An emerging subtype of methicillin-resistant *Staphylococcus aureus* (MRSA), clonal complex (CC) 398, is associated with animals, particularly pigs. We conducted a matched case–control and a case–case study comparing 21 CC398 case-patients with 2 controls randomly selected from the Danish Civil Registry and 2 case-patients infected with MRSA other than CC398. On farms of case-patients, animals were examined for MRSA. Thirteen case-patients reported pig exposure. Living or working on farms with animals was an independent risk factor for CC398 in the case–control (matched odds ratio [MOR] 35.4, 95% confidence interval [CI] 2.7–469.8) and the case–case study (MOR 14.5, 95%CI 2.7–76.7). History of hospitalization was associated with an increased risk only in the case–control study (MOR 11.4, 95% CI 1.4–94.8). A total of 23 of 50 pigs on 4 of 5 farms were positive for CC398. Our results, corroborated by microbiologic testing, demonstrate that pigs are a source of CC398 in Denmark.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming increasingly recognized among persons in the community without established risk factors (1,2). MRSA primarily causes human disease and animals have not, until now, been considered a source of infection.

It has recently become apparent that animals, particularly pigs, can constitute a separate MRSA reservoir and be a source of a novel and rapidly emerging type of MRSA in humans; namely MRSA clonal complex (CC)398 by multilocus sequence typing (MLST) (3). MRSA CC398 consists of 8 MLST types (www.saureus.mlst.net), the predominant type being sequence type (ST)398, and a range of closely related protein A (spa) gene types (i.e., t011, t034, t108, and t1793) (4,5).

Although transmission appears to be primarily between animals, indistinguishable isolates have been found in their human contacts, particularly those with occupational exposure (3–7). MRSA CC398 (ST398) was first detected in 4 pigs and 1 healthy pig farmer in France (3,8). Clinical infection was described in the daughter of a pig farmer in the Netherlands in 2004 (7). That study showed that 23% of pig farmers in a small survey in the same region were seropositive for MRSA CC398.

Denmark has a low incidence of MRSA. In 2006, only 706 new MRSA patients (colonization only or infection) were reported, which corresponds to 13/100,000 population (9). To maintain this status, Denmark has adopted a strict “Search and Destroy” policy, which includes active screening of at-risk persons at admission to a hospital (10). Prompted by reports from the Netherlands, we identified a small (<1% of all MRSA patients) but increasing number of MRSA CC398 human patients after 2003. Furthermore, CC398 was detected in a pig in Denmark in 2006 (11). With an annual production of ≈25 million slaughter pigs (www.dst.dk), Denmark has a large potential reservoir for MRSA CC398.

We report results of an analytical study of MRSA CC398, in parallel with systematic farm and microbiologic investigations, to identify risk factors for MRSA CC398 ac-
Methods

would be expected in the general population. Post hoc interviews to determine if these exposures were higher than previous study has included interviews with control populations to determine if these exposures were higher than would be expected in the general population.

Surveillance

In Denmark, MRSA isolates from all human patients have been referred to Statens Serum Institut since 1988 for characterization and national surveillance. Epidemiologic and clinical information has been obtained prospectively since 1999 on all patients.

Case-Patients and Controls

We conducted a matched case–control study comparing human case-patients with MRSA CC398 during 2004–2007 with 2 population controls. In parallel, we conducted a case–case study comparing the same MRSA CC398 case-patients to 2 case-patients of community-detected MRSA of a type other than CC398 (non-CC398 case-patients). Eligible case-patients were persons with a confirmed diagnosis of MRSA with spa types related to CC398 (carriage or infection) during the study period: October 29, 2003 (first human diagnosed) to May 31, 2007. Where household clusters were identified, secondary case-patients were excluded from the study. Population controls were selected randomly from the Danish Civil Registry System and matched by sex, age, residence in the same region (Zealand, Jutland, or Funen), age group (± 10 years for adults and ± 3 years for persons <18 years of age) according to whether infected or a carrier, and similar time of diagnosis (90% ± 4 months) to limit differential recall bias. When >2 non-CC398 MRSA case-patients were identified within ± 4 months of diagnosis of the case-patient, 2 were randomly selected from the list generated.

