards, indicating the potential for cross contamination and exposure in persons working with pork (Snyder *et al.*, 2012).

Other methicillin-resistant staphylococci could potentially cause food-borne intoxication, but no cases have been reported. Methicillin-resistant *S. intermedius* was detected in camel meat in Jordan (Al-Tarazi *et al.*, 2009). MRSIG strains have been detected in horses, but are not usually present in livestock (Weese and Van Duijkeren, 2010).

Control and prevention

Prevention of staphylococcal infections/intoxication requires strategies to interrupt various modes of transmission. Essentially these control programs include improvements in personal hygiene practices among health care workers and food handlers, decontamination of equipment, surfaces, and clothing, judicious use of antibiotics, proper cooking and storage of foods, and screening programs (Skov *et al.*, 2012).

Hospital and healthcare programs

Increased morbidity and mortality among hospital patients infected with MRSA has led to development of some effective control procedures and strict enforcement of MRSA control policies has been found to decrease rates of MRSA infection in hospitals in 10 European countries (Hansen *et al.*, 2010). An important feature of these MRSA-control programs is screening of patients at admission to ascertain which patients are carriers of MRSA so they can be isolated and treated to prevent transmission to other patients. Intensive programs to improve hygiene and worker training have also been effective in reducing MRSA infections in veterinary hospitals (Bergstrom *et al.*, 2012b).

For example, incidence of MRSA in the Netherlands is extremely low (0.7% in 2008) and this is attributed to

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effective implementation of the national MRSA guidelines in every healthcare setting for more than 20 years. These guidelines recommend prudent and restrictive use of antibiotics and an infection prevention program called 'search and destroy.' All patients and healthcare workers are screened for MRSA and if tests are positive they are isolated and treated to eliminate MRSA. Policies for cleaning and disinfection are also strictly followed (Van Knippenberg-Gordebeke, 2010). A cost–benefit analysis in a Dutch hospital concluded that this program prevented 36 cases of bacteremia annually and 10 deaths and saved >200,000 euros per year (Van Rijen and Kluytmans, 2009).

Similar programs have been implemented in other European countries with similar success in preventing HA infections. However, there has been discussion recently regarding the merits of adopting such a strict control program. The 'search and destroy' programs require significant resources to test all patients, keep them in isolation until test results come back, and continue to isolate them if tests are positive (Butterly *et al.*, 2010). Some have questioned the cost effectiveness of testing all incoming patients and instead recommend testing only certain patient populations, such as those entering intensive care units or those scheduled for surgery. A recent model analyzed the estimated costs of implementing screening and isolation programs at U.S. hospitals, savings in terms of the number of infections prevented, and additional costs incurred by patients with MRSA. Costs for screening all incoming patients and for using PCR methods exceeded those of screening selected high-risk patients, using chromogenic media. This analysis indicated that at hospitals with a relatively high baseline of MRSA infections, all strategies were cost effective, but screening selected patients with PCR had the greatest net benefit (Hubben *et al.*, 2011).

Another strategy to reduce MRSA in healthcare facilities is the reduction of antibiotic use to lower selection pressure for MRSA. This may be a useful procedure as a decrease in antibiotic use in a Taiwanese hospital from 2004 to 2009 was significantly correlated with fewer MRSA infections in patients (Lee *et al.*, 2010). Strengthening general infection control procedures throughout health care facilities can reduce all nosocomial infections. However, because increasing numbers of MRSA infections are acquired outside of health care settings, an effective MRSA control program will need to address prevention of infections arising in the community as well as methods to control infections in hospitals.

A multifaceted infection control system implemented in a Veterans Affairs hospital, emphasizing active surveillance culturing for MRSA and behavior change strategies to encourage workers to adhere to infection control precautions, resulted in sustained decreases in MRSA colonization and infection (Ellingson *et al.*, 2011). Effective implementation of infection control and antibiotic stewardship programs in hospitals requires behavioral changes in supervisors and health care workers. Therefore, attention must be paid to local/national cultural values in order to improve compliance (Borg *et al.*, 2012). Prevalence of MRSA in the environment of different nursing homes varies substantially and investigations demonstrated that frequency of cleaning common rooms and length of time spent cleaning residents' rooms were important factors affecting prevalence (Murphy *et al.*, 2012).

Sanitizers and surface treatments

Several sanitizers can be used to control methicillin-resistant staphylococci. Use of alcohol hand rubs has been significantly correlated with decreasing rates of MRSA infections (Sakamoto *et al.*, 2010; Sroka *et al.*, 2010). Chlorhexidine is also an effective antiseptic, but some strains of MRSA have developed resistance to it (Batra *et al.*, 2010; Zhang *et al.*, 2012). Use of chlorhexidine gluconate impregnated washcloths by marine recruits decreased acquisition of CA-MRSA (Whitman *et al.*, 2012). Mist application of a mixture of chlorine dioxide and a quaternary ammonium compound was found to inactivate MRSA on several environmental surfaces (Callahan *et al.*, 2010). Non-thermal plasmas were shown to be effective in inactivating both planktonic- and biofilm-associated MRSA (Burts *et al.*, 2009; Joshi *et al.*, 2010). Swimming pools containing chlorinated water, biguanide-treated water, or salt water did not permit survival of MRSA (Gregg and Lacroix, 2010).

Certain chemicals added to surface materials exert toxic effects on bacteria. Copper is a known bacteriocide and copper-based biocide solutions (Luna *et al.*, 2010) and copper incorporated into surfaces (Weaver *et al.*, 2010) both effectively killed MRSA. Silver is also bactericidal and a TiO_2–Ag composite completely inactivated MRSA within 24 h (Necula *et al.*, 2009). Data comparing bactericidal effects of Cu- and Ag-containing materials, under different conditions of temperature and humidity, indicate that Cu may be more effective in indoor environments (Michels *et al.*, 2009). A nanocomposite film incorporating a cell wall degrading enzyme has been developed and found to be effective in killing MRSA. This may prove useful in hospitals and other areas where infection control is critical (Pangule *et al.*, 2010).

Since swine are colonized or infected with MRSA fairly frequently, strategies are being devised to protect those with occupational exposure to pigs and other livestock. A preliminary study tested a program based on improving employee hand hygiene and showering, treating wounds, providing clean boots and coveralls, and cleaning and disinfecting showers and eating areas. Results of the small study were inconclusive, but these practices are likely to be effective if used correctly and consistently and a larger trial has been recommended (Larson *et al.*, 2012).

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Prevention of food-borne intoxication

Preventing staphylococcal intoxication by MRSA strains requires the same precautions as for MSSA strains. Efforts should be made to prevent contamination throughout the food production, processing and preparation chain. Adherence to good hygiene practices during slaughter and processing of livestock can significantly reduce contamination of meat and meat products. Workers have been implicated in many outbreaks of foodborne disease. They may shed bacteria and viruses, even when asymptomatic and several weeks after they have recovered from an illness. Improved hygiene precautions, consistently practiced by persons in food preparation and processing would significantly improve safety of foods (Todd *et al.*, 2008; Bystron *et al.*, 2010). Foods also must be cooked properly and refrigerated or kept hot until consumption. A recent analysis of growth requirements noted that *S. aureus* can grow at a water activity of 0.867 and at temperatures as low as 8°C (Valero *et al.*, 2009). This requires education not only of workers in the food industry broadly but also of the general public. Many outbreaks of staphylococcal food intoxication result from allowing foods to remain at ambient temperature, for example at picnics or large banquets, thus giving *S. aureus* time to grow and produce enterotoxins.

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