tiofur for livestock. However, why CMY-2 type class C β-lactamase is predominantly found in livestock in the United States is not clear. The types of antimicrobial agents and their use for livestock in that country may have contributed to its high prevalence of CMY-2 producers, although no statistical data are available about the differences in usage of antimicrobial agents between the United States and Japan. Continuous and prospective investigations of veterinary usage of the antimicrobial agents as well as surveillance of antimicrobial-resistance seem necessary for preventing the emergence and further proliferation of antimicrobial-resistant bacteria in livestock.

The CTX-M-2 producers were not considered to reflect a clonal expansion of an *E. coli* strain carrying $bla_{CTX\text{-}M\text{-}2}$ because five distinct RAPD patterns and plasmid profiles were identified in the nine isolates. These findings suggest that stealthy plasmid-mediated dissemination of $bla_{CTX\text{-}M\text{-}2}$ gene among *E. coli* strains might be under way with the continuous consumption of the third-generation cephalosporin for veterinary use. Conjugal transfer of R-plasmid might occur in the intestinal tract, which is the main habitat of ESBL producers (17,39). Both strains GS553 and GS554 were isolated from the same fecal sample and produced the same β-lactamase, but they were different in terms of RAPD analysis and plasmid profile. Frequencies of transfer of the isolates were high (Table 3). These results suggested that conjugal transfer of the R-plasmids also occurred in the intestinal tract of cattle. Therefore, the possibility of further transfer of the resistance profile of *E. coli* to expanded-spectrum cephalosporins to other pathogenic bacteria such as *Salmonella* spp. and diarrheagenic *E. coli* should not be ignored.

The isolates in this study did not correspond to the serotypes of pathogenic *E. coli*, and they did not possess the virulence factors assayed. However, lack of virulence factors might contribute to subclinical increase of healthy carriers of these strains and might promote their dissemination among both cattle and human. Especially in livestock, environmental contamination and transmission among individual animals by these strains could expand rapidly because of their breeding system. Therefore, CTX-M-2 producers may well be disseminated even further in cattle farms hereafter. Although nosocomial bacteria that produce extended-spectrum class A β-lactamases have thus far been considered to emerge only among in humans, our study suggested that CTX-M-2 producers could potentially emerge in livestock and that cattle might be an original reservoir of CTX-M-2 producers. Therefore, active and continuous surveillance and strategic countermeasures are necessary for antimicrobial-resistant bacteria, including those strains producing such β-lactamases as CTX-M-type, CMY-type (37,40) and metalloenzymes (16) in livestock, especially in countries where these producers have emerged in human populations.

This work was supported by grant nos. H12-Shinko-19 and H12-Shinko-20 from the Ministry of Health, Labor and Welfare, Japan.

Mr. Shiraki is a microbiological specialist in the isolation and identification of pathogenic microorganisms from foods, environments, and clinical samples. His major research interests are epidemiology and the mechanisms of antimicrobial resistance in life-threatening bacteria. He conducts microbiological diagnostic tests of microbial pathogens isolated from clinical institutions and works in the Department of Infectious Diseases, Gifu Prefectural Institute of Health and Environmental Sciences.

References

1. Knothe H, Shah P, Kromery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection 1983;11:315–7.
2. Rasheed JK, Anderson GJ, Queenan AM, Biddle JW, Oliver A, Jacoby GA, et al. TEM-71, a novel plasmid-encoded, extended-spectrum β-lactamase produced by a clinical isolate of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2002;46:2000–3.
3. Rasheed JK, Anderson GJ, Yigit H, Queenan AM, Domenech-Sanchez A, Swenson JM, et al. Characterization of the extended-spectrum β-lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC 700603), which produces the novel enzyme SHV-18. Antimicrob Agents Chemother 2000;44:2382–8.
4. Ishii Y, Ohno A, Taguchi H, Imajo S, Ishiguro M, Matsuzawa H. Cloning and sequence of the gene encoding a cefotaxime-hydrolyzing class A β-lactamase isolated from *Escherichia coli*. Antimicrob Agents Chemother 1995;39:2269–75.
5. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. CTX-M-type β-lactamases: an emerging group of extended-spectrum enzymes. Int J Antimicrob Agents 2000;14:137–42.
6. Gniadkowski M, Schneider I, Palucha A, Jungwirth R, Mikiewicz B, Bauernfeind A. Cefotaxime-resistant *Enterobacteriaceae* isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. Antimicrob Agents Chemother 1998;42:827–32.
7. Bonnet R, Sampaio JL, Labia R, De Champs C, Sirot D, Chanal C, et al. A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob Agents Chemother 2000;44:1936–42.
8. Sabaté M, Tarragó R, Navarro F, Miró E, Vergés C, Barbé J, et al. Cloning and sequence of the gene encoding a novel cefotaxime-hydrolyzing β-lactamase (CTX-M-9) from *Escherichia coli* in Spain. Antimicrob Agents Chemother 2000;44:1970–3.
9. Simarro E, Navarro F, Ruiz J, Miró E, Gómez J, Mirelis B. *Salmonella enterica* serovar Virchow with CTX-M-like β-lactamase in Spain. J Clin Microbiol 2000;38:4676–8.
10. Bradford PA, Yang Y, Sahm D, Grope I, Gardovska D, Storch G. CTX-M-5, a novel cefotaxime-hydrolyzing β-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. Antimicrob Agents Chemother 1998;42:1980–4.
11. Nordmann P. Trends in β-lactam resistance among Enterobacteriaceae. Clin Infect Dis 1998;27(Suppl 1):S100–6.

