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A Study on Postoperative Enteritis Caused by Methicillin-Resistant *Staphylococcus aureus*

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Abstract: We investigated the production of staphylococcal enterotoxin (SE) with respect to coagulase types by methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 138 strains of MRSA, which were isolated from clinical materials in the surgical ward between 1983 and 1990, were studied. Coagulase type IV strains produced SE A only, whereas coagulase type II strains were classified into four groups by SE production: SE B producing strains (32.7%), SE C producing strains (29.8%), SE B and C coproducing strains (12.5%), and SE A and C coproducing strains (25.0%). Almost all of the organisms (nine of ten) which were isolated from the feces of patients with MRSA enteritis were SE A and C coproducing strains. The coincidence in time of the prevalence of MRSA enteritis and the isolation SE A and C coproducing strains also demonstrated that these strains caused MRSA enteritis. Although SE C producing strains and SE A and C coproducing strains were simultaneously prevalent in 1990, the former tended to be sensitive while the latter tended to be resistant to minocycline. Considering the variety of antibiotic sensitivity in coagulase type II strains, it is thus considered to be of critical importance for epidemiologic purposes to further characterize isolates by SE typing.

Key Words: methicillin-resistant *Staphylococcus aureus* (MRSA), enteritis, enterotoxin, coagulase, epidemiologic study

Introduction

Recently enteritis caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has attracted attention as a new refractory postoperative infection in Japan.[1–4] MRSA enteritis is characterized by high fever, abdominal distension, and diarrhea leading to severe dehydration and shock, multiorgan involvement, and a sharp decrease in the peripheral blood leukocyte count.[5] Though infections due to MRSA have been prevalent in our ward since 1984,[6] we had not encountered patients with MRSA enteritis until just recently. However, ten cases of this disease were observed in 1990. In a recent study, we investigated the production of staphylococcal enterotoxins (SE), which are considered to be a major cause of food poisoning,[7] in order to determine why the prevalence of this disease increased in 1990.

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Materials and Methods

Bacterial Strains

We investigated 138 strains of MRSA isolated from patients admitted to our department between 1983 and 1990: 95 were isolated from intra-abdominal drains, 19 from sputum, 11 from feces, 9 from skin and soft tissue, and 4 from blood.

Determination of Antibiotic Minimum Inhibitory Concentration (MIC)

Antibiotic sensitivities of bacteria were evaluated using the MIC measured by the standard method of the Japan Society of Chemotherapy.[8] We measured the MICs of the following antibiotics: methicillin (DMPPC), gentamicin (GM), clindamycin (CLDM), minocycline (MINO), and ofloxacin (OFLX).

Characteristics of Patients with MRSA Enteritis

We observed ten patients with postoperative MRSA enteritis in 1990. In our department 503 cases (gastroenterological surgery, 276 cases) were operated on in 1990, and the rate of outbreaks of MRSA infection and MRSA enteritis among all operations was 7.2% and 2.0%, respectively.

