Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates have been known to be a cause of serious nosocomial disease in humans in Japan and other countries, showing resistance to many β-lactam and other antibiotics. The major cause of the drug resistance is ascribed to the production of penicillin-binding protein (PBP) 2', which is encoded by the mecA gene and which results in a low affinity for β-lactam antibiotics (11, 17, 31, 41). Other than MRSA isolates, isolates of coagulase-negative staphylococci from patients, such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis*, have been found to be methicillin resistant and to possess mecA and PBP 2' (18, 20, 38). The methicillin resistance in those coagulase-negative staphylococci has been considered to be controlled by the same mechanism as that which controls methicillin resistance in MRSA (20).

In animals, the first report of the isolation of MRSA appeared in 1975, in which it was reported to have been isolated from the milk of mastitic cows (5). At that time the MRSA isolates were suspected of being human contaminants because of their bacteriologic characteristics, and since then, the existence of MRSA in animals has been considered improbable. Naturally, there has been no report of the isolation of methicillin-resistant coagulase-negative staphylococci. It cannot be denied on the one hand that no serious effort has been made to detect MRSA or methicillin-resistant coagulase-negative staphylococci from animals.

Coagulase-negative staphylococci, including many species such as *Staphylococcus hyicus* (4, 35), *Staphylococcus gallinarum* (7), *Staphylococcus arlettae* (27), *Staphylococcus chromogenes* (10), *Staphylococcus xylosus* (28, 29), and *S. epidermidis* (28, 29), have commonly been isolated from the nares and skin of chickens. In most cases those coagulase-negative staphylococci were first isolated from healthy chickens, and their taxonomic positions were discussed apart from the pathogenicities. Although coagulase-negative staphylococci in chickens have generally been accepted as harmless inhabitants, it has gradually become clear that they manifest pathogenicity under suitable conditions. From dermatitis and tenosynovitis, coagulase-negative species of *S. hyicus*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *S. epidermidis*, and so on, were isolated (15, 26). Those coagulase-negative staphylococcal infections in chickens appear to be opportunistic.

We describe here the isolation of methicillin-resistant coagulase-negative staphylococci from the nares and skin of healthy chickens and discuss their pathogenic and epidemiologic roles. The mecA gene was detected by two methods based on the PCR technique, which has often been a useful tool for the detection of the mecA gene (13, 18, 21, 40), and the DNA sequence of a PCR fragment which was obtained from one of the methicillin-resistant coagulase-negative staphylococcal isolates was determined. This may be the first demonstration of the existence of the mecA gene in coagulase-negative staphylococci from animals.

**MATERIALS AND METHODS**

*Isolation of methicillin-resistant staphylococci.* A total of 280 healthy chickens (White Leghorn) in three flocks (flocks A to C) from a single farm in Hyogo Prefecture were used (Table 1). Flock A consisted of 35 4-week-old chickens. Flock B consisted of 65 3- to 4-week-old chickens. One hundred eighty chickens in flock C were introduced at 1 week of age and were reared in our laboratory, and 20 to 40 chickens were tested weekly until they were 8 weeks of age. Isolation of staphylococci was carried out from swabs taken from the nares and skin. The swabs were incubated for 48 h at 35°C with heart infusion broth (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) containing 7.5% NaCl. A portion of the broth was then spread onto a medium selective for MRSA, MS medium (19a), consisting of nutrient agar (Nissui), 25 µg of ceftizoxime (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) per ml, 1% mannitol, 3.0% NaCl, and 0.004% bromo-

