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Technical Note

A New and Practical Method for Transmission Electron Microscopy Analysis of *Proteus mirabilis*

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INTRODUCTION

Proteus mirabilis is rod-shaped Gram-negative bacteria that have distinguishing features like swarming motility and urease activity.[1] Transmission electron microscopy (TEM) is used to examine very small structures (0.1 nm) via short-wavelength electrons.[2] Although there are various protocols for TEM analysis of *Proteus* species, the materials needed for the preparation of the samples cannot easily be found in the microbiology laboratory settings.[3] In this study, we aimed to delineate a new simplified protocol for TEM imaging of *Proteus mirabilis* isolates to the researchers working at clinical microbiology laboratories.

METHODS

Ten *Proteus mirabilis* clinical isolates were included in the study. The bacterial isolates were stored at −20°C and thawed and subcultured onto 5% sheep blood agar prior to testing. After 24 hours of incubation at 36°C, one loopful (10 μL) of bacteria from growing colonies was transferred into a tube containing 2 mL Mueller Hinton broth (MHB; Merck, Germany) and incubated at 36°C for 3 hours. Then, 1 mL of the bacterial suspension was re-suspended inside 5 mL of 2.5% glutaraldehyde solution; the suspension was kept at room temperature for 10 minutes, then centrifuged at 3000 g for 5 minutes in a 15 mL falcon tube. The supernatant on the surface was discarded, and 1 mL 2.5% glutaraldehyde solution was added to the pellet. After 20 minutes, the pellet was removed from the bottom of the tube with the help of a Pasteur pipette. Concurrently, the pellet was embedded into 96-well microplates which had been filled with freshly prepared (>45°C; about to solidify) 1 mL Mueller Hinton agar (MHA; Merck, Darmstadt, Germany). The pellet was mixed with the agar using a sterile injector needle for 5 seconds. Then, the microplate was covered and left to solidify for 2 hours at room temperature. Afterward, using the needle and a scalpel tip, the solidified agar piece was scraped from the wall of the well and placed on a sterile slide as a mold. It was divided into 1 mm^3 piece with the scalpel tip. The pieces were stored in falcon tubes containing 15 mL of phosphate buffer at 4°C, which was then examined by the TEM laboratory the next day. The post-fixation was applied to samples with 1% osmium tetroxide solution for 1 hour at 4°C. Later, dehydration of the samples was achieved by graded series of alcohol, and a transparency process was applied with propylene oxide. Subsequently, the samples were embedded in epoxy resin in embedding capsules and polymerized in a 60°C incubator for 24 hours. From the blocks obtained, sections of 70 nm thickness were taken on copper grids with an ultra-microtome, and the sections were contrasted with uranyl acetate and lead citrate. The contrasting sections were examined and photographed by TEM (Jeol® JEM1011, Japan).

DISCUSSION

Since TEM imaging is not very common in clinical microbiology laboratories, when microbiologists need to obtain electron microscopic images of bacteria, they search for different protocols and try to adapt those protocols to their studies. For microscopic examination under TEM, suspensions of bacteria must be supported on a thin film of plastic, carbon, or a combination of the two applied to the surface of an electron microscope specimen grid.[4] In our study, we tested MHB and MHA, frequently used media in microbiology laboratories, as dilution and fixation media for TEM, and the results were satisfactory. We were able to get clear and detailed TEM images of *P. mirabilis* (Figure 1 and 2). Organelles such as nucleoids, ribosomes, cell membrane, and

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Figure 1. Detailed transmission electron microscopy image of *Proteus mirabilis* bacteria.

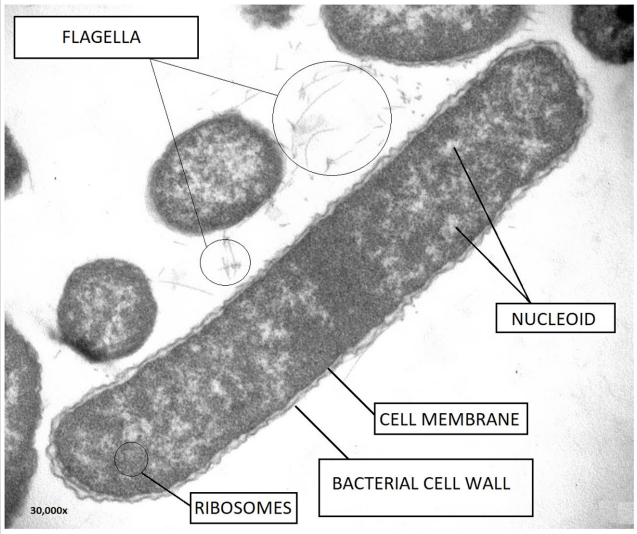
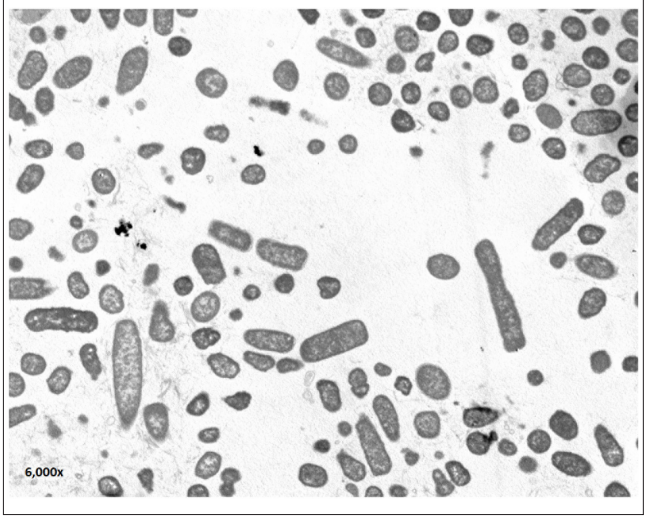


Figure 2. Transmission electron microscopy image of a group of *Proteus mirabilis* bacteria.



cell wall can be observed clearly under a microscope (Figure 1). In our protocol, *Proteus* bacteria were scattered adequately that they did not touch each other, but so close that a few of them could be observed in each frame (Figure 2). We think that this protocol can easily be repeated and applied to the other members of the order Enterobacterales using the equipment that can be found in clinical microbiology laboratory settings.

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