12. Hirakata Y. Extended-spectrum β-lactamases (ESBLs) producing bacteria [in Japanese]. Nippon Rinsho 2001;59:694–700.
13. Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum β-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. FEMS Microbiol Lett 2000;184:53–6.
14. Kurokawa H, Yagi T, Shibata N, Shibayama K, Kamachi K, Arakawa Y. A new SHV-derived extended-spectrum β-lactamase (SHV-24) that hydrolyzes ceftazidime through a single-amino-acid substitution (D179G) in the omega-loop. Antimicrob Agents Chemother 2000;44:1725–7.
15. Arakawa Y, Ike Y, Nagasawa M, Shibata N, Doi Y, Shibayama K, et al. Trends in antimicrobial-drug resistance in Japan. Emerg Infect Dis 2000;6:572–5.
16. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, et al. Multifocal outbreaks of metallo-β-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. Antimicrob Agents Chemother 1996;40:349–53.
17. Komatsu M, Aihara M, Shimakawa K, Yamanaka T, Matsuo S. Detection of extended-spectrum β-lactamases producing *Enterobacteriaceae* in feces [in Japanese]. Kansenshogaku Zasshi 2000;74:250–8.
18. Kawakami S, Ono Y, Yamamoto M, Matumura M, Okamoto R, Inoue M, et al. Extended-spectrum β-lactamase (ESBL) produced by *Escherichia coli* and *Klebsiella pneumoniae* isolated from Teikyo University Hospital—the second report. [in Japanese] Kansenshogaku Zasshi 2000;74:24–9.
19. Bates J, Jordens Z, Selkon JB. Evidence for an animal origin of vancomycin-resistant enterococci. Lancet 1993;342:490–1.
20. Fone DL, Barker RM. Associations between human and farm animal infections with *Salmonella typhimurium* DT104 in Herefordshire. Commun Dis Rep CDR Rev 1994;4:R136–40.
21. Endtz HP, Mouton RP, van der Reyden T, Ruijs GJ, Biever M, van Klingeren B. Fluoroquinolone resistance in *Campylobacter* spp isolated from human stools and poultry products. Lancet 1990;335:787.
22. Teshager T, Domínguez L, Moreno MA, Saénz Y, Torres C, Cardeñosa S. Isolation of an SHV-12 β-lactamase-producing *Escherichia coli* strain from a dog with recurrent urinary tract infections. Antimicrob Agents Chemother 2000;44:3483–4.
23. Bradford PA, Petersen PJ, Fingerman IM, White DG. Characterization of expanded-spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. J Antimicrob Chemother 1999;44:607–10.
24. Gannon VPJ, D'souza S, Graham T, King RK, Rahn K, Read S. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J Clin Microbiol 1997;35:656–62.
25. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988;10:867–78.
26. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Ninth informational supplement, M100-S9. Wayne (PA): The Committee; 1999.
27. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard M7-A4. Wayne (PA): The Committee; 1997.
28. Nakamura T, Uchida S, Heijyo H, Masuda M, Takahashi H, Komatsu M, et al. A SHV-derived extended-spectrum β-lactamase (SHV-12) produced by an *Escherichia coli* recovered from wound abscess in post operative case with rectal carcinoma [in Japanese]. Kansenshogaku Zasshi 2000;74:112–9.
29. Radice M, González C, Power P, Vidal MC, Gutkind G. Third-generation cephalosporin resistance in *Shigella sonnei*, Argentina. Emerg Infect Dis 2001;7:442–3.
30. Thomson KS. Controversies about extended-spectrum and AmpC β-lactamases. Emerg Infect Dis 2001;7:333–6.
31. Bauernfeind A, Stemplinger I, Jungwirth R, Ernst S, Casellas JM. Sequences of β-lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other β-lactamases. Antimicrob Agents Chemother 1996;40:509–13.
32. Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, et al. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N Engl J Med 2000;342:1242–9.
33. Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, et al. Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC β-lactamase. JAMA 2000;284:3151–6.
34. Casin I, Breuil J, Brisabois A, Moury F, Grimont F, Collatz E. Multidrug-resistant human and animal *Salmonella typhimurium* isolates in France belong predominantly to a DT104 clone with the chromosome- and integron-encoded β-lactamase PSE-1. J Infect Dis 1999;179:1173–82.
35. Winokur PL, Brueggemann A, DeSalvo DL, Hoffmann L, Apley MD, Uhlenhopp EK, et al. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. Antimicrob Agents Chemother 2000;44:2777–83.
36. Kariuki S, Gilks CF, Kimari J, Muyodi J, Waiyaki P, Hart CA. Plasmid diversity of multi-drug-resistant *Escherichia coli* isolated from children with diarrhoea in a poultry-farming area in Kenya. Ann Trop Med Parasitol 1997;91:87–94.
37. Yan JJ, Ko WC, Chiu CH, Tsai SH, Wu HM, Wu JJ. Emergence of ceftriaxone-resistant *Salmonella* isolates and rapid spread of plasmid-encoded CMY-2-like cephalosporinase, Taiwan. Emerg Infect Dis 2003;9:323–8.
38. Deshpande L, Pfaller MA, Jones RN. In vitro activity of ceftiofur tested against clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* including extended spectrum β-lactamase producing strains. Int J Antimicrob Agents 2000;15:271–5.
39. Ohkawa T, Yoshinaga M, Ikarimoto N, Miyanohara H, Miyata K, Doi Y, et al. Characterization of *Klebsiella pneumoniae* and *Escherichia coli* strains that produce CTX-M-2-type broad spectrum β-lactamase isolated from a child with leukemia. Pediatr Infect Dis J 2002;21:260–2.
40. Doi Y, Shibata N, Shibayama K, Kamachi K, Kurokawa H, Yokoyama K, et al. Characterization of a novel plasmid-mediated cephalosporinase (CMY-9) and its genetic environment in an *Escherichia coli* clinical isolate. Antimicrob Agents Chemother 2002;46:2427–34.

