NOTES

Ultrastructure of Small Colony Variants of a Methicillin-resistant Staphylococcus aureus

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A methicillin-resistant strain of Staphylococcus aureus was isolated from two patients in the same ward of the Seattle Veterans Administration Hospital. This strain, called the Seattle variant, caused a fatal pneumonia in one of the patients and has been the object of a detailed laboratory investigation (R. J. Bulger, Ann. Internal Med., 67:81, 1967). The results indicate that the mechanism of resistance to methicillin was probably a combination of “innate” resistance and excessive penicillinase production, similar to that of methicillin-resistant strains studied elsewhere in the world (S. Seligman, J. Gen. Microbiol. 42:315, 1966; Y. Chabbert et al., Rev. Franc. Etudes Clin. Biol. 10:495, 1965; C. F. Gravenkemper, J. L. Brodie, and W. M. M. Kirby, J. Bacteriol. 89:1005, 1965; K. Ericksen and I. Ericksen, Acta Pathol. Microbiol. Scand. 62:399, 1964). The great majority of cells surviving exposure to high concentrations of methicillin were small colony variants similar to those described by Seligman. They often became visible to the naked eye as tiny, translucent colonies only after 36 to 48 hr of incubation at 37 C. This small colony variant seems to bear a close resemblance to the antibiotic-resistant G forms reported in the past with a variety of organisms (R. Wise and W. Spink, J. Clin. Invest. 33:1611, 1964). In the literature on G forms, there has been a debate as to whether or not these organisms were filterable and whether they might be L forms. In a study of methicillin-resistant staphylococci, M. Barber (J. Gen. Microbiol. 35:183, 1964) noted slow-growing colonies, whose cells appeared to have irregular size and shape and took Gram stain poorly, suggesting a defective cell wall.

These factors prompted us to examine the following cells of the Seattle strain with the electron microscope: (i) wild-type parent strain unexposed to antibiotic; (ii) normal-sized colonies surviving in high concentrations of methicillin; and (iii) methicillin-resistant small colony variants.

Colonies on agar were fixed for 1 to 4 hr in the following fixative solutions: (i) 2% osmium tetroxide in s-collidine buffer; (ii) 2.5% glutaraldehyde in 0.1 M sodium cacodylate; (iii) Karnovsky’s fixative (M. Karnovsky, J. Cell Biol. 27:137A, 1965); and (iv) a mixture of glutaraldehyde and osmium tetroxide in s-collidine (B. F. Trump and R. E. Bulger, Lab. Invest. 15:368, 1966). The colonies were rapidly dehydrated in ethyl alcohol, embedded in Epon epoxy resin (J. H. Luft, J. Biophys. Biochem. Cytol. 9:409, 1961).

Fig. 1. Electron micrograph showing morphology of a multiple-resistant strain of Staphylococcus aureus, which is typical of normal ultrastructure as described for staphylococci by previous authors. X 82,000.

Fig. 2. Electron micrograph of small colony variant. See text for specific description of each labeled part. Note the circumferential substructure (free arrow) of layer C. Fixed in Karnovsky’s fixative. X 61,000.

Fig. 3. Electron micrograph of cells from a normal colony of the wild-type Seattle strain fixed in a combination of glutaraldehyde and osmium tetroxide. X 5,600.

Fig. 4. Electron micrograph of cells from small colony variant of the Seattle strain, fixed simultaneously and in the same manner as cells in Fig. 3. Magnification is the same in both Fig. 3 and 4; the larger size of the cells of the small colony variant may be noted after this procedure. X 5,600.

Fig. 5. Electron micrograph of small colony variant fixed in 2% osmium tetroxide buffered with s-collidine again showing morphology similar to that found with other fixatives. This fixation clearly demonstrates the layer of radial filaments (A), the circumferential substructure of cell wall (arrow), and vesicular profiles (V). X 59,000.

Fig. 6. Electron micrograph of a small colony variant fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate and postosmicated showing similar morphology. X 92,000.
Fig. 1–6
stained sequentially with uranyl acetate and lead tartrate (G. Millonig, J. Biophys. Biochem. Cytol. 11:736, 1961), and viewed in an RCA-EMU-3G or AEI-6B electron microscope.

The bacteria of each of the three groups studied by us demonstrated a morphology similar to that reported by A. Suganuma (Ann. N. Y. Acad. Sci. 128:26, 1965; J. Cell Biol. 21:290, 1964) who studied a number of strains of \textit{S. aureus} (Fig. 1).

The bacteria appeared to be surrounded by the following series of electron-dense and lucent layers (Fig. 2, 5, 6): (A) a layer of radial filaments (Fig. 5); (B) a dense layer thought to be a cell wall component; (C) a less dense layer also interpreted to be a cell wall component; (D) a dense layer interpreted as cell membrane (J. R. G. Bradfield, Symp. Soc. Gen. Microbiol., 6th, p. 296–317, Cambridge University Press London, 1956), as cell wall (R. G. E. Murray, W. H. Francombe, and B. H. Mayall, Can. J. Microbiol. 5:641, 1959), or as a combination of the two (A. Suganuma, Ann. N. Y. Acad. Sci. 128:26 1965); (E) a less dense layer; and (F) a dense layer difficult to demarcate from the cytoplasm. The bacterium also contains a nuclear area (N), dense granules (Gr), and vesicular profiles (V) of low density which are suggestive of mesosomes.

Only minor variations were seen with the use of several fixatives, although an external layer of radial filaments and the circumferential substructure of layer C (Fig. 5) can be described, because we have used a variety of fixatives not generally applied to bacteria.

In some cases, the cells of the small colony variant appeared to be larger in size than the other types studied (Fig. 3, 4), and were seen more often with a complete cell wall dividing the bacterium into two parts. The significance of these observations is unknown.

It can therefore be concluded that the cell wall of the methicillin-resistant small colony variant is intact and that on morphological grounds it can be distinguished from protoplasts or L forms (L. Dienes, J. Bacteriol. 93:693, 1967).

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