Data Collection

After written informed consent was obtained, case-patients, controls, and non-CC398 case-patients were interviewed by using a structured telephone-administered questionnaire. Questions captured demographic and clinical data as well as information on known/identified risk factors for MRSA (including hospitalization and other medical exposures, contact sports, and travel) and hypothesized farm and animal exposures for MRSA CC398 (including living or working on a farm, exposure to farm animals, contact with farm workers, preference for eating, and contact with pets) in the year before case-patient diagnosis. Data were double-entered into Epidata version 3.1 (Statens Serum Institut, Copenhagen, Denmark) and collected and handled according to the requirements of the Danish Data Protection Agency. The study did not require ethical approval.

Statistical Analysis

Univariable and multivariable conditional logistic regression analyses were conducted to estimate matched odds ratios (MORs). Unmatched logistic regression was used for variables for which MORs could not be calculated because not enough controls were exposed to MRSA. Multivariable conditional logistic regression analysis included significant variables (based on a p value of 0.05) from univariable analysis. Stepwise exclusion was used, and variables were tested for significance by using the likelihood ratio test. Stata version 9.2 software (StataCorp, College Station, TX, USA) was used for all analyses.

Farm Investigations

For case-patients, controls, and non-CC398 case-patients reporting contact with production animals, the farm owner was contacted. If the owner consented, nasal swabs were taken from 10 randomly selected animals (from 10 different pens where possible) and tested for MRSA.

Microbiologic Analysis

Human isolates were tested by using PCR to identify the mecA gene (13), pulsed-field gel electrophoresis (PFGE) with the Harmony protocol (14), spa typing (15), and staphylococcal cassette chromosome mec (SCCmec) typing (16). Isolates were also tested for lukF/lukS genes encoding Panton-Valentine leukocidin (PVL) (17). Results of PFGE and spa typing were interpreted by using BioNumerics version 4.6 software (Applied Maths, Sint-Martens-Latem, Belgium). Because spa typing is acknowledged as being a proxy for MLST, the MLST clonal complex annotation was inferred on the basis of spa types. One of the human isolates was typed by MLST for confirmation. Furthermore, a random selection of 7 isolates were tested by PCR for the exotoxin genes tst, eta, and etb encoding staphylococcal toxic shock syndrome toxin 1, exfoliative toxin A, and exfoliative toxin B, respectively (18).

Animal swabs were plated directly on CHROM-MRSA agar (Becton Dickinson, Heidelberg, Germany) and blood agar (containing 5% bovine blood) and subsequently placed in selective broth (tryptic soy broth, 2.5% salt, aztreonam [20 mg/L], and cefoxitin [3.5 mg/L]; SSI Diagnostika, Hilleroed, Denmark). After incubation for 24 hours, the CHROM-MRSA agar was inspected for putative MRSA. Subcultivation on CHROM-MRSA was conducted with samples from the enrichment broth. Possible MRSA
colonies were subcultivated on blood agar plates, identified by PCR for the mecA gene (13), and subjected to spa typing and SCCmec typing.

Human and animal isolates underwent antimicrobial drug susceptibility testing by using disc diffusion (D) with Neosensitabs (Rosco, Taastrup, Denmark) on Danish blood agar (SSI Diagnostika) or microbroth dilution (M) (19). Susceptibility tests were performed for tetracycline (D and M), erythromycin (D and M), streptomycin (D and M), kanamycin (D), norfloxacin (D), pencillin (D), clindamycin (D), fusidic acid (D), rifampicin (D), cefoxitin (D), clindamycin (D), spectinomycin (M), sulfadiazine (M), tiamulin (M), and trimethoprim (M).

Results

Descriptive Epidemiology

Thirty-one case-patients with MRSA with spa types related to MRSA CC398 were detected from October 29, 2003, through February 16, 2007. Of these, 6 were excluded from the study because they were secondary case-patients (3 family clusters). Of the remaining eligible case-patients, we were unable to interview 4 because of a death (n = 1) and refusal to participate (n = 3). The questionnaire was therefore administered to 21 of 25 primary case-patients. Median age of the case-patients was 29 years (age range 8 months to 80 years), and 13 (62%) were female. Three case-patients reported having Dutch relatives, and 2 case-patients had a connection to the People’s Republic of China; 1 case-patient was an adopted child from China and another case-patient had adopted a child from China.

Ten case-patients (48%) reported having had an infection, of which all were skin and soft tissue infections. Moreover, sinusitis developed in 1 case-patient, and a severe invasive infection with multiorgan failure after knee surgery developed in another case-patient.

Univariable Analysis

Several exposure variables related to farms and animals were associated with CC398 in the case–control and case–case studies (Table 1). Case–control analysis also identified 4 medical-related risk factors (Table 1). No association was found in the case–control and case–case studies for the following exposures: travel 12 months before diagnosis, working in the healthcare sector, contact with primary healthcare sector (doctor, specialist), visiting an emergency department, presence of a person in the household with a skin condition, presence of a person in the household with staphylococcal infection, smoking daily, contact sports, swimming in public pools, and being an adopted child.