Address for correspondence: Yutaka Shiraki, Department of Infectious Diseases, Gifu Prefectural Institute of Health and Environmental Sciences, 1-1 Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan; fax: +81-583-80-2131; email: shiraki-yutaka@pref.gifu.lg.jp

Pediatrics International (2003) **45**, 238–245

Feature Article

Prevention of methicillin-resistant *Staphylococcus aureus* infections in neonates

HIROYUKI KITAJIMA

Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan

Abstract Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in neonatal intensive care units (NICU) and normal newborn nurseries in Japan were investigated, and various methods of preventing transmission were evaluated.

In the late 1980s, MRSA which had spread in adult wards also invaded NICU. Very low birthweight or premature infants had become the targets of MRSA infection and this has now become a serious problem. Recent reports have revealed that 87% of major NICU in Japan have suffered from MRSA infections. However, we have found that preventive measures can greatly reduce the risk of a newborn being infected by a carrier, while also controlling the disease caused by MRSA infection. Recently, MRSA infections in normal newborn nurseries have also become a serious problem in pediatric departments. Methicillin-resistant *Staphylococcus aureus* which can colonize in the newborn baby just after birth, is passed on to the newborn by carrier medical staff. It was found to be of great importance that infant's mothers hold and nurse their babies immediately after birth, and start breast-feeding while still in the delivery room. Furthermore, the most appropriate and ideal newborn nursery is one where mother and child are roomed together and there is little intervention by the hospital. In neonatal care, it is of utmost importance to treat carriers of MRSA bacteria, and to inhibit the spread of the bacterium in babies by taking standard precautionary measures.

Key words methicillin-resistant *Staphylococcus aureus* (MRSA), mupirocin, newborn, surveillance.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was reported for the first time in Britain in 1961 and an increase in infections was seen in western countries in the 1970s, which soon became a very serious problem. In the 1980s, the infection rate decreased rapidly with the well-controlled use of antibiotics in each country. However, an exponential increase in the isolation rate of MRSA could be seen in most Japanese hospitals (Fig. 1).[1]

The race to develop new cephem antibiotics against Gram-negative bacilli and the wide use of these new antibiotics in adults as a preventive measure caused a resistance to the Gram positive cocci. In recent years, one of the dominant strains of MRSA (type II coagulase) which possessed a toxic shock syndrome toxin–1 (TSST–1) has been the most frequently isolated strain from hospitalized patients (Fig. 2).[1]

Correspondence: H Kitajima, Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594–1101, Japan. Email: kitajima@mch.pref.osaka.jp

Received 13 December 2002.

Coagulase is one of the most virulent factors of *Staphylococcus aureus* (*S. aureus*) and can be grouped into eight different coagulase types.[2] The coagulase type II strain has increased sharply since 1983, while type IV has decreased (Fig. 2).[3] Moreover, the type II strain is the most toxic because it has various enterotoxins and/or toxic shock syndrome toxin–1 (TSST–1) which are rarely seen in other strains.[1]

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections, especially neonatal TSS-like exanthematous disease (NTED), became a large problem in NICU (neonatal intensive care units) in the 1990s. The pathogenesis of NTED was elucidated by Takahashi *et al.*[4] who revealed that NTED is induced by the superantigen TSST–1 produced by MRSA.[5] It is thought that these β-lactam resistant strains of MRSA spread rapidly in the adult population in the mid 1980s, and consequently spread from adult wards to NICUs by rotating medical staff who were carriers.

In the 1980s, aminobenzyl-penicillin (AB-PC) and gentamicin (GM) were used for initial treatment in NICU, and these were originally the world-wide standard. However, newborn babies who enter NICU in an aseptic state are easy targets for colonization by the bacteria already present in the ward.

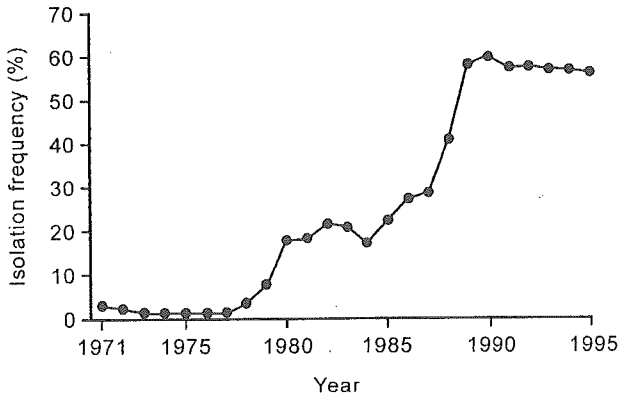

Fig. 1 Annual changes in the proportion of Methicillin-resistant *Staphylococcus aureus* (MRSA) in all *Staphylococcus aureus* isolates from most general hospitals in Japan. A rapid increase in MRSA has been observed since the early 1980s, reaching a rate of about 60%.

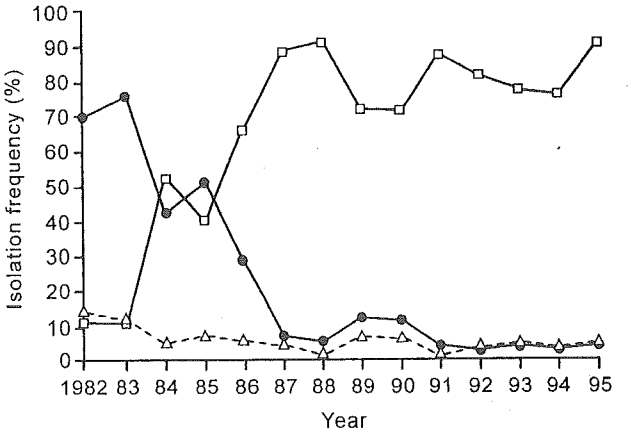

Fig. 2 Annual changes in the proportion of coagulase types of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in Japan. A significant increase of type II strains and a decrease in other types since the mid-1980s has been noted. (□) Type II; (△), Type III; (■), Type IV.

