JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 846–847
0095-1137/94/$04.00+0

Vol. 32, No. 3

Discrimination of Epidemic and Nonepidemic Methicillin-Resistant *Staphylococcus aureus* Strains on the Basis of Protein A Gene Polymorphism

H. M. E. FRÉNAY,[1] J. P. G. THEELEN,[1] L. M. SCHOULS,[1] C. M. J. E. VANDENBROUCKE-GRAULS,[2] J. VERHOEF,[2] W. J. VAN LEEUWEN,[1] AND F. R. MOOI[1]*

Molecular Microbiology Unit and Laboratory of Bacteriology and Antimicrobial Agents, National Institute of Public Health and Environmental Protection, 3720 BA Bilthoven,[1] and Eijkman-Winkler Institute of Medical Microbiology, University Hospital, Utrecht,[2] The Netherlands

Received 22 September 1993/Returned for modification 18 November 1993/Accepted 17 December 1993

The X region of the protein A gene of *Staphylococcus aureus* contains a highly polymorphic sequence which is composed of repeats of 24 bp. We used amplification by PCR to investigate whether this region could be used to discriminate between epidemic and nonepidemic methicillin-resistant *S. aureus* (MRSA) strains. Most epidemic MRSA strains (24 of 33) harbored more than seven repeats, while most nonepidemic MRSA strains (10 of 14) contained seven or fewer repeats. It is conceivable that a longer X region results in a better exposition of the Fc-binding region of protein A, thereby facilitating colonization of host surfaces and contributing to the epidemic phenotype.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a persistent and increasing cause of nosocomially acquired infections in many hospitals in the world. Some MRSA strains are able to spread rapidly among patients, and these strains have been designated epidemic to differentiate them from strains that lack this capacity (4). The phenomenon of epidemic strains was also described for methicillin-sensitive *S. aureus* (MSSA) strains by Williams in 1959 (9). The question of why some *S. aureus* strains show an epidemic spread after introduction in the hospital whereas others do not has not yet been answered.

The aim of the present study was to identify a genetic marker to discriminate epidemic from nonepidemic MRSA strains. Such a prognostic marker would allow selective implementation of strict measures to prevent dissemination of MRSA strains within hospitals. It may also provide insight into the bacterial factors involved in epidemic spread. In the present study, epidemic MRSA strains were defined as having caused hospital outbreaks involving at least two patients or staff members in one or more hospitals. MRSA strains that were isolated only once were defined as nonepidemic or sporadic.

The majority (i.e., 33) of the 47 MRSA strains used for this study were isolated during an ongoing MRSA surveillance study in Dutch hospitals that was initiated by the National Institute of Public Health and Environmental Protection. These strains were not epidemiologically related and were generally imported by patients after a stay in a hospital in another country. According to the criteria mentioned above, 19 of the 33 strains were classified as epidemic MRSA strains, whereas 14 were classified as nonepidemic MRSA strains. In addition to the 33 strains of the Dutch survey, 14 well-documented epidemic MRSA strains from England and Wales (EMRSA-1 through EMRSA-14) were included in this study (5). Most of the 47 strains belonged to different phage types, as was determined with the International set of phages and two sets of supplementary phages (8a). A supplement with details on the origins and phage types of the MRSA strains is available upon request.

Phage typing has been used in attempts to discriminate between epidemic and nonepidemic MRSA strains. However, although there is a preponderance of certain phage types in epidemic strains (5), the association of phage type and epidemic character is not rigorous enough to be of prognostic value. Another method to characterize MRSA strains is to focus on polymorphic DNA regions (2). Such polymorphisms can be detected by amplification of a hypervariable DNA region(s) and subsequent digestion with restriction endonucleases. We selected the gene for protein A (*spa*) for our studies, because it is a surface protein known to carry polymorphic regions (1). The *spa* gene is composed of approximately 2,150 bp and harbors a number of functionally distinct regions (Fig. 1): an Fc-binding region, the so-called X region, and, at the C terminus, a sequence required for cell wall attachment (7). The Fc-binding region is composed of five 160-bp repeats. The X region contains a varying number of 24-bp repeats (3, 8). This repetitive region is highly polymorphic, and we investigated whether it could be used to discriminate epidemic from nonepidemic strains. The X region from a number of strains was amplified by PCR. The PCR product is cleaved in three fragments with *Rsa*I, two of which are composed of 214 and 35 bases, respectively. The third fragment contains the repetitive DNA, and from its size the number of repeats can be estimated (Fig. 1). The numbers of complete repeats in the X region appeared to vary from 3 to 15 between different strains (Fig. 1).