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Methicillin-resistant coagulase-negative staphylococci were isolated from the nares and skin of 1- to 8-week-old healthy chickens in three flocks from a farm. Isolation of methicillin-resistant coagulase-negative staphylococci was positive for 72 (25.7%) of the 280 chickens tested, with the frequency varying from 2.2 to 100% according to flock. A total of 45 appropriate isolates were selected and subjected to identification. Of the 45 methicillin-resistant coagulase-negative staphylococcal isolates selected, 37 were identified as *Staphylococcus sciuri*, 5 were identified as *Staphylococcus epidermidis*, and 3 were identified as *Staphylococcus saprophyticus*. The distribution of the species was different among the flocks. Comparative analysis of the Smal-digested chromosomal DNA by pulsed-field gel electrophoresis revealed that the isolates could have originated from a single clone of each of *S. sciuri* and *S. saprophyticus* and three clones of *S. epidermidis*. By two methods based on the PCR technique, the mecA gene was detected in all five representative isolates of each methicillin-resistant coagulase-negative staphylococcal clone. The nucleotide sequence of a PCR fragment obtained from an isolate of *S. sciuri* was completely identical to the corresponding region of mecA genes reported in human methicillin-resistant *Staphylococcus aureus* isolates and *Staphylococcus epidermidis* isolates. The representative methicillin-resistant coagulase-negative staphylococcal isolates were resistant to many β-lactam antibiotics, and some isolates were also resistant to macrolide and aminoglycoside antibiotics. This is the first evidence of the existence of methicillin-resistant coagulase-negative staphylococci from animals possessing the mecA gene.
cresol purple at pH 7.2, and the mixture was incubated for 48 h at 30 °C. Yellow or white colonies were picked and tested for catalase. Catalase-positive colonies were Gram stained, simultaneously transferred to sensitivity disk agar-N (a modified Mueller-Hinton agar; Nissui) containing 12.5 μg of methicillin (Sigma Chemical Co., St. Louis, Mo.) per ml, and incubated for 18 h at 35 °C. Gram-positive cocci with irregular clusters which were capable of growing on the agar medium containing methicillin were tentatively regarded as methicillin-resistant staphylococci. Methicillin-resistant isolates were further tested for lysostaphin (50 and 200 μg/ml; Sigma) susceptibility and coagulation of rabbit plasma. Identification of the isolates was done with the API-Staph system (BioMerieux S.A., Marcy l’Etoile, France), and the identities were confirmed by some additional characteristics such as novobiocin resistance (1.6 μg/ml; Sigma); the presence of hemolysins; hyaluronidase; and acid production from cellulose, arabinose, ribose, xylose, glycerol, salicin, melezitose, sorbitol, and turanose.

PFGE. The procedures for pulsed-field gel electrophoresis (PFGE) analysis of SmaI-digested chromosomal DNA followed the method of George and Kloos (9). The DNA fragments were separated in a 1% agarose (SeaKem GTG; FMC Corp., Rockland, Maine) gel slab by using the CHEF-DRII system (Bio-Rad, Hercules, Calif.). Electrophoresis was performed in 0.5× Tris-borate-EDTA buffer with a ramped pulse time of 5 to 40 s at 6 V/cm for 22 h. The gel was stained with ethidium bromide (2 μg/ml) for 20 min, destained in distilled water for 60 min, and observed with a transilluminator (model TDM-20; UVP Inc., San Gabriel, Calif.).

PCR primers and amplification conditions. The PCR technique was used to detect the mecA gene (41a). Primers were designed to amplify a 258-bp fragment of the mecA gene. One of the primers (5′-GAAGGTATCATCTTGTACCC-3′) corresponds to nucleotides 375 to 394 of the skin strain, and the other primer (5′-GAAGGTATCATCTTGTACCC-3′) corresponds to nucleotides 613 to 632 of the ats strain. DNA was extracted by a standard method. Half a loopful of bacteria was harvested from an agar culture onto a glass fiber filter-paper disc (1.0 mm in diameter), transferred to a glass fiber filter-paper disc (2.5 mm in diameter), and incubated for 18 h at 30 °C. The filter-paper discs were incubated for 18 h at 35 °C. The following antibiotics were used: benzylpenicillin, which was from Meiji Seika Co. Ltd., Tokyo, Japan; tobramycin, which was from Shionogi & Co. Ltd., Osaka, Japan; kanamycin, which was from Banyu Pharmaceutical Co. Ltd., Tokyo, Japan; and tetracycline, which was from Sigma; cefmetazole, which was from Sankyo Co. Ltd., Tokyo, Japan; and tetracycline, which was from Sigma; cefazolin and ceftizoxime, which were from Sigma.