Moreover, a hospital setting in which there are regular rotations of medical staff between the NICU and geriatric or adult wards where MRSA cases are rampant may also contribute to the infiltration of MRSA into the NICU. In such cases, carrier staff could easily introduce MRSA into NICU. It is therefore necessary when considering preventive measures against MRSA infection, to take into account the entire hospital and its operating system in order to establish the origin(s) of the infection. In fact, from a bacteriologic point of view, it can be said that the care of newborn in an aseptic state by anyone other than its mother is extremely dangerous. The most preferable and safest method of care of a newborn is for it to be handled by others only after it has first been in touch with common germs and flora, which is established by initial contact and care with the newborn's mother. Taking these various points into consideration, the most desirable preventive measures against transmission are discussed in the present report.

Epidemiologic data of MRSA infection in NICU

The present study is a report on data about MRSA infections in neonates of 60 NICU, including the NICU at the author's own hospital,[6] and preventive measures taken against MRSA infections. The effects these measures have on MRSA infections is reviewed.

Incidence of MRSA infection in neonates

A nation-wide investigation of MRSA infections in NICU was first reported by Shimura in 1995.[7] Acting as a research group for the Ministry of Health and Welfare, Shimura and colleagues collected data from 3250 very low birthweight (VLBW) infants of 77 NICU in which MRSA was acquired at the hospital. According to this report, the total infection rate in VLBW was 11.8% and the severe MRSA infection rate was 4.2%. The most prevalent causative bacterium of hospital acquired infection was MRSA (34%), followed by *Pseudomonas aeruginosa* (9.4%) and *Candida* species (3.8%). The cause of infection was unknown in 32.2% of cases. The same pattern of causative organisms for hospital-acquired infections in NICU was found again in 1997 by Shimura *et al.*,[8] and in 2001 by Kitajima.[6] In 2002, Sai *et al.*[9] revealed the high frequency of MRSA infection compared with other hospital-acquired infections, from investigations of 200 NICU facilities throughout Japan. This report revealed that 87% of major NICU in Japan suffered from MRSA infections.

Based on the results of the survey of nosocomial infections in 60 standard NICU in Japan (total admission number of VLBW: 2939), the MRSA infection rate was able to be estimated.[6] The average number of VLBW infants admissions in 2000 was 48.9 (±21.7 : average ±SD), and the average nosocomial infection rate for theses admissions was 12.4% (±9.7%) with an average MRSA infection rate of 5.1% (±5.7%). The most prevalent causative bacterium of hospital acquired infection was MRSA (150/358 : 41.9%) followed by *Pseudomonas aeruginosa* (3.6%), *Candida* species (4.7%), CNS (5.3%) and unknown causes (26.3%). The rate of MRSA infection among nosocomial infections varied among the NICU from 0 to 100% (total mean = 41.3%). Of the NICU which had fewer than 40 VLBW admissions a year, two-thirds showed an infection rate of 0%. However, in NICU where there were more than 60 VLBW admissions a year, the MRSA infection rate was 20–80% of total nosocomial infections (Fig 3).

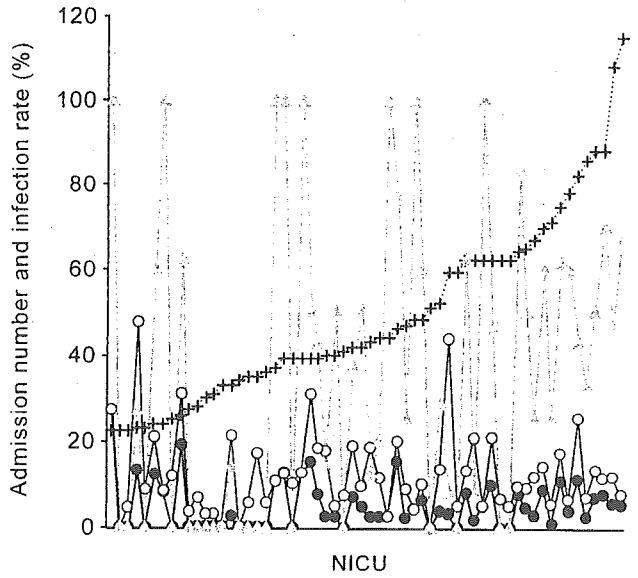

Fig. 3 Admission number and nosocomial infection rate of very low birthweight (VLBW) infants in neonatal intensive care units (NICU). (+), admission number of VLBW; (○), total nosocomial infection rate; (●), MRSA infection rate (%); (▲), MRSA/total × 100 (%).

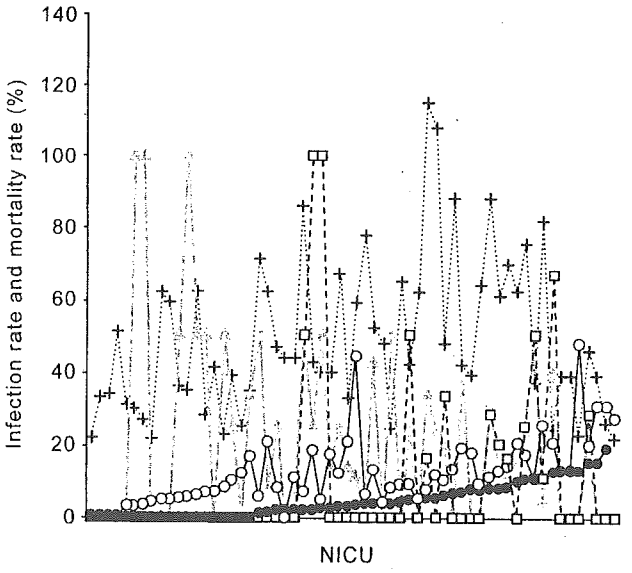

Fig. 4 Nosocomial infection rate and mortality rate of very low birthweight (VLBW). (+), admission number of VLBW; (○), total nosocomial infection rate; (▲), mortality rate by nosocomial infection; (●), MRSA infection rate of VLBW; (□), mortality rate by MRSA infection.

