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THE IDENTIFICATION OF THIN FIMBRIAE ON THE CELL WALL SURFACE OF *ACINETOBACTER JUNII* AT THE ULTRASTRUCTURAL LEVEL

WYKAZANIE OBECNOŚCI CIENKICH FIMBRII NA ŚCIANIE KOMÓRKOWEJ BAKTERII *ACINETOBACTER JUNII* NA POZIOMIE MIKROSKOPU ELEKTRONOWEGO

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ABSTRACT. Ultrastructural studies were performed to detect the presence of thin fimbriae on the cell wall surface of Acinetobacter junii. Three methods were used. Firstly, thin sections were contrasted with uranyl acetate and lead citrate. In this method only electron dense amorphous material could be seen surrounding the cell wall of Acinetobacter junii. Afterwards, the second method of staining was applied by using 1 % uranyl acetate only. In this method thin fimbriae were observed. Using ruthenium red the presence of flocculent layer and thin strands resembling fimbriae were demonstrated as well. In our opinion, staining with 1 % uranyl acetate and ruthenium red are useful in estimation of thin fimbriae on the cell wall surface of Acinetobacter junii.

KEY WORDS. Acinetobacter junii, electron microscope, thin fimbriae, staining methods

Introduction

Numerous report have illustrated the increasing nosocomial problem induced by Acinetobacter species. These organisms fequently found in soil, water and have been also isolated from the hospital environments as well as from food and animals. Acinetobacter species are gram-negative bacteria that may be resident on human skin. According to the latest taxonomic changes of the genus Acinetobacter, seventeen genetic species have been recognised, eleven of them, can be differentiated by phenotypic properties. It has been shown that most isolating strains from hospital infections belong to *Acinetobacter baumannii* and *Acinetobacter junii* (Towner et al 1991).

In this study we report on the use of three methods of staining for ultrastructural examination of thin fimbriae on the cell wall surface of *Acinetobacter junii.*

Materials and methods

Bacterial strains and growth conditions. Two strains of *Acinetobacter junii* (AJU), which were isolated from groin (321 AJU) and from secretion of nasal cavity (554 AJU), were used. The strains were identified by means of API 20 NE System (Bio-Mérieux). These organisms were cultured on the Tryptic Soy Agar medium (Bio-Mérieux) and incubated at 22° C for 48 hours. For studies at the electron-microscope level 5 ml portions of isotonic salt solution were transferred on the surface of the solid cultures of the 321 AJU and 554 AJU and bacterial cells of these strains in tubes for centrifugation were collected. The cell suspensions were centrifuged at 1 200 g for 10 minutes. The deposits of bacterial cells were rapidly fixed in 3 % glutaraldehyde in 0,1 M cacodylate buffer pH 7,4 for three hours at 4° C. The bacterial cells were sedimented at 1 200 gr for 10 minutes and then they were washed overnight at 4°C in the same buffer and postfixed for one hour in 2 % OsO_4 in 0,1 M cacodylate buffer pH 7,4. The samples were dehydrated in ethanol and acetone and embedded in Epon 812. Thin sections were stained as follows. They were contrasted with uranyl acetate and lead citrate. Several specimens were stained only with 1 % uranyl acetate. In a few other cases ruthenium red was used before embedding in Epon 812. 1 ml 3,6 % glutaraldehyde was mixed with 40 mg ruthenum red (Sigma) in 20 µl distilled water and was added to sediment of the bacterial strains of 312 AJU and 554 AJU. The samples were fixed for three hours at 4° C. The bacterial cells were sedimented at 1200 g for 10 minutes and washed in cacodylate buffer and postfixed in 5 % OsO_4 in cacodylate

buffer pH 7,4 and treated as described above. All preparation were examined by electron microscope JEM 100 CX.

Results

Three staining methods were estimated for using them in evaluation of structures on the cell wall surface of the two strains of *Acinetobacter junii*. The samples were especially examined for the presence of thin fimbriae. Electron dense amorphous material could be seen adjacent to the cells by using uranyl acetate and lead citrate to stain thin section (fig. 1). There was no evidence of any structures resembling fimbriae. Then, thin sections of the two bacterial strains 331 AJU and 554 AJU were stained using only 1 % uranyl acetate. In this method cells of bacteria were observed to possess fimbriae (fig. 2). When bacterial cells were fixed in the presence of ruthenium red, electron-dense cell surface layers were formed. The outer surface was covered with a flocculent layer of ruthenium red positive material (fig. 3). The sections of ruthenium red staining samples also showed the presence of thin strands resembling fimbriae (fig. 4). The electron microscopic study on these bacteria by using 1 % uranyl acetate staining and the ruthenium red one revealed cell wall associated structures resembling thin fimbriae in peritrihael disposition. This result indicated that these two methods can be used to identify structures on the cell wall surface of bacteria.