RESULTS

Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. Methicillin-resistant coagulase-negative staphylococci were isolated from the skin and/or nares of 72 (25.7%) of the 280 chickens tested (Table 1). The frequency of isolation varied according to the flock, as follows: flock A, 3 of 35 chickens (8.6%); flock B, 65 of 65 chickens (100%); and flock C, 4 of 180 chickens (2.2%). A total of 45 isolates which consisted of all isolates from flock A (3 isolates) and flock C (5 isolates) and 37 isolates randomly selected from flock B (17 from the skin and 20 from the nares) were identified by the API-Staph system and according to some additional characteristics. Three isolates from flock A were identified as S. sciuri, 33 isolates from flock B were identified as S. sciuri, 3 isolates from flock B were identified as Staphylococcus saprophilicus, 1 isolate from flock B was unidentifiable, and 5 isolates from flock C were identified as S. epidermidis.

Subsequently, the clonality of the isolates of each species was examined by PFGE analysis for comparison of SmaI-digested chromosomal DNA (Fig. 1). All 36 isolates of S. sciuri and the 1 unidentifiable isolate showed the same patterns. The unidentifiable isolate was hence regarded as S. sciuri. All three isolates of S. saprophilicus showed the same fragment patterns, which were distinct from those of S. sciuri. The five isolates of S. epidermidis were differentiated into three fragment patterns. The isolates of S. sciuri and those of S. saprophilicus could have originated from clones of each of those species, while those of S. epidermidis could be from three clones. Representative strains were then selected for each clone: S. sciuri SK1 from the nares, S. saprophilicus SK4 from the skin, and S. epidermidis SK6 from the nares and SK4 from the skin. The five representative strains were used for further study.

Detection of the mecA gene from chicken methicillin-resistant coagulase-negative staphylococci. We examined the five representative strains of S. sciuri, S. saprophilicus, and S. epidermidis for the mecA gene by a PCR technique (Fig. 2). PCR amplified the mecA gene from each of the five strains. The size
of the fragment containing the mecA gene was estimated to be 258 or 259 bp on the basis of the corresponding regions of the mecA genes from human MRSA and S. epidermidis isolates (24, 31, 42). All of the strains also showed a mecA-specific enzymatic reaction by the ED-PCR method (37).

The nucleotide sequence of the PCR fragment from S. sciuri SK1 was determined and was compared with the known sequence of the mecA gene (Fig. 3). The fragment was 259 bp long, and the sequence was completely identical to those of mecA genes from some strains of human MRSA and S. epidermidis isolates (24, 42). Comparison of the mecA gene sequence that we determined with that of the mecA gene from human MRSA strain TK784 (31; EMBL database accession number Y00688). The differences in the nucleotide sequences of the corresponding regions of the mecA genes from human MRSA strains TK784 (31), BB270 (24; EMBL database accession number X52503), 27r (42), 67 (42), and 1561 (42) and methicillin-resistant S. epidermidis WT55 (24; EMBL database accession number X52592) are indicated in the rectangles, and those of the PCR fragment from S. sciuri SK1 from a chicken are indicated in the rectangles on the last row. Solid lines bound to the rectangles denote the identities of the nucleotide sequences. The nucleotide sequence of the region of SK1 is completely identical to those of 27r, 67, 1561, and WT55.

TK784 (31), which was the first mecA gene sequence to be reported, showed differences in three positions.

**MICs of antibiotics for chicken methicillin-resistant coagulase-negative staphylococci.** The MICs of a series of antibiotics were determined for the five methicillin-resistant coagulase-negative staphylococcal strains (Table 2). S. sciuri SK1 was resistant to β-lactam antibiotics (benzylpenicillin, methicillin, oxacillin, cloxacillin, carbencillin, cephalaxin, cefmetazole, and ceftizoxime) and tetracycline and was susceptible to all other antibiotics tested.

