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Short Communication

# Nanoscale effects of antibiotics on *P. aeruginosa*

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**Abstract**

Studying living bacteria at the nanoscale in their native liquid environment opens an unexplored landscape. We focus on *Pseudomonas aeruginosa* and demonstrate how the cell wall is biophysically affected at the nanoscale by two reference antibiotics (ticarcillin and tobramycin). The elasticity of the cells drops dramatically after treatment (from 263 ± 70 kPa to 50 ± 18 and 24 ± 4 kPa, respectively on ticarcillin- and tobramycin-treated bacteria) and major micro- and nano-morphological modifications are observed (the surface roughness of native, ticarcillin- and tobramycin-treated bacteria are respectively 2.5, 0.8, and 4.4 nm for a surface area of 40,000 nm²). Thus the nanoscale approach in liquid is valid and can be extended.

***From the Clinical Editor:*** *Pseudomonas aeruginosa* cell wall was demonstrated to be biophysically affected at the nanoscale by two reference antibiotics, ticarcillin, and tobramycin, with the elasticity dropping dramatically after treatment.


*Key words:* *Pseudomonas aeruginosa*; Atomic Force Microscopy; Ticarcillin; Tobramycin; Elasticity; Bacterial cell wall

For 25 years, Atomic Force Microscopy (AFM) has emerged as a valuable tool in microbiology.[1] Recently it has been used to study the effects of antimicrobial drugs on living microorganisms.[2] An advantage of AFM is the possibility to work in liquid on living cells. Nevertheless, sample immobilization is a challenge[3] and explains why in most publications the bacteria were air dried. Here, we focused on the dreadful bacteria *Pseudomonas aeruginosa*[4] and overcome the immobilization problem by taking advantage of electrostatic interactions between a positively charged surface and the negatively charged bacteria. *P. aeruginosa,* is implicated in 10% of nosocomial infections in France. This pathogen, resistant to several antibiotics and antiseptics, has a great capacity for acquiring new resistance mechanisms under selective antibiotic pressure.[5] Therefore, understanding the effects of antibiotics on these bacteria has become a necessity. We studied two reference antibiotics, ticarcillin and tobramycin, which are highly active on *P. aeruginosa*. They have known action mechanisms and are widely used in terapeutics, unlike other molecules, e.g. colistin wihch effects were recently studied.[6] Ticarcillin is a β-lactamin which inhibits the bacterial transpeptidases and transglycosylases responsible for the assembly of the cell wall peptidoglycan.[6] Tobramycin is an aminoglycoside that works by binding to the 30S and 50S bacterial ribosome to prevent formation of the 70S complex. As a result, mRNA cannot be translated into protein.[7] These two different mechanisms of action should therefore produce different effects on the bacterial cells. In this study, our purpose was to understand better the antibiotics' effects on the cell wall of *P. aeruginosa* at the nanoscale.

We chose to explore the effects of tobramycin and ticarcillin on *P. aeruginosa* ATCC 27853. We used AFM (details appear in Supplementary Material 1) to explore bacterial cell wall modifications. We recorded images of single bacteria (Figure 1) and images at higher resolution on the top of the cells to qualitatively explore the impact of tobramycin and ticarcillin on