Clinical manifestations and mortality rates

Shimura reported that in 1994, in a study of 3250 VLBW infants, 138 (4.2%) were severely affected with MRSA infections. Of these MRSA infections, the following infection rates were found: (i) 75 sepsis (mortality rate: 16%); (ii) 28 pneumonia (32.1%); (iii) 11 enterocolitis including necrotizing enterocolitis (27.3%); (iv) 8 skin lesions (25%); (v) 7 arthritis/osteomyelitis (0%); (vi) 4 abscesses (0%); (vi) 3 meningitis/ventriculitis (0%); and (viii) 2 other lesions (0%).[7] The author of the present study found similar infection rates occured in 2000.[6] Of 2939 VLBW infants studied, 150 (5.1%) had MRSA infections at the following rates: (i) 45 sepsis (mortality rate: 22.2%); (i) 50 pneumonia (2.0%); (iii) 14 enterocolitis including necrotizing enterocolitis (35.7%); (iv) 6 skin lesions (0%); (v) 3 arthritis/osteomyelitis (0%); (vi) 1 abscess (0%); (vii) 3 meningitis/ventriculitis (33.3%); (viii) 31 NTED (0%); and (ix) 5 other lesions (0%).

The data of the latter study revealed that MRSA mainly affected neonates with extremely low birthweight (ELBW). Sepsis (ELBW/VLBW: 35/45) decreased, but milder pneumonia (39/50) increased, although enterocolitis (10/14), arthritis (3/3) and meningitis (3/3) did not change. However, NTED (5/31) which was not reported in 1994, became a significant problem in VLBW neonates in 2000.

The mean mortality rates due to all nosocomial infections of VLBW and due to MRSA infection were 17.6 ± 26.7% (mean ± SD) and 14.9 ± 24.5%, respectively.[6] High infection mortality rates of over 50% were seen in NICU with low MRSA infections rates (between 2.5 and 6% of total nosocomial infections). In these NICU the cause of death due to infection may be attributed to organisms other than MRSA. The mortality rate of each causative bacterium of hospital acquired infection was as follows: MRSA (13%), *Pseudomonas aeruginosa* (46%), *Candida* species (53%), CNS (21%) and unknown causes (11.7%) (Fig. 4).

Essential principals of prevention of MRSA transmission

If there have been no problems in the delivery of a newborn baby, it is of great importance that the mother immediately holds the newborn to establish the mother's original bacterial florae onto the child. These common bacterial florae would work to prevent the colonization of more alien and harmful pathologic bacteria. Strict adherence to the following seven guidelines would significantly help prevent nosocomial infections. These guidelines should generally be applied to all newborns in the NICU, except guideline number two, which concerns infants no longer in the NICU.

1 Skin contact and early breast-feeding (for the mouth and intestinal tract) by the mother should be carried out immediately after delivery.
2 The practice of rooming the mother and infant in the same room as well as placing the beds side by side should be

implemented. This can assist in the reduction of horizontal infection from other caregivers.

3 The growth of *bifido bacteria* in the intestines of the neonates should be promoted, by immediate maternal care and the feeding of breast milk.

4 The practice of having staff wash their hands with disinfectant immediately before and after contact with newborns and before and after bathing infants, should be enforced. The bathtub should be disinfected after each use.

5 All instruments (thermometers, stethoscopes, incubators etc.) should be thoroughly sterilized, and be used in the care of only one infant at a time.

6 In the case of a nosocomial MRSA infection, carrier staff should immediately be screened and treated with mupirocin nasal cleaning.

7 The noses and ears of infected MRSA patients in NICU should immediately be cleansed with mupirocin, and their skin cleansed with an acidic water shower at the same time.

Methods to prevent MRSA infections in neonates

As a defence against MRSA infection, it is of utmost importance to establish similar but non-toxic bacterial species as normal bacterial flora on the newborn immediately after birth.

(1) Bacterial flora of the nose and throat

The embryo in the womb exists in a germ-free state but becomes exposed to bacterial flora upon passing through the mother's birth canal. Immediately after delivery, the newborn infant's mouth should be placed on the mother's nipple in order to receive the bacterium on the mother's skin, and particularly, to induce the flow of the mother's breast milk which should be given at the earliest possible stage. *Streptococcus* species and *Neisseria*, which breed most easily among the various bacteria present, can then rapidly colonize. Newborns who were breastfed while rooming together with their mothers had a rapid increase in such common α or Á-*Streptococcus* in their mouths, according to data of Fukuda *et al.*[11]

Significantly, it was proven that the precolonization of the common α- and/or Á-*Streptococcus* inhibited the settling of MRSA in the noses and mouths of newborns, according to a report of Uehara *et al.*[12] Moreover, Nakamura has reported that the colonization rate of MRSA in the mouths of ELBW infants could be significantly lowered by spreading the mother's breast milk over and into the mouths of such infants immediately upon entering the NICU.[13]

(2) Bacterial flora of the skin

A common flora of the skin is *Staphylococcus epidermidis* which can be found on every adult. The most serious flora for newborns are coagulase positive *Staphylococcus aureus* such as MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA). All of these bacterial strains establish themselves in each in the hair follicles of the host and compete to survive.[14] In a report on NTED, newborns delivered by Caesarean section were found to be infected earlier and at a higher rate than infants of natural vaginal delivery.[15] This clearly shows that MRSA can colonize much more easily in aseptic skin than in skin already colonized with *Staphylococcus epidermidis* derived from the mother through vaginal birth. It can also be seen that, in the case of NTED, the MRSA bacteria carriers are healthcare personnel and that there is clearly horizontal infection.

It can therefore be deduced that one of the most effective preventive methods is to thoroughly occupy the hair follicles with a non-toxic similar bacterial strain to keep the toxic strain from being established. However, regardless of whether an infant is born by Caesarean section or natural vaginal delivery, skin to skin contact between a newborn and its mother should be established immediately upon birth in the delivery room so that the mother's beneficial *Staphylococcus epidermidis* bacteria can be transferred to the child at the earliest possible moment. This has been referred to as 'Kangaroo care' or 'Touch care' and should be begun in the delivery room. This could also be considered the first stage in infection control for a newborn baby's skin.