**TABLE 2. MICs of antibiotics for representative strains of methicillin-resistant coagulase-negative staphylococci isolated from chickens**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. sciuri SK1</th>
<th>S. saprophyticus SK4</th>
<th>S. epidermidis SK6</th>
<th>S. epidermidis SK42</th>
<th>S. epidermidis SK44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylpenicillin</td>
<td>25</td>
<td>&gt;100</td>
<td>100</td>
<td>≤0.39</td>
<td>100</td>
</tr>
<tr>
<td>Methicillin</td>
<td>&gt;100</td>
<td>100</td>
<td>25</td>
<td>25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12.5</td>
<td>&gt;100</td>
<td>100</td>
<td>3.13</td>
<td>100</td>
</tr>
<tr>
<td>Carbencillin</td>
<td>100</td>
<td>&gt;100</td>
<td>25</td>
<td>6.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>1.56</td>
<td>12.5</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>3.13</td>
<td>50</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Cephalaxin</td>
<td>100</td>
<td>&gt;100</td>
<td>25</td>
<td>25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>25</td>
<td>100</td>
<td>6.25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Cefmetazole</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>25</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12.5</td>
<td>25</td>
<td>3.13</td>
<td>1.56</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.39</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>12.5</td>
<td>&gt;100</td>
<td>≤0.39</td>
<td>25</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.78</td>
<td>&gt;100</td>
<td>≤0.39</td>
<td>100</td>
<td>≤0.39</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>0.78</td>
<td>0.78</td>
<td>&gt;100</td>
<td>1.56</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>50</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤0.39</td>
<td>≤0.39</td>
<td>0.78</td>
<td>0.78</td>
<td>50</td>
</tr>
</tbody>
</table>
ble to erythromycin and the aminoglycoside antibiotics (kanamycin, gentamicin, and tobramycin). S. saprophyticus SK4 was resistant to benzylpenicillin, methicillin, oxacillin, cloxacillin, ampicillin, carbenicillin, cephalothin, cephalaxin, cefazolin, cefmetazole, cefetizoxime, tetracycline, doxycycline, erythromycin, lincomycin, and clindamycin. Three strains of S. epidermidis, SK6, SK42, and SK44, showed different resistance patterns: SK6 was resistant to benzylpenicillin, methicillin, oxacillin, cloxacillin, ampicillin, carbenicillin, cephalaxin, cefmetazole, cefetizoxime, tetracycline, erythromycin, and kana
mycin; SK42 was resistant to methicillin, oxacillin, cephalaxin, cefetizoxime, tetracycline, erythromycin, and kana
mycin; and SK44 was resistant to benzylpenicillin, methicillin, oxacillin, cloxacillin, ampicillin, carbenicillin, cephalaxin, cefmetazole, cefetizoxime, tetracycline, erythromycin, lin
comycin, and clindamycin. Other characteristics of chicken methicillin-resistant coagulase-negative staphylococci. PCase production and phage typing were performed. All except one of the five methicillin-resistant coagulase-negative staphylococcal strains (S. epidermidis SK42) produced PCase. The phage types of three strains of S. epidermidis could be determined at 100× the routine test dilution, and the strains could be differentiated into three phage patterns by using the phages of Pulverer et al. (23): phage type Ph9 for strain SK6, phage type Ph14/Ph15 for strain SK42, and phage type Ph9/Ph12/Ph13 for strain SK44. S. sciuri SK1 and S. saprophyticus SK4 were both untypeable.

The MICs of methicillin for the five strains were determined at temperatures of 30, 35, and 43°C. With the elevation of temperature the MICs for all isolates were reduced. Namely, the MICs for S. sciuri SK1 decreased from >100 to 50 μg/ml, those for S. saprophyticus SK4 decreased from >100 to 12.5 μg/ml, those for S. epidermidis SK6 decreased from 25 to 1.5 μg/ml, those for S. epidermidis SK42 decreased from 25 to 3.13 μg/ml, and those for S. epidermidis SK44 decreased from >100 to 6.25 μg/ml.