To study the degree of instability of the X region, we analyzed a number of MRSA isolates obtained from a patient with cystic fibrosis. These strains were isolated in 1987, 1989, and 1992 and were indistinguishable with respect to phage type and antibiogram. All three strains revealed the same number of repeats in the X region (i.e., 12 repeats). Also, during subculturing of strains in vitro we have not been able to detect a change in the number of repeats in the X region. These observations indicate that this region, although polymorphic, is sufficiently stable to allow discrimination of epidemiologically unrelated isolates.

* Corresponding author. Mailing address: Molecular Microbiology Unit, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Phone: 31 30 743091. Fax: 31 30 292957.

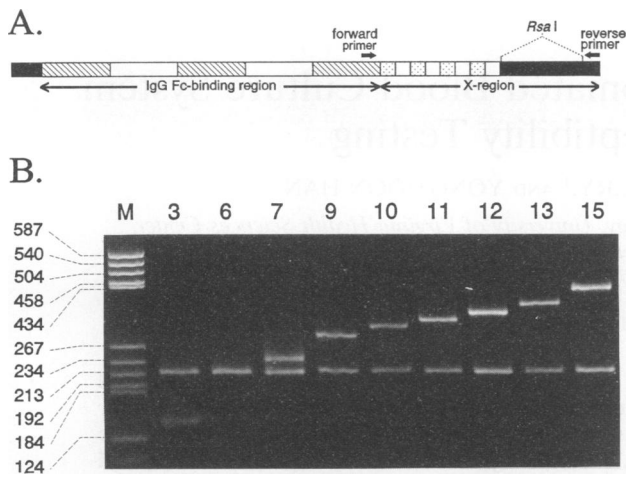

FIG. 1. (A) Physical map of the protein A. The locations of the forward primer (5′ TGTAAAACGACGGCCAGTGCTAAAAAGC TAAACGATGC 3′) and reverse primer (5′ CAGGAAACAGCTAT GACCCCACCAAATACAGTTGTACC 3′) are indicated. (B) Analysis of the X region with PCR. After PCR, DNA was cleaved with *Rsa*I and separated on 3% NuSieve GTG agarose (FMC) in 0.5× Tris-borate-EDTA. The smallest fragment (35 bases) generated by cleaving the PCR product with *Rsa*I is not visible on the gel. The numbers of complete repeats are indicated above the lanes. Molecular weight standards are indicated on the left (Marker V; Boehringer).

Subsequently, we determined whether there was a correlation between the number of repeats and the epidemic character of MRSA strains. In most (24 of 33) of the epidemic MRSA strains, we found more than seven 24-bp repeats in the X region of the *spa* gene (Fig. 2). Ten of the 14 nonepidemic strains showed seven or fewer repeats (Fig. 2). Thus, there was a highly significant correlation (chi-square test [$P < 0.005$]) between the number of repeats and the epidemic character of strains; strains with more than seven repeats in the X region

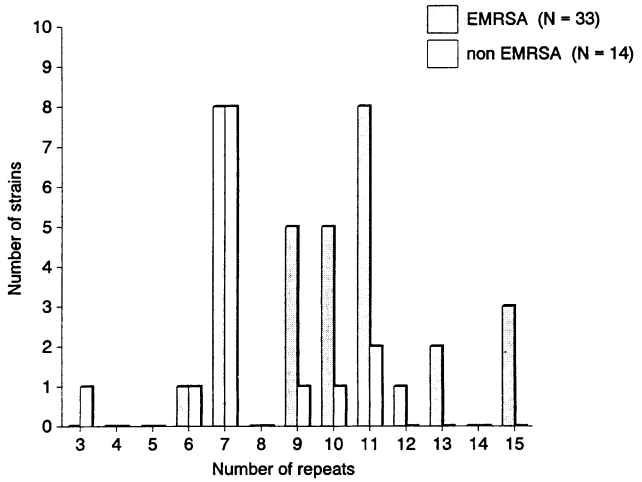

FIG. 2. Histogram showing the distribution of the numbers of repeats in epidemic (EMRSA) and nonepidemic (non-EMRSA) strains.