(3) Hand washing before and after touching babies

A strict policy of hand-washing before and after contact with newborns is the most important step in infection control, yet it is one of the least practiced. Even without the use of a disinfectant, washing the hands only with running tap water can still considerably lessen the risk of infection. However, because many strains of MRSA have a tolerance to chlorhexidine gluconate and other similar disinfectants in soap, hospitals should be made aware that using such disinfectants or soaps are not an effective means of prevention and are only as good as using tap water.

(4) Use of gloves

When gloves are used as an infection control measure when handling newborns, the MRSA isolation rate was found to decrease, according to several reports.[16,17] However, even a temporary expulsion of MRSA from the ward (i.e. MRSA no longer detected in the ward) is difficult unless strict adherence to the preventive measures described in the present report are also carried out.

(5) How to remove MRSA from the carrier neonates

Once MRSA colonizes in a newborn, the baby can easily become a long-term carrier, especially in ELBW infants. This can occur when no normal or common bacteria to defend against the MRSA strains are present on or around the newborn. Recently, mupirocin has been found to be effective

Table 1 Treatment of MRSA carriers of the patients and staff

Treatment number	Date begining treatment period (1 week)	Objectives		MRSA carriers		Data		
		patients	staff	patients	staff	MRSA eradicated	MRSA not eradicated (NEW)	Discharged
1	02.11.1998	39	74	13	1	5	6	2
2	14.12.1998	37	74	17†	0	4	10 (7‡)	3
3	22.02.1999	43	81	18†	2	6	8 (3‡)	4
4	08.04.1999	40	–	7†	–	2	5 (2‡)	0
5	21.05.1999	37	–	6†	–	0	3 (1‡)	3
6	23.06.1999	38	19	11†	2	6	4 (1‡)	1

†including new carriers, ‡MRSA newly isolated patients. MRSA, Methicillin-resistant *Staphylococcus aureus*, NEW, number of new carriers.

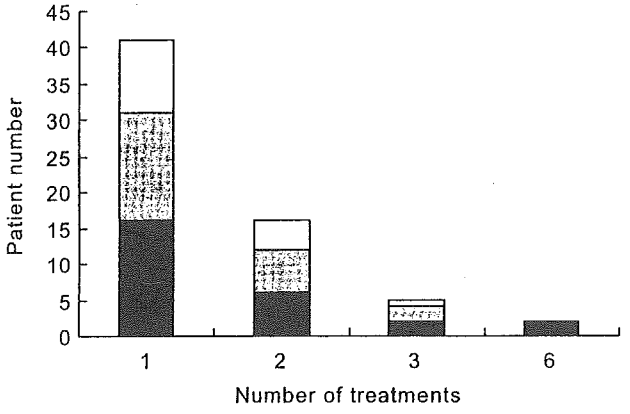

Fig. 5 Effectiveness of Mupirocin and acid water shower for eradication of Methicillin-resistant *Staphylococcus aureus* (MRSA) from the colonized patients. (■), MRSA not eradicated; (□), MRSA eradicated; (□), discharged.

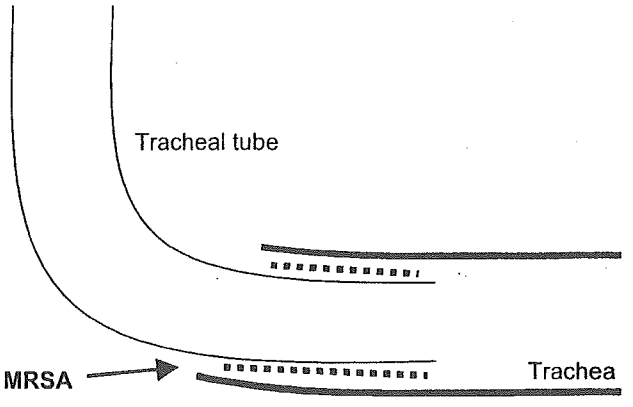

Fig. 6 Methicillin-resistant *Staphylococcus aureus* (MRSA) can survive and grow beneath the tracheal tube, and can colonize beneath the endo-tracheal tube, possibly producing some kind of toxins or growth inhibitors. It is difficult to eradicate MRSA from this site; however, mupirocin ointment applied to the surface of the tracheal tube is very effective in killing MRSA.

in eradicating MRSA from carrier babies. Supporting evidence showing the same results regarding the eradication of MRSA from newborns has also been found. The guidelines described in the present report were found to have an eradication rate of approximately 50% for MRSA (Table 1 and Fig. 5). In patient treatments 4 and 5, the eradication rates were very low at 29.5% (2/7) in treatment 4, and 0% (0/3) in treatment 5. Up until these treatments we had used a benzalconeum chloride solution as a disinfectant for the skin. However, remaining MRSA strains in our NICU were resistant to these disinfectants including chlorhexidine gluconate, as described later in the sterilization of incubators. Thereafter we used an acid water shower after bathing in treatment 6. The eradication rate improved, reaching up to 60% (6/10). Cleaning around the tracheal tube with mupirocin ointment is especially effective in tracheotomy patients. However, the bacterium beneath the endo-tracheal canula can hide within the mucous membrane, making it difficult to eradicate, as shown in Fig. 6. When the tracheal tube is replaced, mupirocin ointment should be thinly spread outside the section to be inserted into the infant. In the case of two infants at our NICU with tracheotomy for over 2 years (shown in Fig. 5; not eradicated cases after 6 treatments), MRSA could be eradicated finally by using this method in conjunction with our usual procedures. However, mupirocin-resistant strains of MRSA have already been reported for topical long-term use in NICU in Japan, so an interval of 2 months or more should be allowed before the next disinfection round for all carriers. Of course, it is necessary to obtain approval for such treatment from the parents of the infant concerned.