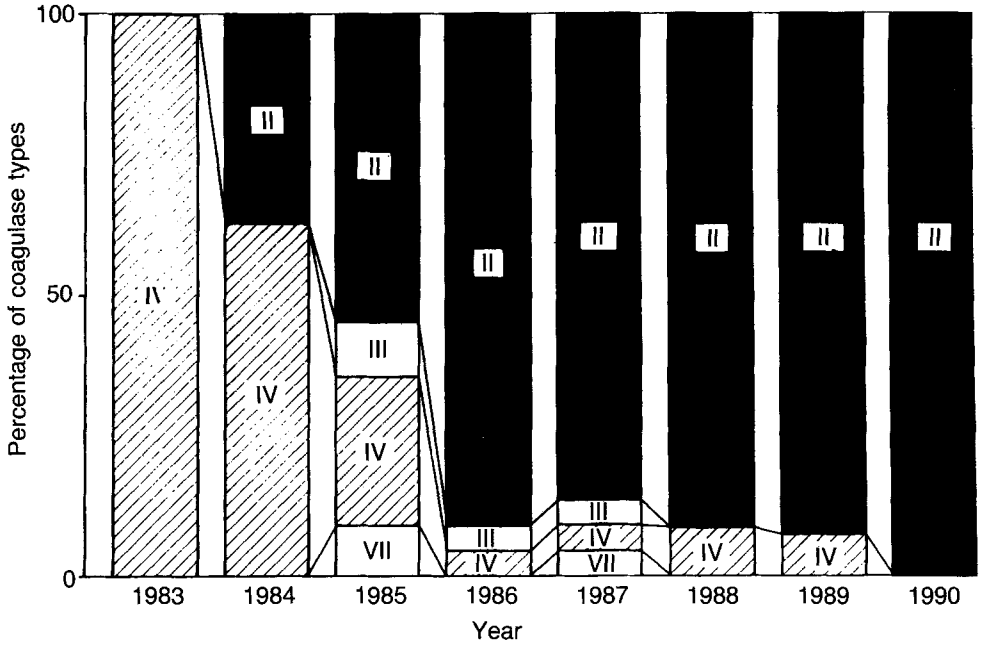

Fig. 1. Annual changes in frequency of coagulase types of methicillin-resistant *Staphylococcus aureus*. Type IV strains (*hatched area*) were predominantly isolated until 1984. After 1986 most strains belonged to type II (*black area*)

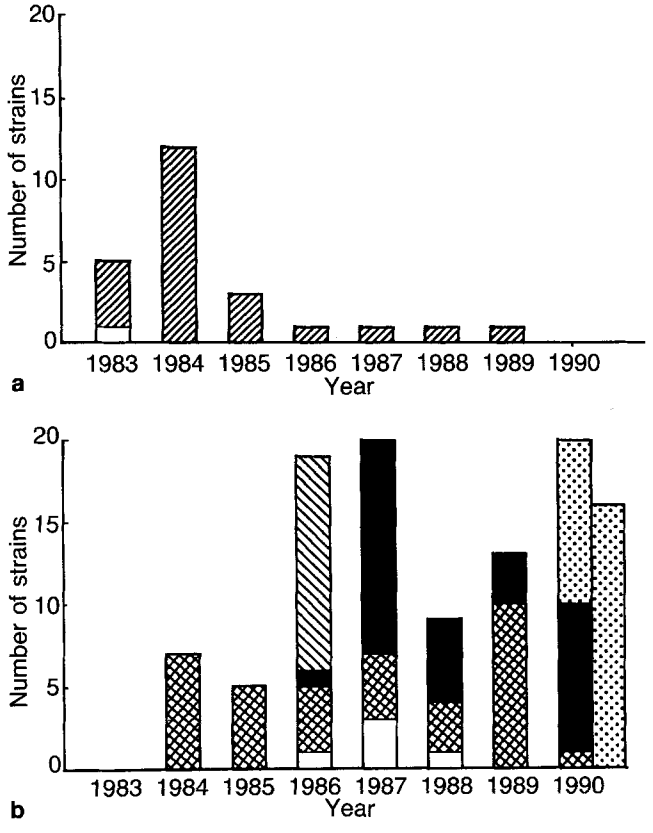

Fig. 2a,b. Enterotoxin production by isolates of coagulase type II and IV MRSA. **a** Coagulase type IV strains produced SE A only. **b** Coagulase type II strains were classified into four groups according to enterotoxin production: SE B producing strains, SE C producing strains, SE B and C coproducing strains, and SE A and C coproducing strains. □, SE A; □, SE B; ■, SE C; □, SE BC; □, SE AC; □, not detected; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *SE*, staphylococcal enterotoxin

The mean age of the MRSA enteritis patients was 70.2 years (range, 54–88 years). Nine of the ten cases were males and one female. All patients survived. Four of the ten were severe cases, and demonstrated toxic shock syndrome (TSS)-like symptoms except for normally associated skin disorders. *S. aureus* was typically the predominant organism in the stool culture. The primary diseases were as follows: five cases of gastric cancer, one case of gastric ulcer, one case of biliary duct cancer, one case of colon cancer, one case of esophageal cancer, and one case of lung cancer. All cases not following a gastrectomy were treated with histamine H_2 receptor antagonist.