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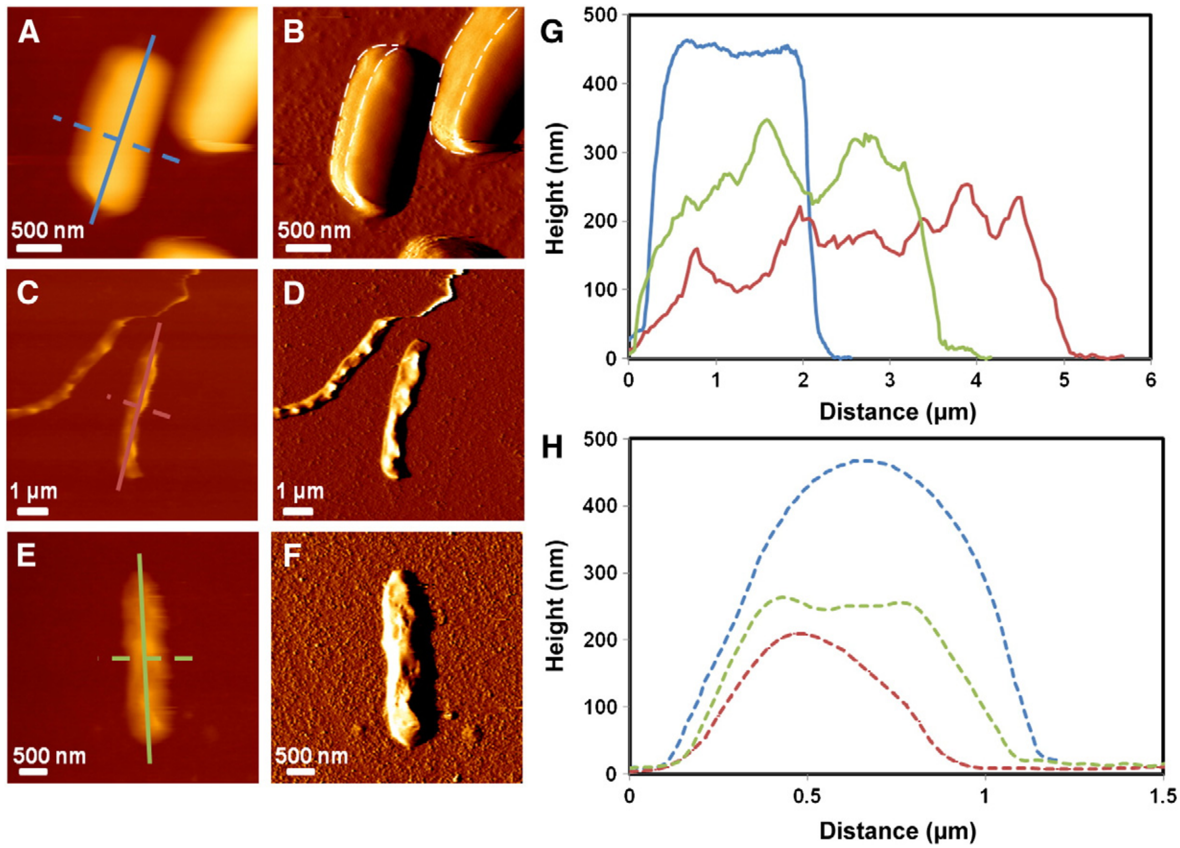


Figure 1. Imaging of bacteria. Height images (z range = 1μm) recorded on **(A)** native, **(C)** ticarcillin- and **(E)** tobramycin- treated cell. **(B, D and F)** Vertical deflection images correspond to the height images. **(G)** Vertical cross-sections taken along the solid lines of native (*blue*), ticarcillin- (*green*) and tobramycin-treated cell (*red*). **(H)** Horizontal cross-sections taken along the dashed lines.

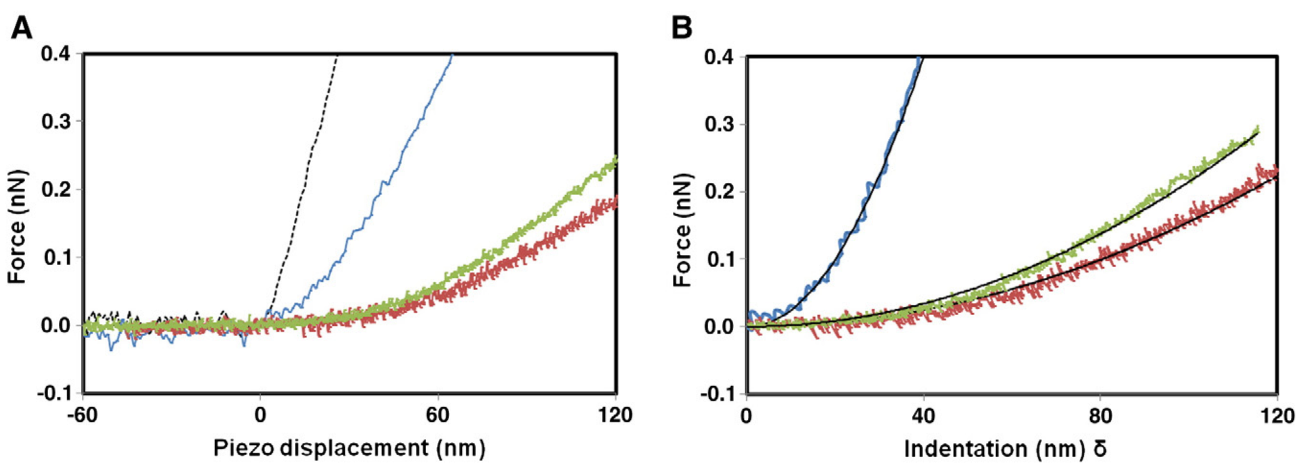


Figure 2. Mechanical properties of native (*blue*), ticarcillin- (*green*) and tobramycin-treated (*red*) bacteria. **(A)** Representative force-displacement curves recorded on the glass slide (*dashed line*) and on single cells. The lines between the dashed lines and the colored lines represent the indentation (δ). **(B)** Force-indentation curves obtained from the curves showed in panel A. Colored lines show the data, whereas the black lines show the theoretical fits (Hertz model) used to extract Young modulus values.