Discussion

The pathogenesis of Acinetobacter is unknown, despite of important increase in hospital infections for the last years. Previous studies indicated that the bacterial adhesion to eucariotic cells is the important virulence factor (Beachey 1981, Daifuku and Stamm 1986, Sharon 1987). Understanding this association is connected with the presence of fimbriae covered bacterial cells, which should be solved. This study was based on establishment the best method, which can be used in evaluation of structures on the cell wall surface of *Acinetobacter junii*. The observations with electron microscope clearly showed that first method, by using uranyl acetate and lead citrate to stain thin sections, is inadequate to show fimbriae on the cell wall surface of *Acinetobacter junii*. Our results indicated that cell wall associated fimbriae were demonstrated when only uranyl acetate was used to stain thin sections. The bacterial cells were observed to have fimbriae.

It was noted by microscopical examination that they were thin fimbriae. Rosenberg et al. (182) received these same results using 0,5 % solution of uranyl acetate. They distinguishend two types of fimbriae. Our findings were also similar to the results achieved by Henrichsen and Blom (1975). In general, our experience corresponds to the other data, which indicated that fimbriae have been demonstrated also on the cell wall surface of different bacteria by using negative staining (DeFlaun et al. 1990, Gottschalk et alt. 1990, Hazlett et a. 1991, Ramphal et al 1991). Furthermore, our study also showed that the cells of *Acinetobacter junii,* which were fixed in the presence of ruthenium red had thin strands resembling fimbriae. Our observations at the utrastructural level were in agreement with other authors data obtained by ruthenium red staining (Miller and Ahearn 1987, Scott et al. 1989).

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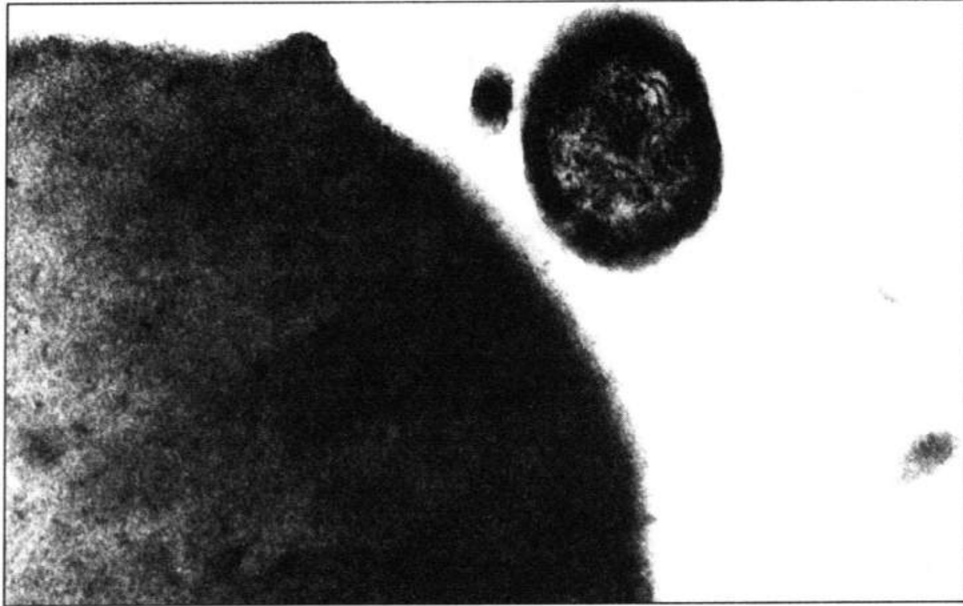

Fig. 1. Electron micrograph of uranyl acetate and lead citrate staning
Fimbriae are not shown, only electron dense amorphous material is appered on the bacterial cell surface (arrow) x 85.000