tended to be epidemic, while the presence of seven or fewer repeats was indicative of a nonepidemic character. However, not all strains were found to comply with this simple rule. In nine epidemic MRSA strains, we found six and seven repeats in the X region. Four nonepidemic MRSA strains which contained 9, 10, or 11 24-bp repeats also were exceptions to the rule.

Several explanations may be put forward for these exceptions. First, the definition of the epidemic phenotype may be subject to error. For example, the implementation of stringent hygienic measures may have prevented an epidemic strain from spreading, with the result that it was classified as nonepidemic. Conversely, nonepidemic strains may become epidemic under favorable conditions such as poor hygiene or the presence of patients with skin disorders. Another explanation is that multiple loci on the chromosome may contribute to the epidemic character, and the X region of the *spa* gene is associated with only one of these loci.

The correlation between the number of repeats and the epidemic phenotype may simply reflect an association of the polymorphic *spa* region with other not yet identified loci involved in epidemic spread. Alternatively, the correlation may be based on a functional link between the number of repeats in the X region and the ability of MRSA strains to disseminate. There is evidence for a role of immunoglobulin G-binding proteins in skin infections (6), and it is conceivable that a longer X region allows a more-favorable exposition of the Fc-binding regions at the cell surface, thereby facilitating infection of the skin or other not yet identified sites important for epidemic spread. These aspects are currently under investigation.

We thank R. R. Marples, B. D. Cookson, and J. Richardson from the Staphylococcus Reference Laboratory in London, United Kingdom, for kindly providing the EMRSA strains. We also acknowledge Edith Peters and Annelies Bunschoten for their excellent technical assistance.

REFERENCES

1. **Colbert, D., A. Anilionis, P. Gelep, J. Farley, and R. Breyer.** 1984. Molecular organization of the protein A gene and its expression in recombinant host organisms. J. Biol. Response Modif. **3:**255–259.
2. **Goh, S.-W., S. K. Byrne, J. L. Zhang, and A. W. Chow.** 1992. Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. **30:**1642–1645.
3. **Guss, B., M. Uhlén, B. Nilsson, M. Lindberg, J. Sjöquist, and J. Sjödahl.** 1984. Region X, the cell-wall-attachment part of staphylococcal protein A. Eur. J. Biochem. **138:**413–420.
4. **Hospital Infection Society and British Society for Antimicrobial Chemotherapy.** 1990. Revised guidelines for the control of epidemic methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **16:**351–377.
5. **Marples, R. R., and S. Reith.** Methicillin-resistant *Staphylococcus aureus* in England and Wales. 1992. Commun. Dis. Rep. **2:**25–29.
6. **Raeder, R., and M. D. P. Boyle.** 1993. Association between expression of immunoglobulin G-binding proteins by group A streptococci and virulence in a mouse skin infection model. Infect. Immun. **61:**1378–1384.
7. **Schneewind, O., P. Model, and V. A. Fischetti.** 1992. Sorting of protein A to the staphylococcal cell wall. Cell **70:**267–281.
8. **Uhlén, M., B. Guss, B. Nilsson, S. Gatenbeck, L. Philipson, and M. Lindberg.** 1984. Complete sequence of the staphylococcal gene encoding protein A. A gene evolved through multiple duplications. J. Biol. Chem. **259:**1695–1702.

8a. **van Leeuwen, W. J.** Unpublished data.

9. **Williams, R. E. O.** 1959. Epidemic staphylococci. Lancet **i:**190–195.