*P. aeruginosa* surface topology. We then recorded force maps of 20 × 20 force curves on the same area of the cells. The force curves were recorded in the same conditions with calibrated cantilevers. The force-distance curves recorded on the bacteria were treated to subtract the cantilever deflection on a solid surface (see Figure 2). The distance difference, at a given force, between a curve recorded on glass and on the bacteria is called indentation (δ). The indentation curves were then fitted to the Hertz model,

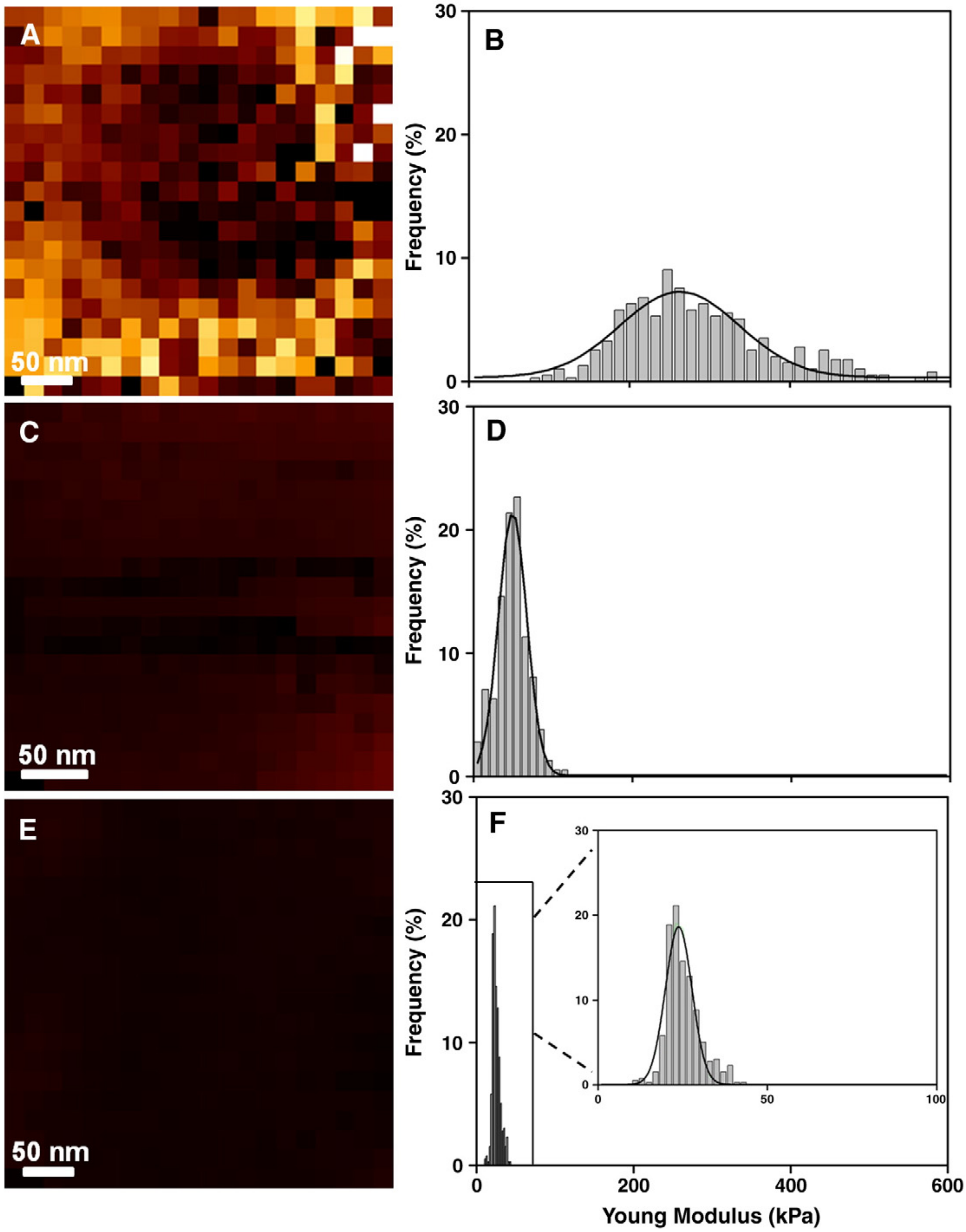


Figure 3. Mapping of bacteria surface elasticity. **(A)** elasticity map (z-range = 600 kPa) recorded on a native, **(C)** ticarcillin- and **(E)** tobramycin-treated cell. **(B, D and F)** Distribution of Young Modulus values (n = 399 force curves) corresponding to the elasticity maps. In **F** the insert presents more clearly the E repartition.

which links force (F) as a function of the elastic modulus (E) and the square of the indentation (δ) for a conical indenter.