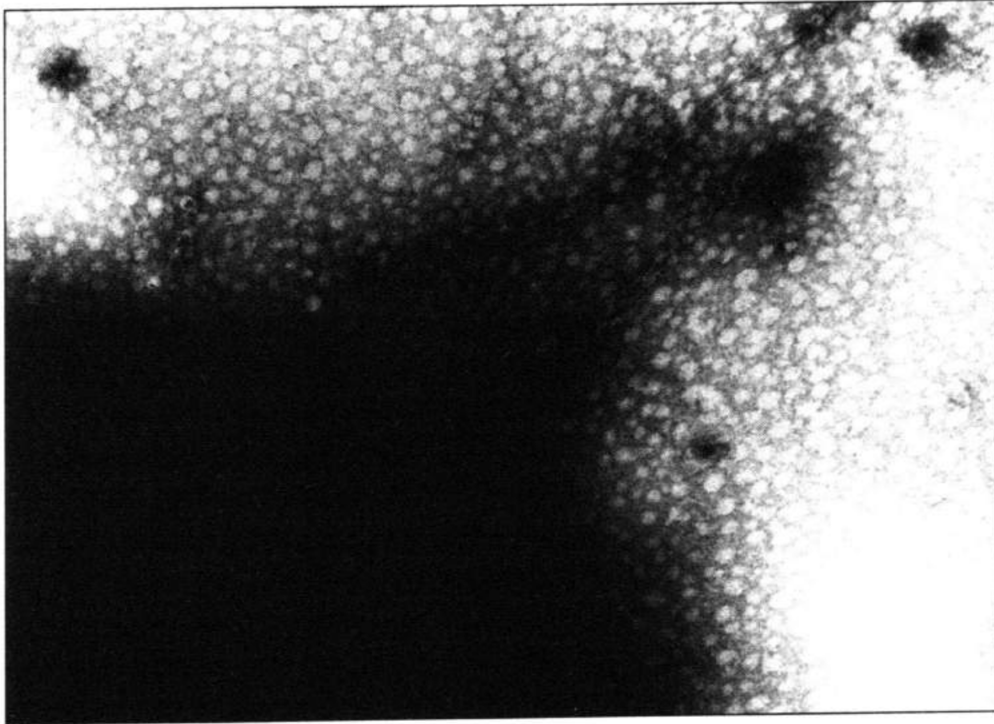

Fig. 2. Preparation stained with 1 % uranyl acetate. The arrows point to thin fimbriae. x 300.000

Fig. 3. Transmission electron micrograph of *Acinetobacter junii*. In the presence of ruthenium red, flocculent layer was seen (arrows) x 92.000

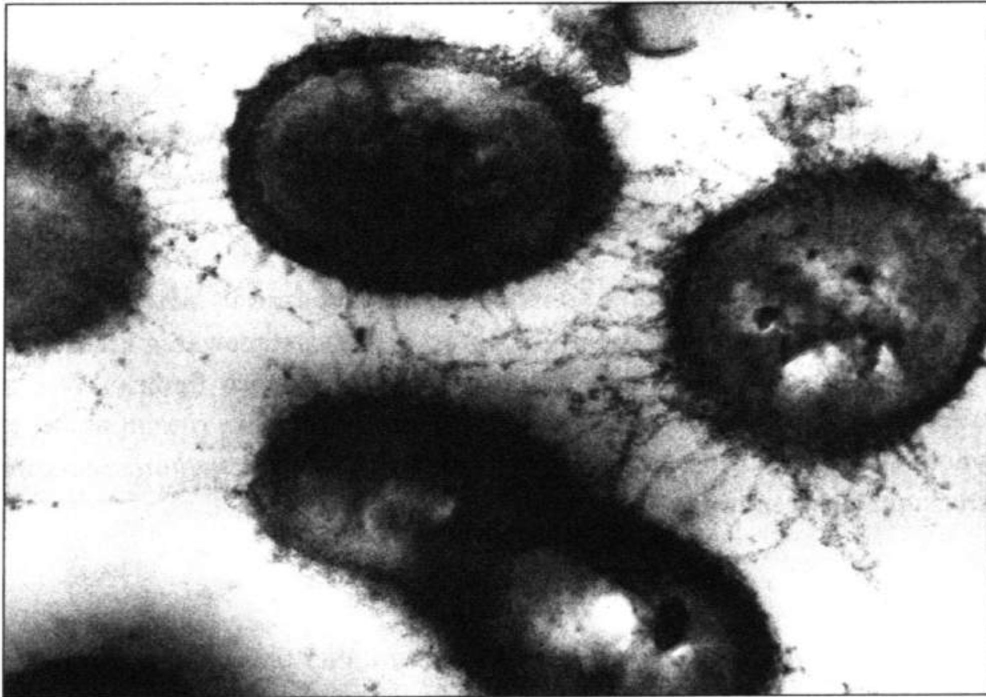

Fig. 4. The preparation was stained during fixation with ruthenum red. Arrows designate thin strands resembling fimbriae x 95.000.

The photographs were taken at the Electron-Microscope Unit in the Department of Pathomorphology, University School of Medical Sciences in Bydgoszcz

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Streszczenie

Przeprowadzono badania ultrastrukturalne, których celem było wykazanie obecności cienkich fimbrii na ścianie komórkowej bakterii *Acinetobacter junii*. Zastosowano trzy metody kontrastowania. Po pierwsze, skrawki barwiono octanem uranylu i cytrynianem ołowiu. Przy zastosowaniu tej metody obserwowano jedynie bezpostaciowy materiał otaczający komórki bakterii. Następnie zastosowano kontrastowanie samym 1 % octanem uranylu. Ta metoda umożliwiła wykrycie cienkich fimbrii. Stosując czerwień rutenu jako trzecią metodę barwienia, stwierdzono na powierzchni komórek bakteryjnych występowanie kłaczkowatej warstwy, jak również nitkowate struktury przypominające fimbrie. W naszej ocenie metoda z 1 % octanem uranylu i metoda z czerwienią rutenu okazały się przydatne w wykryciu obecności fimbrii u bakterii *Acinetobacter junii* na poziomie mikroskopu elektronowego.