Coagulase Typing of S. aureus

Clinical isolates were cultured with constant shaking for 24 h in a heart infusion broth (Eiken KK) and centrifuged at 3,000 rpm for 30 min. Anticoagulase sera (Type I–VIII) were added to the supernatant and incubated at 37°C for 1 h. The coagulase type of *S. aureus* was determined by the corresponding coagulase antiserum which inhibited the coagulation of normal rabbit plasma.

Analysis for Production of SE by MRSA

S. aureus was grown with constant shaking for 24 h in a heart infusion broth at 37°C. Detection of SE in the supernatant fluid recovered after centrifugation (3,000 rpm, 30 min) was performed using the reversed passive latex agglutination (RPLA) technique with polystyrene latex particles coupled with immuno-

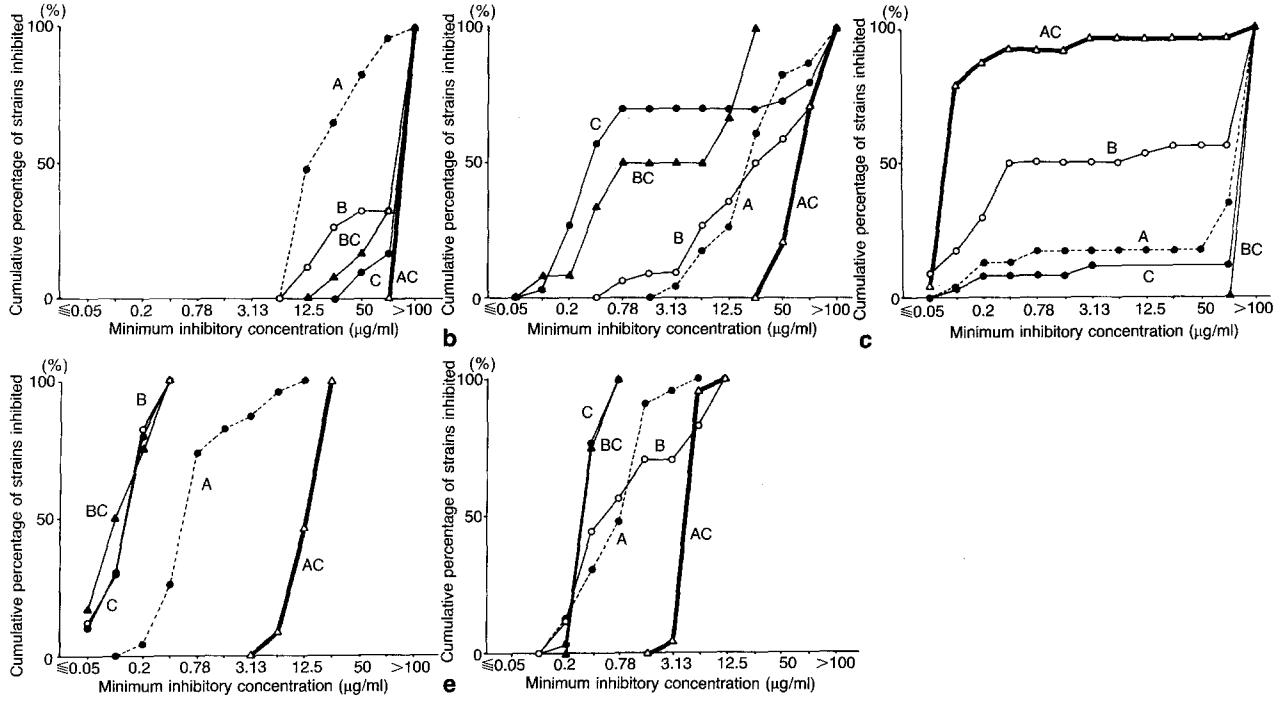

Fig. 3a–e. Antibiotic susceptibility of each enterotoxin type. **a** Methicillin. **b** Gentamicin. **c** Clindamycin. **d** Minocycline. **e** Ofloxacin. Characteristics of antibiotic sensitivity varied with each SE type. In the SE A and C coproducing strains, all strains were highly methicillin-resistant, and tended to be resistant to minocycline and ofloxacine. Clindamycine was the only antibiotic that was effective to this type strain. *Solid circles, dotted line*, SE A (n = 23); *open circles*, SE B (n = 34); *solid circles, solid line*, SE C (n = 31); *open triangles*, SE AC (n = 26); *solid triangles*, SE BC (n = 13); *MRSA*, methicilline-resistant *Staphylococcus aureus*; *SE*, staphylococcal enterotoxin

globulins of anti SE A–D rabbit hyperimmune sera fractionated by affinity chromatography.[9]