$$F = \frac{2E\ \tan\alpha}{\pi(1-\mathrm{v}^2)}\delta^2 \qquad (1)$$

In equation (1), α is the tip opening angle (36°) and $\nu$ the Poisson ratio assumed to be 0.5.

Figure 1 presents height and deflection AFM images of *P. aeruginosa* in (A) native condition, after (C) ticarcillin and (E) tobramycin treatment. The native cell shows a smooth surface. The cells are 2 μm long (blue line, Figure 1, *G*), 1.2 μm large (blue dashed line, Figure 1, *H*) and 450 nm high (blue lines, Figure 1, *G* and *H*). Due to this relatively higher height, convolution artifacts can be seen on the deflection image in Figure 1, *B* (white dashed lines). Figure 2 is a comparison of the

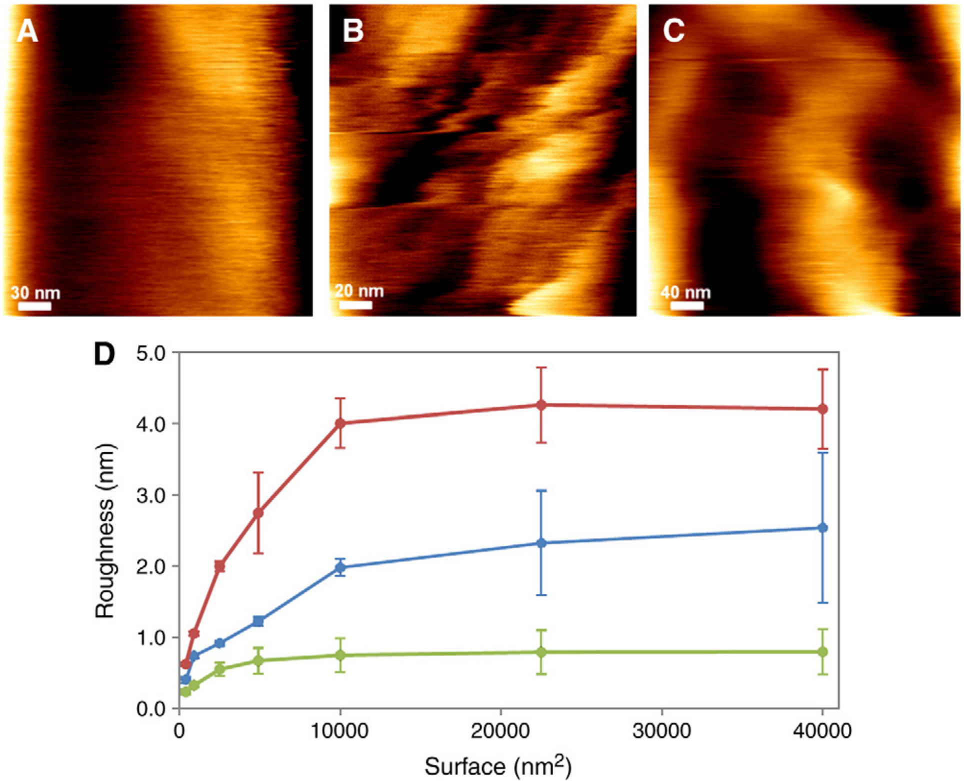


Figure 4. Imaging of bacteria cells. Height images recorded on **(A)** native, **(B)** ticarcillin- **(C)** tobramycin-treated cell. **D** shows the roughness measured on the height images of native (blue), ticarcillin- (green) and tobramycin-treated cells (red).