DISCUSSION

The present study shows that chickens harbor methicillin-resistant coagulase-negative staphylococci, including S. sciuri, S. saprophyticus, and S. epidermidis, at a considerable rate, although isolates from chickens on only a single farm were examined. Staphylococci of these three species have been isolated from chickens or chicken house air (25, 26, 28, 29) and may not be particular to the chickens of the farm. The presence of the mecA gene in the methicillin-resistant coagulase-negative staphylococcal isolates was revealed by two PCR methods for which pairs of primers were used to amplify the different regions of the mecA gene, with confirmation of the results performed by DNA sequencing of a PCR fragment from an isolate of S. sciuri. The isolates were resistant to many β-lactam antibiotics and the methicillin MICs for the isolates were reduced at higher temperatures, which are analogous to the characteristics of human MRSA isolates and methicillin-resistant coagulase-negative staphylococci (1, 2, 38). The methicillin resistance of methicillin-resistant coagulase-negative staphylococci from chickens may be due to the emergence of PBP 2', which is encoded by the mecA gene.

Methicillin-resistant S. sciuri strains were first detected in chickens of flock A at a relatively low frequency but were then detected at a high frequency in chickens of flock B tested 2 months later. The PFGE analysis may indicate that a single clone of the species spread on the farm. Colonization with the clone might have occurred. On the contrary, when testing was done 1 year later, S. epidermidis isolates were found only in 1- to 2-week-old chickens of flock C and were not found thereafter. The S. epidermidis isolates might have been in transit and did not colonize the chickens of the flock. The pathogenic role of the methicillin-resistant coagulase-negative staphylococcal isolates is unclear. Because coagulase-negative staphylococcal infections in chickens are considered to be opportunistic (15, 26), the isolates from the present study could be pathogenic under the appropriate conditions. The chickens tested did not reveal any signs of disease at the time of testing.

It is difficult to define whether the methicillin-resistant coagulase-negative staphylococcal isolates were human contaminants. However, because of the epidemiologic aspects of the S. sciuri isolates described above, it cannot be considered that the isolates of the species were merely contaminants. With regard to S. epidermidis, three clones were found at one time, as proved by PFGE analysis. It appears unlikely that five clones of methicillin-resistant coagulase-negative staphylococci consisting of three species were directly transmitted by humans or that those isolates originated from humans and adapted to the chickens on the farm. The farm may not be affected by human methicillin-resistant staphylococci like a hospital.

A question arises as to when and from where methicillin-resistant coagulase-negative staphylococci or the mecA gene was introduced into staphylococci in chickens. This unanswered question is the same as the case for MRSA and methicillin-resistant coagulase-negative staphylococci that infect humans. With regard to humans, the dominant hypothesis is of the horizontal transfer of the mecA gene between staphylococcal species because of the following evidence: (i) the methicillin resistance protein, PBP 2', detected in MRSA and species of methicillin-resistant coagulase-negative staphylococci has a constant electrophoretic migration rate, whereas those of the other PBPs differ according to species (20, 33); (ii) the nucleotide sequences of the mecA gene between strains of S. aureus and S. epidermidis are well conserved (24); (iii) the mecA gene is carried by a mobile genetic element (32, 36, 38); (iv) strains of MRSA have many divergent phylogenetic lineages defined by analysis of allelic variation at chromosomal enzyme loci (19).

It may be possible that a chicken coagulase-negative staphylococcus once acquired the mecA gene from a human methicillin-resistant staphylococcus and that the gene was prevailed among chicken staphylococci. This unan-
swered question is the same as the case for MRSA and methicillin-resistant coagulase-negative staphylococci that infect humans. With regard to human, the dominant hypothesis is of the horizontal transfer of mesicillin-resistant staphylococci between staphylo
coccal species because of the following evidence: (i) the methicillin resistance protein, PBP 2', detected in MRSA and species of methicillin-resistant coagulase-negative staphylococci has a constant electrophoretic migration rate, whereas those of the other PBPs differ according to species (20, 33); (ii) the nucleotide sequences of the mecA gene between strains of S. aureus and S. epidermidis are well conserved (24); (iii) the mecA gene is carried by a mobile genetic element (32, 36, 38); (iv) strains of MRSA have many divergent phylogenetic lineages defined by analysis of allelic variation at chromosomal enzyme loci (19).