Mupirocin application schedule:

1 For treatment of the nasal cavity, 3 applications per day for 3 days or for up to 7 days
2 For the external ear cavity, one application per day for 7 days

3 For the external surface of the endo-tracheal tube, once per tube change
4 For cleaning of the skin, an acid water shower once per day after bathing for 7 days.

Acquired subglottic stenosis or tracheomalacia in some intubated patients have been seen and they are very troublesome in NICU. Recently Yamada *et al.*[18] reported that MRSA with TSST-1 positive strain caused acquired subglottic stenosis in two term infants who were intubated for 10 and 13 days, respectively. They showed symptoms of NTED within their first week of life and both later required a tracheotomy. They also reported that the MRSA strains produce a toxin, named as an inhibitor of epidermal cell differentiation factor. In the past 21 years we have only had two cases of acquired tracheomalacia in 2500 babies (mainly VLBW infants weighing less than 1500 g at birth) in our NICU. Both infants were very immature at 25 weeks of gestation and carried MRSA (coagulase type II) in the trachea for a few days of life, but they did not show respiratory infections and had no traumatic intubations. It is the opinion of the author that preventive measures can only inhibit the growth of MRSA beneath the tracheal tube.

(6) Bacterial flora of the intestines

When the aseptic fetus passes through the birth canal, it is able to receive vertical transmissions of such common bacteria as *Lactobacillus*, *Bifidobacterium*, and *Peptostreptococcus* that are present in the canal. Moreover, one specific *bifidobacteria* common in obstetric hospitals or wards, which is prevalent due to the giving of mother's milk, may initiate a horizontal infection. However, for neonates taken to the NICU and separated from the mother at birth, colonization of *bifidobacteria* is delayed, especially if routine and strict hand washing is practiced. In these cases, we artificially give ELBW infants *bifidobacteria* which are able to colonize easily in infants inside incubators so that beneficial intestinal flora can be established.[19] However, this does not work as a defence against MRSA infection of the skin or nasal cavity. Because MRSA has no affinity with the mucous membrane of the intestinal tract, even if the *Staphylococcus* originally has affinity with the skin and the mucous membrane of oral and nasal cavity, MRSA seems to disappear from the stool when eradication from the skin, mouth and nose is carried out.

However, for neonates with a surgically made stoma, it is very difficult to eradicate MRSA established on the skin around the opening of the stoma, especially when there is constant stool passage. Although it is believed that the most effective procedure is to clean the skin around the surgical opening with mupirocin, unfortunately, there is no data to provide definite evidence as yet.

Medical staff and MRSA

It is imperative that medical personnel are not carriers of MRSA. The following is a discussion of the hospital settings in which MRSA can proliferate and how to address these concerns.

(1) Easy transmission of MRSA

Methicillin-resistant Staphylococcus aureus, which can colonize in the nose of medical staff, can easily be transmitted to infants in their care. Even if medical staff are not directly involved in the care of carrier babies, items such as ball-point pens or the various medical instruments around the carrier patient can be the bacterial source of MRSA. One way to prevent infection is for staff members to always wear surgical masks when caring for MRSA carrier patients.[20]

(2) New medical staff members are particularly vulnerable

Even just a few carrier staff can spread MRSA to babies in the NICU. However, they usually carry MRSA only temporally in comparison with MSSA, as shown in our data. MSSA can be carried for periods of over 2 years. Particularly worrisome are new staff members who can easily become MRSA carriers because they fail to adhere to the strict hand washing procedures, especially if such procedure were not enforced in their previous workplace. Without due care in overcoming such unsafe hygiene practices, these new staff members can easily become carriers through contact of their face, hair and eyeglasses with the carrier-infant.

(3) An examination of the MRSA carrier state

One's MRSA carrier state can be easily investigated by a simple culture kit which is commercially available (MRSA check, Nikken Biomedical Co., Tokyo, Japan). New members of staff should always be screened for MRSA before being allowed to enter the NICU because it is possible that the infection originated in their previous workplace.

(4) Elimination of MRSA carriers in the NICU

If the screening culture is positive, the carrier staff should apply mupirocin ointment on the nasal cavity, in accordance with procedures described in the drug manual. Moreover, due to heightened awareness and care after an infection, relapses are usually very rare.

These guidelines outlined here were found to be the most effective way to eliminate MRSA carriers in the NICU.[21]

Close surveillance of MRSA contamination in the environment

(1) Separation of all items

Fundamentally, all items or instruments used for the care of the patient should be kept in isolation from other patients.

For example, tapes, stethoscopes, clinical thermometers, measuring tools, scissors, Vaseline or glycerin, TcPO2/CO2 sensor gels, etc. Also, ballpoint pens used for flow sheet charts should be kept separately for each patient.

(2) Contamination check of incubators and medical instruments

To evaluate whether the disinfectant used to clean the incubators is effective or not, the degree of contamination should be examined by using swabs to wipe the various parts as well as the entire incubator before and after daily cleansing. The isolation rate of resistant bacteria will reveal the effectiveness of the disinfectant used.

(3) Sterilization of incubators

Sterilization should be carried out after the exchange of incubators and before the next use. However, if MRSA can still be detected even after the guidelines proposed here have been carried out, then the sterilization process is obviously ineffective. Methicillin-resistant *Staphylococcus aureus* has already been shown to be resistant to some disinfectants such as chlorhexidine gluconate and benzalconeum chloride, which are used daily in many NICU. It can therefore be seen that these disinfectants are of no use in cleaning the incubators before their weekly exchange. The most effective method of sterilizing incubators is the use of formalin gas. Recently, a new gas sterilizer was made available (Ecopalzer of Mediate Co., Kyoto, Japan) which decomposes the formalin used in the sterilization process efficiently in water and carbon dioxide using heat and electrolysis, which is safe and does not pollute the environment.