Table 1. Statistically significant associations by univariable analysis for infection with MRSA CC398, Denmark*

| Exposure variable | No. (%) case-patients exposed, n = 21 | Odds ratio (95% confidence interval) for MRSA infection |
|-------------------|--------------------------------------|-------------------------------------------------------|
|                   | Case–control study, population controls, n = 42 | Case–case study, non-CC398 case-patients, n = 39 |
| Animal and farm-related exposures | | |
| Lived or worked on farm with animals | 14 (67) | 22.1 (2.9–170.3) | 11.6 (2.6–51.7) |
| Worked with animals or meat | 11 (50) | 16.2 (2.0–127.8) | |
| Worked on farm with animals | 10 (48) | | |
| Lived on farm with animals | 9 (43) | 6.9 (1.5–32.8) | 7.9 (1.7–36.7) |
| Exposure to pigs | 13 (62) | | |
| Exposure to cattle | 6 (29) | | |
| Exposure to other farm animals (hens, goats, sheep) | 7 (33) | 11.1 (1.4–92.4) | 5.9 (1.2–28.8) |
| Provided antimicrobial drugs to animals | 10 (48) | | |
| Contact with farm workers | 16 (76) | 5.2 (1.4–19.3) | |
| Contact with farmer | 14 (67) | 3.2 (1.0–10.6) | |
| Contact with veterinarian | 7 (33) | 6.3 (1.3–30.7) | 6.6 (1.4–31.8) |
| Lived in countryside | 13 (62) | 7.2 (1.5–33.8) | 5.2 (1.4–18.9) |
| Had cat in home | 11 (50) | 3.2 (1.0–10.6) | 3.4 (1.1–9.9) |
| Used manure in garden | 7 (33) | 3.2 (0.9–11.0) | 6.6 (1.4–31.8) |
| Visited farm, zoo, or stables | 12 (57) | 0.8 (0.3–2.4) | 4.7 (1.3–17.4) |
| Medical-related exposures | | |
| Admission to hospital in 12 mo before diagnosis | 13 (62) | 6.8 (1.9–24.4) | 1.5 (0.4–5.2) |
| Someone in household with chronic condition | 12 (57) | 3.8 (1.2–12.5) | 2.0 (0.8–9.3) |
| Antimicrobial drug use in 12 mo before diagnosis | 9 (43) | 3.4 (1.0–11.5) | 2.6 (0.8–9.2) |
| Contact with person with skin sore or other skin infection | 5 (26) | 8.6 (1–74.9) | 0.6 (0.2–1.9) |

*MRSA, methicillin-resistant Staphylococcus aureus; CC, clonal complex.
†p<0.01, by unmatched analysis.
because of evidence of prolonged MRSA carriage (21), questions related to exposures referred to a period of 1 year before patient diagnosis. This lengthy recall period is a limitation of this study. However, all questions related to memorable activities and lifestyle choices; any bias introduced is therefore thought to be minimal.

Our finding that living or working on a farm with animals is associated with CC398 acquisition reinforces results of studies in France, the Netherlands, and Canada that indicated that CC398 is transmissible from animals to humans (3,4,6,7). Also in the Netherlands, a CC398 prevalence of 3.9% in 179 veterinarians has been de-
the human case-patient's infection was diagnosed, samples were obtained months, in some cases years, after lived on farms where these pigs were located. These or only differed by 1 additional antimicrobial drug class, was 0.7% (4/576) (comparison, the prevalence of CC398 among delegates prevalence was in German and Dutch delegates (23). In comparison, the prevalence of CC398 among delegates attending several national animal conferences in Denmark was 0.7% (4/576) (24).

Pig isolates from contact herds were indistinguishable, or only differed by 1 additional antimicrobial drug class, from the isolates from human case-patients who worked or lived on farms where these pigs were located. These samples were obtained months, in some cases years, after the human case-patient's infection was diagnosed, findings that lend weight to the hypotheses that CC398 carriage in animals is unlikely to be transient and that animals are a reservoir of CC398. Although we isolated CC398 from 23 pigs on 4 of 5 pig farms, but not from cattle farms, this type may not be exclusive to pigs. CC398 has been shown to have a prevalence of 39% in pigs in the Netherlands (25). However, it has also been found in horses and dogs in Germany and Austria (5) and in cattle and poultry in the Netherlands (26,27). These findings are particularly interesting because S. aureus is usually host specific (28).