The present study shows that chickens harbor methicillin-resistant coagulase-negative staphylococci, including S. sciuri, S. saprophyticus, and S. epidermidis, at a considerable rate, although isolates from chickens on only a single farm were examined. Staphylococci of these three species have been isolated from chickens or chicken house air (25, 26, 28, 29) and may not be particular to the chickens of the farm. The presence of the mecA gene in the methicillin-resistant coagulase-negative staphylococcal isolates was revealed by two PCR methods for which pairs of primers were used to amplify the different regions of the mecA gene, with confirmation of the results performed by DNA sequencing of a PCR fragment from an isolate of S. sciuri. The isolates were resistant to many β-lactam antibiotics and the methicillin MICs for the isolates were reduced at higher temperatures, which are analogous to the characteristics of human MRSA isolates and methicillin-resistant coagulase-negative staphylococci. The nucleotide sequence of a PCR fragment from a representative strain of S. sciuri was completely identical to the corresponding region of some of the known mecA genes from human MRSA and S. epidermidis isolates (24, 42). The horizontal transmission of the mecA gene may be true for human staphylococci; however, which organism acquired the mecA gene first, MRSA or methicillin-resistant coagulase-negative staphylococci? We would like to include chicken methicillin-resistant coagulase-negative staphylococci as part of this question and then present the hypothe
sis that chickens are a reservoir for mecA as a cause of human disease.

On the other hand, a selective medium (MS medium) was used for the isolation of methicillin-resistant staphylococci in the present study. The reason was that cephaloridine is one of the inducers of PBP 2' (39) and that there are methicillin-susceptible S. aureus isolates in which mecA gene transcription could not be induced by methicillin but could be induced by other β-lactam antibiotics (12). Although colonies of every culture were not tested, colonies of gram-positive and catalase-negative cocci were observed in almost all of the cultures. Enterococi, which are commonly isolated from the chicken intestinal flora (6), are known to be uniformly resistant to cephalo anti
biotics (8), and enterococci other than methicillin-resistant
coagulase-negative staphylococci might be suspects for those colonies.

We attempted to isolate S. aureus strains from the nares and skin of 130 chickens (1 to 4 weeks of age), including some of the chickens (flocks A and B) used for the isolation of methicillin-resistant coagulase-negative staphylococci in the present study (24a). S. aureus strains were isolated from 103 (79.2%) of the 130 chickens. All of the isolates did not grow on an agar medium containing either 12.5 μg of methicillin per ml or 6.25 μg of oxacillin per ml and were regarded as methicillin susceptible. In the course of the present study, no coagulase-positive Staphylococcus isolate was selected on the selective medium. These facts suggest that the mecA gene could rarely be transferred from methicillin-resistant coagulase-negative staphylococci to S. aureus isolates in chickens. It is considered that on a few occasions human S. aureus isolates have acquired the mecA gene (16).

With respect to animal MRSA isolates Devrieze and Hommez (5) reported the isolation of MRSA from the milk of mastitic cows in 1975. From the results of phage typing and as a result of some of the biological characteristics that would later be adopted as key characteristics for biotyping S. aureus isolates (3), they concluded that the MRSA isolates might be transmitted incidentally by humans. There has been no subsequent report on animal MRSA isolates. No MRSA isolates were found among our collection of S. aureus strains isolated from chickens in Japan between 1970 and 1993 or from Argentina, Belgium, Bulgaria, Great Britain, or Northern Ireland (n = 511), pigs (n = 56), mice (n = 48), the milk of mastitic cows (n = 6), meats (n = 111), and fish (n = 19) (data not shown). It seems likely that MRSA isolates may not be inhabitants of animals. However, recently, MRSA strains have been isolated from race horses with uertis (1a) and bulk milk (11a) in Japan. The origins of these MRSA isolates are not known; i.e., there is no evidence that they were transmitted by humans or that they acquired the mecA gene from animal methicillin-resistant coagulase-negative staphylococci.

Although we have studied the ecology and epidemiology of animal staphylococci for years (14, 28–30), the present study is the first attempt to detect methicillin-resistant coagulase-negative staphylococci showing that they were isolated from the chickens on a farm. Methicillin-resistant coagulase-negative staphylococci could be distributed extensively in chickens or other animals. We are now starting a survey of methicillin-resistant coagulase-negative staphylococci in animals with the expectation of trying to obtain an understanding of the distribution and origin of the mecA gene.

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REFERENCES


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