Results

Changes in Coagulase Type in MRSA, 1983–1990

Predominantly MRSA type IV strains were isolated prior to 1984. Type II became apparent in 1984, and after 1986 most MRSA were coagulase type II (Fig. 1). Among 138 MRSA isolates, 109 (79%) were type II, 24 (17.4%) were type IV, 3 (2.2%) were type III, and 2 (1.5%) were type VII.

SE Production by MRSA

Among coagulase type II and IV, 127 (95.5%) of 133 strains produced SE. Coagulase type IV strains produced only SE A (SE type A, 100%), whereas coagulase type II strains were classified into four groups by SE production: SE B producing strains (SE type B, 32.7%), SE C producing strains (SE type C, 29.8%), SE B and C coproducing strains (SE type BC, 12.5%), and SE A and C coproducing strains (SE type AC, 25.0%). Nine of ten organisms which were isolated from the feces of the patients with MRSA enteritis were determined to be SE type AC, while one was SE type C.

Each SE type of MRSA tended to be epidemic: SE type A strains were prevalent from 1983 to 1985, SE type B strains from 1984 to 1989, SE type BC strains in 1986, SE type C strains from 1987 to 1990, and SE type AC strains in 1990 (Fig. 2).

Antibiotic Susceptibility of MRSA of each Enterotoxin Type

In the SE type AC, all strains were highly methicillin-resistant (>100 μg/ml), and tended to be resistant to minocycline and ofloxacin. Clindamycin was the only antibiotic that was effective against the SE type AC strains, although these showed multiple high resistance to the other antibiotics tested. SE type C, B, and BC strains were sensitive to minocycline, and type C and BC strains also revealed high sensitivity to both ofloxacin and gentamicin. The SE type A strains were more sensitive to methicillin than the other strains of the coagulase type II group (SE type B, C, BC, and AC). However type A strains were less sensitive to minocycline than the other strains of the coagulase type II group, except for type AC (Fig. 3).

Discussion

Diseases resulting from the consumption of foods may normally be classified as intoxications or infections.[7] Usually staphylococcal food-borne disease is an intoxication owing to the ingestion of a toxin formed by certain strains of *S. aureus*, although MRSA enteritis is classified as an infection. Food-borne infections result from the ingestion of microorganisms which induce a reaction in the host tissue by penetration into the intestinal mucosa or by production of SE within the lumen of the colonized bowel, as is the case in MRSA enteritis.[7] *S. aureus* is a normal inhabitant of the gastrointestinal tract and can be found in small numbers as part of normal bowel flora in up to 10% of healthy people.[5] However, in certain situations, the organism may over multiply in the bowel and eventually bring on disease.

Considering these facts, particular environments may be necessary for *S. aureus* to keep producing toxin within the intestine. Gastric juice with a high pH following gastrectomy or administration of a histamine H_2 receptor antagonist is a serious factor thought to cause MRSA enteritis.[10] *S. aureus* ingested through the naso-oral route would not be killed by gastric juice of lower acidity, and when it moves downward and proliferates in the lower digestive tract, MRSA enteritis can then easily occur.

The postoperative utilization of antibiotics is another important factor in the causation of MRSA enteritis, because *S. aureus* is not a strong competitor and its growth is limited by the microorganisms present in the intestinal flora. In addition, postoperative paralysis of the intestine may give the MRSA a chance to produce toxins in the bowel, since the patients excreting MRSA in their stool tended not to develop any disease when their normal bowel movement remained intact.[11]

In the series reported by Gutman et al.,[12] all patients with *S. aureus* enterocolitis also had an indwelling stomach tube. The introduction of *S. aureus* into the gastrointestinal tract through the use of drainage or feeding tubes, with trauma caused to the mucosa by the indwelling foreign body, may therefore be important factors common to the pathogenesis of this disease.