elastic properties of native and treated cells. Figure 2, *A* shows representative raw data, whereas Figure 2, *B* shows the force versus indentation curve. In Figure 2 the plain black lines are the Hertz model fitted to the indentation curves. On those curves it is clear that both ticarcillin and tobramycin induce a dramatic decrease of the bacterial cell wall elasticity. In Figure 3 this obvious observation is quantified with force volume. Native cells have a Young modulus of 261 ± 70 kPa (Figure 3, *B*), whereas for ticarcillin- and tobramycin-treated cells, the histograms (Figure 3, *D* and *F*) show that the modulus drops, respectively, to 24 ± 4 kPa and 50 ± 18 kPa (for statistic see Supplementary Material 2). On the elasticity maps in Figure 3, *A*, *C* and *E*, each pixel represents an elastic modulus. The brighter the pixel is, the higher the Young modulus is. The maps are homogeneous and confirm that the elasticity decreases between the native and treated cells. From Figure 4, high-resolution height images of the cells' surface and a roughness analysis, we learn that the native cells are smooth, homogeneous, and bulging whereas the tobramycin-treated cells are rougher. Surprisingly, the Power Spectral Density (PSD) analysis of ticarcillin-treated cells show a decrease of the surface roughness. Moreover, bacteria growing in presence of ticarcillin form filaments. This morphology, already observed by Scanning Electron Microscopy,[8] can be explained by the fact that β-lactamins, like ticarcillin, activates the SOS system of the bacteria, therefore inhibiting the cell division.[9,10] The Young modulus of ticarcillin-treated cells is reduced to 50 ± 18 kPa (Figure 3), which indicates that ticarcillin affects the bacterial cell wall. This observation is consistent with the mechanism of action of the antibiotic that inhibits the biosynthesis of the peptidoglycan. Therefore the bacteria are not able to yield a rigid cell wall, leading to the decrease of the Young modulus.

Tobramycin binds to the bacterial ribosome, which leads to the synthesis of abnormal proteins. These proteins are then incorporated to the bacterial cell wall, which loses its integrity. We show that tobramycin-treated cells have a deformed cell wall (Figure 1), and a Young modulus highly reduced in comparison with native cells (Figure 3).

This set of data demonstrates that looking at the nanobiophysical properties of bacteria treated by antibiotics is rich in information. A straightforward extension of this study will be (i) to work with *P. aeruginosa* strains resistant to ticarcillin or/and tobramycin; and (ii) to analyze the effects of innovative antimicrobial agents. Because of the method and data presented here, we are confident that real and major advances in both fundamental and applied microbiology could be done.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.nano.2011.09.009.

## References


1. Dufrêne YF, Müller DJ. Atomic Force Microscopy as a multifunctional molecular toolbox in nanobiotechnology. Nat Nanotech 2008;3:261-9.
2. Dorobantu LS, Gray MR. Application of atomic force microscopy in bacterial research. Scanning 2010;32:74-96.
3. El Kirat K, Burton I, Duprès V, Dufrêne YF. Sample preparation procedures for biological atomic force microscopy. J Microscopy 2005;218:199-207.
4. Hauser AR, Ozer EA. *Pseudomonas aeruginosa*. Nat Rev Microbiol 2011;9 [Poster].
5. Strateva T, Yordanov D. *Pseudomonas aeruginosa*: a phenomenon of bacterial resistance. J Med Microbiol 2009;58:133-48.
6. Mortensen NP, Fowlkes JD, Sullivan CJ, Allison DP, Larsen NB, Molin S, et al. Effects of colistin on surface ultrastructure and nanomechanics of *Pseudomonas aeruginosa* cells. Langmuir 2009;25: 3728-33.
7. Jehl F, Chomarat M, Weber M, Gérard A. De l'antibiogramme à la p. rescription. Edition Biomérieux. France: Marcy L'étoile; 2004.
8. Prior RB, Warner JF. Morphological alterations of *Pseudomonas aeruginosa* by ticarcillin: a scanning electron microscope study. Antimicrob Agents Chemother 1974;6:853-5.
9. Schlacher K, Goodman M. Lessons from 50 years of SOS DNA-damage-induced mutagenesis. Nat Rev Mol Cell Biol 2007;8:587-94.
10. Blaquez J, Gomez-Gomez JM, Oliver A, Juan C, Kapur V, Martin S. PBP3 inhibition elicits adaptative responses in *Pseudomnoas aeruginosa*. Mol Microbiol 2006;62:84-99.