In 2002, a nation-wide survey of NICU was conducted by the author of the present study. Multiple-regression analyses of the data revealed that the treatment of MRSA carrier patients and medical staff with mupirocin as well as the sterilization of incubators after each use, were the two main factors that were effective in the reduction of the rate of MRSA infections.[6]

Future perspective

Of the 60 NICU facilities investigated via questionnaire, 12 had zero MRSA carrier patients for 1 month or more within the previous year.[6] These NICU showed average admission numbers of 268 neonates, 18 ELBW and 50 VLBW infants per year. Seven of these NICU did not have a single MRSA infection among the VLBW infants, while the remaining five NICU had very low MRSA infection rates (12%, 8%, 6%, 4% and 2%). This indicates that seven NICUs of the 60 investigated were able to completely eradicate MRSA from their NICU. This should be looked upon with great optimism and it should be of great value to evaluate the methods and practises of these NICU.

Acknowledgments

This work was supported by the grant for 'Risk assessments and preventive measures for nosocomial infections including MRSA one in newborns and infants' from the Ministry of Health and Welfare, Japan.

We thank the neonatologists of the 90 hospitals in Japan who answered our questionnaire survey, and Ms Sumiyo Ono for preparing the manuscript.

References

1 Inoue M, Kuga A, Shimauchi C, Yano H, Okamoto R. Why do antimicrobial agents become ineffectual? *Yonsei Med. J.* 1998; **39**: 502–13.
2 Ushioda H, Terayama T, Sakai S, Zenyouji H, Nishiwaki M, Hidano A. Coagulase typing of *Staphylococcus aureus* and its application in routine work. In: Jeljaszewicz, J, Gustav, J (eds). *Staphylococcal Infections*, Fischer Verlag, Stuttgard, 1981; 10: 77–83.
3 Inoue M, Nonoyama M, Okamoto R, Ida T. Antimicrobial activity of arbekacin, a new aminoglycoside antibiotic, against methicillin-resistant *Staphylococcus aureus*. *Drugs Exp. Clin. Res.* 1994; **20**: 233–9.
4 Takahashi N, Nishida H, Kato H, Imanishi K, Sakata Y, Uchiyama T. Exanthematous disease induced by toxic shock syndrome toxin I in the early neonatal period. *Lancet* 1998; **351**: 1614–19.
5 Takahashi N, Kato H, Imanishi K, Miwa K, Yamanami S, Nishida H, Uchiyama T. Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen. *J. Clin. Invest.* 2000; **106**: 1409–15.
6 Kitajima H. Settings of surveillance system for nosocomial infections in the NICU. Annual report. *Risk assessments and preventive measures for nosocomial infections including MRSA one in newborns and infants* 2001; 35–9.
7 Shimura K. Surveillance of severe infections in high risk neonates and study of prevention of them. Annual report. *Studies for management of high risk neonates* 1995; 18.
8 Shimura K, Usukura Y, Igarashi T *et al.* Investigation of neonatal infections; Study of severe infections in extremely low birth-weight infants. Annual report. *Studies for management of high risk neonates* 1997; 23.
9 Sai N, Tkahashi N, Nishida H. MRSA infection and an attitude survey for the protective measurements. *J. Jpn. Pediatr. Soc.* 2001; **105**: 1123–5.
10 Kitajima H, Principles of infection control. Care of the normal newborn. *Perinatal Care* 2002; **1**: 300–6.
11 Fukuda M, Matsuo K, Etou M *et al.* Effects of breast milk, breast feeding and skin-ship of mother and infant on nosocomial infection (2nd report) Breast feeding and interaction of bacteria in the breast milk and oral bacteria. *J. Japan Soc. for Premature Newborn Medicine* 1997; **9**: 369.
12 Uehara Y, Kikuchi K, Nakamura T *et al.* Inhibition of methicillin-resistant *Staphylococcus aureus* colonization of

oral cavities in newborns by viridans group streptococci. *Clin. Infect. Dis.* 2001; **15**: 1399–407.
13 Nakamura T. Studies of colonization of MRSA and normal bacterial flora in the upper airway of extremely low birth weight infants. Annual report. *Risk assessments and preventive measures for nosocomial infections including MRSA one in newborns and infants.* 2001; 27–30.
14 Shishido H. Points of preventive measures of MRSA infection. *Iyaku Journal Company, Osaka* 1992; p 48.
15 Takahashi N, Nishida H, Hoshi J, Ino M, Tsukano K, Sakata Y, Takeda Y. A new viral disease in the neonatal period. *Acta Neonatologica Japonica* 1994; **30**: 626.
16 Oshiro M, Takahashi R, Nishikawa H *et al.* Reduction of neonates colonized with Methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. Effect of glove precaution. *J. Jpn Pediatr. Soc.* 1998; **102**: 1171–5.
17 Hasegawa K, Tokuda S, Hada S, Murata M, Yoshioka H. Control of Methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit: Effectiveness of wearing disposable gloves. *Acta Neonatologica Japonica* 2001; **37**: 474–8.
18 Yamada Y, Sugai M, Woo M, Nishida N, Sugimoto T. Acquired subglottic stenosis caused by methicillin resistant *Staphylococcus aureus* that produce epidermal cell differentiation inhibitor. *Arch. Dis. Child. Fetal Neonatal Ed.* 2001; **4**: F38–9.
19 Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. *Arch. Dis. Child. Fetal Neonatal Ed.* 1997; **6**: F101–7.
20 Kitajima H. Control of nosocomial infections. Symposium of perinatal infections. *Acta Neonatologica Japonica* 1993; **9**: 77–82.
21 Sobajima H. Studies of colonization of MRSA in the nose of medical staff and follow up after treatment with a mupirocin ointment. Annual report. *Risk assessments and preventive measures for nosocomial infections including MRSA one in newborns and infants* 2001; 31–3.