Although we have just mentioned several factors which cause MRSA enteritis, these alone do not explain the prevalence of MRSA enteritis in our ward in 1990. There were no particular changes regarding these factors which influenced conditions of the host between the period starting around 1989 and continuing until after 1990. Therefore, we investigated from the parasitical aspect why the prevalence of MRSA enteritis increased in 1990. SE type AC strains were isolated from the feces of almost all the patients who developed MRSA enteritis. The coincidence in time of prevalence of MRSA enteritis and the isolation SE type AC strains also demonstrated that these strains caused MRSA enteritis. Generally, SE A is most commonly implicated in food-borne outbreaks with SE D next in the order of frequency.[13] In a previous study we reported that the SE A positive strains in MRSA were a more potent producer of SE which was considered to be responsible for enteritis than the others, and that all SE C positive MRSA strains also produced toxic shock syndrome toxin-1 (TSST-1).[14] Therefore, we hypothesized that SE A and C coproducing strains tended to cause enteritis associated with TSS-like symptoms.

Isono et al.[2] reported that coagulase type IV strains produced SE A, and coagulase type II strains produced SE C. However, in our study SE production of coagulase type II strains were more complicated, although the same result was obtained with coagulase type IV. Coagulase type II were classified into four groups by SE production: SE B, SE C, SE both B and C, or SE both A and C producing strains. We considered that this classification of MRSA by SE enables us to achieve a further detailed analysis of MRSA.

Some preventive measures were adopted to decrease the incidence of MRSA enteritis. Preoperative patients made it a rule to gargle with providone-iodine after admission, and povidone-iodine gel was also applied intranasally to eliminate any coincidental MRSA carriage. We also kept the postoperative utilization of H_2 receptor antagonist to a minimum.

Little information is available on the optimal management of MRSA enteritis other than the oral administration of vancomycin and appropriate intravenous antibiotic treatment. We recommend the use of gamma globulin and fresh frozen plasma for enteritis patients, because of their high titer of antibody to TSST-1.[15] The sera from patients with TSS has been shown to have lower levels of antibodies to TSST-1 than to control sera.[16] As another possible treatment, we have previously reported that the early administration of steroid hormone was also effective in treating the pathological changes observed in MRSA enteritis.[11]

MRSA enteritis was characterized by an association with TSS-like symptoms except for normally associated skin disorders. It was reported that TSST-1 and SE were often coproduced by MRSA,[14] and these toxins were strong inducers of experimental TSS.[17–20] TSST-1 and SE have been shown to be potent stimulators or interleukin-1 (IL-1) and tumor necrosis factor (TNF) released by human monocytes.[21–23] The massive release of these monokines has been implicated in the mediation of many symptoms of TSS. The release of IL-1 by TSST-1 was shown to be blocked by hydrocortisone (from 21,896 cpm to 1,099 cpm in a lymphocyte activating assay) but not by indomethacin (24,225 cpm).[19] We hypothesized that the efficacy of

steroid hormones in MRSA enteritis was due to the blockage of IL-1 release.

Antibiotic sensitivity varied with the SE type. Among SE type AC, all strains were highly methicillin-resistant *S. aureus*, and tended to be resistant to minocycline and ofloxacin which have previously been considered to be effective against MRSA.[6] Clindamycin was the only antibiotic that was effective against SE type AC strains. Ubukata et al.[24] reported gentamicin-sensitive, tobramycin-resistant strains of MRSA, and they showed restriction maps of the regions coding for methicillin and tobramicin resistance on chromosomal DNA. We have previously reported that strains of this type were detected in our institution beginning in the latter half of 1986,[6] and in this study they have been classified as either SE type C or type BC. Considering the variety of antibiotic sensitivities among coagulase type II strains, it is thus considered to be of critical importance for epidemiologic purposes to further characterize isolates by SE typing.

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