Our study has highlighted other important human epidemiologic aspects of CC398. The clinical picture for CC398, with ≈50% of case-patients having had an infection and all reporting skin and soft tissue infections, is similar to that seen in community-acquired MRSA isolates in general (29,30). There was a serious invasive infection with MRSA reported in a man in Denmark after surgery on his knee; arthritis and multiorgan failure also developed (31). Serious complications from CC398 have also been described in other reports including ventilator-associated pneumonia (5) and endocarditis (32). Because no statistical association was found with travel abroad, this finding indicates that CC398 is endemic among pigs in Denmark. Nevertheless, there was an overrepresentation of case-patients or their family members who had had contact with another country, 2 with adopted children from China and 3 case-patients with relatives from the Netherlands. Likewise, in the Netherlands, a child adopted from China was found to be an MRSA CC398 carrier in 2004 (12). Three family clusters were identified in the present study, which indicates that CC398 can be transmitted from person to person. This finding is not surprising because of the adaptability of MRSA. Its potential for transmission between humans has also been observed in the Netherlands (4,7). MRSA has been isolated from dairy products, beef, chicken and pork (33–37) and although foodborne transmission is plausible (34), the risk is thought to be low. Preference for eating pork was not associated with being a CC398 case-patient in our study.

A high degree of variability in the types of CC398 (resistance patterns, spa types, and SCCmec types) suggests that this type is either rapidly evolving or emerging from a hitherto unrecognized reservoir. In the latter case, CC398 must have been introduced into Denmark on more than 1 occasion or by various routes to explain the high degree of variance. When one considers the rapid adaptability of MRSA, it may only be a matter of time before we see an increased prevalence of CC398 in humans, including those in hospitals as has been recently reported in the Netherlands (12). A high prevalence of tetracycline resistance in CC398 patients in contact with pigs has also been observed in the Netherlands; this finding suggests that use of tetracyclines and possibly other antimicrobial drugs in food animals is selecting these multidrug-resistant bacteria (25). Two case-patients who were positive for PVL had direct connections to China. To our knowledge, there are no published reports of CC398 patients in China but isolates from pigs have recently been reported from Singapore, with Indonesian origin (38).

In conclusion, transmission of CC398 from a zoonotic reservoir to humans could undermine existing MRSA control programs. We therefore recommend increased awareness among healthcare professionals that animals are a possible source of MRSA infection and that the potential for person-to-person spread exists. To limit further spread, pig farmers may warrant screening and isolation on admission to hospitals as has been implemented in the Netherlands (39). However, further studies are required to better understand the human and veterinary epidemiology of this emerging zoonosis. Areas of study should include size of the reservoir in pigs, whether other animals constitute a res-

| Case-patient no. | Date of diagnosis | Resistance pattern | spa type | Contact herd | No. isolates | Resistance pattern | spa type |
|------------------|------------------|--------------------|----------|--------------|--------------|--------------------|----------|
| 1                | 2005 Mar         | tet, ery, cli, str, tmp | t034     | A            | 9            | tet, ery, cli, str, spe, tmp | t034     |
| 2                | 2005 Oct         | tet, tmp           | t034     | B            | 5            | tet, tmp           | t034     |
|                  |                  |                    |          |              | 4            | tet, str, tmp      | t034     |
|                  |                  |                    |          |              | 1            | tet, str, tmp      | t034     |
| 3                | 2006 Oct         | tet, ery, cli, str, spe, tmp | t034 | C            | 1            | tet, ery, cli, str, spe, tmp | t034 |
|                  |                  |                    |          |              | 2            | tet, str, spe, tmp | t034 |
| 4                | 2006 Nov         | tet, kan, str, spe, tmp | t034     | D            | 1            | tet, ery, cli, kan, str, spe, tmp | t034 |

*spa, staphylococcal protein A; tet, tetracycline; ery, erythromycin; cli, clindamycin; str, streptomycin; tmp, trimethoprim; spe, spectinomycin; kan, kanamycin.
ervoir of CC398, and how frequently CC398 is transmitted from animals to humans and from humans to humans. The European Union baseline survey on the prevalence of MRSA in breeding pigs, initiated in January 2008, is an important step in addressing the first of these points (40).

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Ms Lewis is a fellow with the European Programme for Intervention Epidemiology Training at the Statens Serum Institut in Copenhagen. Her primary research interest is intervention epidemiology, particularly in relation to zoonoses.

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