Supplementary Information

Synergism in the Antibacterial Action of Ternary Mixtures Involving Silver Nanoparticles, Chitosan and Antibiotics

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The UV-Vis spectra of the aqueous suspensions of chitosan at 2.2 g L^{-1} in 1.0% v/v of HAc (pH 3.0), 1.0% v/v of HCl (pH 2.5) and 1.0% v/v of formic acid (HForm) (pH 3.5) are presented in Figure S1. The higher scattering (background intensity) observed for aqueous suspension of chitosan in HForm medium shows this is not a good solubilizing agent and the lower scattering in HAc medium led to its choice as the appropriate acid for the solubilization of chitosan. The HCl was also a good solubilizing agent, but it was avoided because chloride ion adsorbs strongly on silver surface, being capable to compete with the other adsorbates when chitosan is used together with AgNP suspension in the biological analyses.

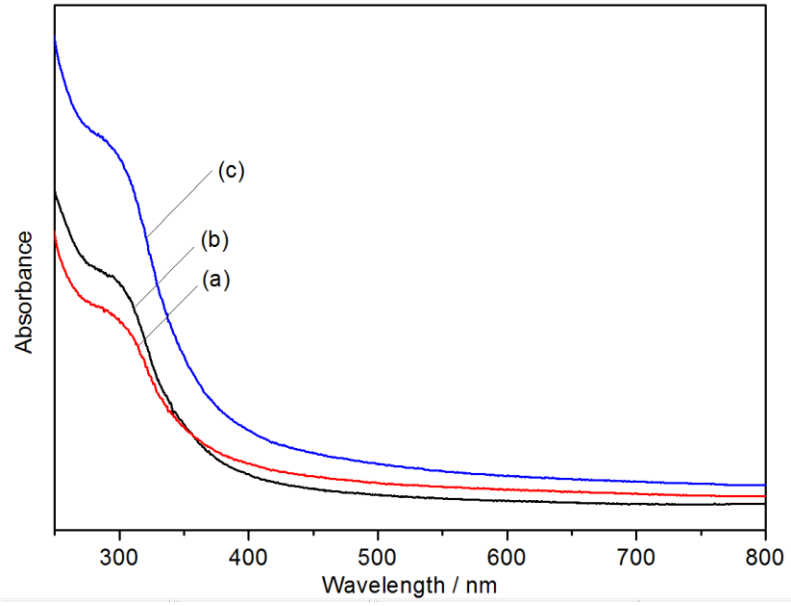

Figure S1. UV-Vis spectra of aqueous suspensions of chitosan at 2.2 g L^{-1} solubilized at 1.0% v/v aqueous solutions of (a) HAc; (b) HCl and (c) HForm.

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Figure S2 shows the UV-Vis spectra of seven samples of AgNP-Chit with different pH, which varied from acidic to neutral. The small changes of the spectral patterns suggest the variations in the size distributions of the silver nanoparticles were negligible. It is noteworthy that in pH 7.0 the AgNP-Chit suspension was stable for few hours due to the deprotonation of chitosan, with its loss of solubility in water. The spectra of chitosan 2.2 g L^{-1} aqueous solution at pH 6.0 (Figure 2h) and pH 3.0 (Figure 2i) show the system is stable in this pH range. Remembering that the final concentrations of silver and chitosan in the AgNP-Chit suspension were ca. 0.2 g L^{-1}.

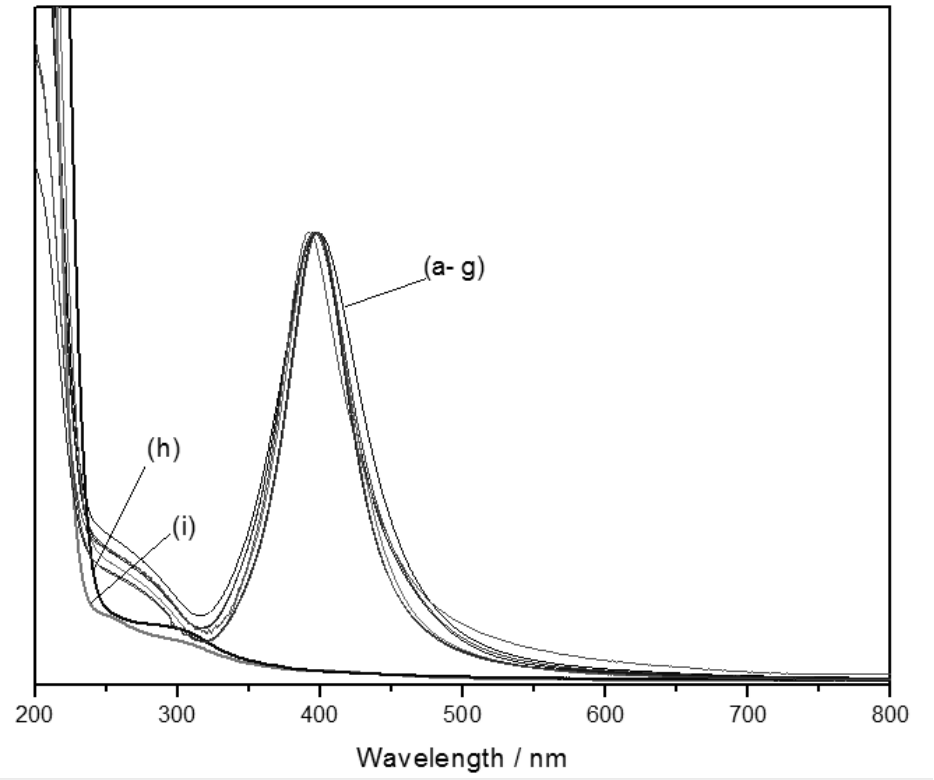

Figure S2. UV-Vis spectra of AgNP-Chit aqueous suspension at pH 3.0 (a); pH 4.5 (b); pH 5.0 (c); pH 5.5 (d); pH 6.0 (e); pH 6.5 (f); pH 7.0 (g); and Chit 2.2 g L^{-1} aqueous suspension in Ac^- (pH = 6.0) (h) and in HAc 1% v/v (pH = 3.0) (i).

Figure S3 shows SEM images of AgNP-Chit. A large number of small nanoparticles can be observed, with diameters around 10 nm. The presence of chitosan is interfering in the quality of images due to the dielectric character of the organic polymer.

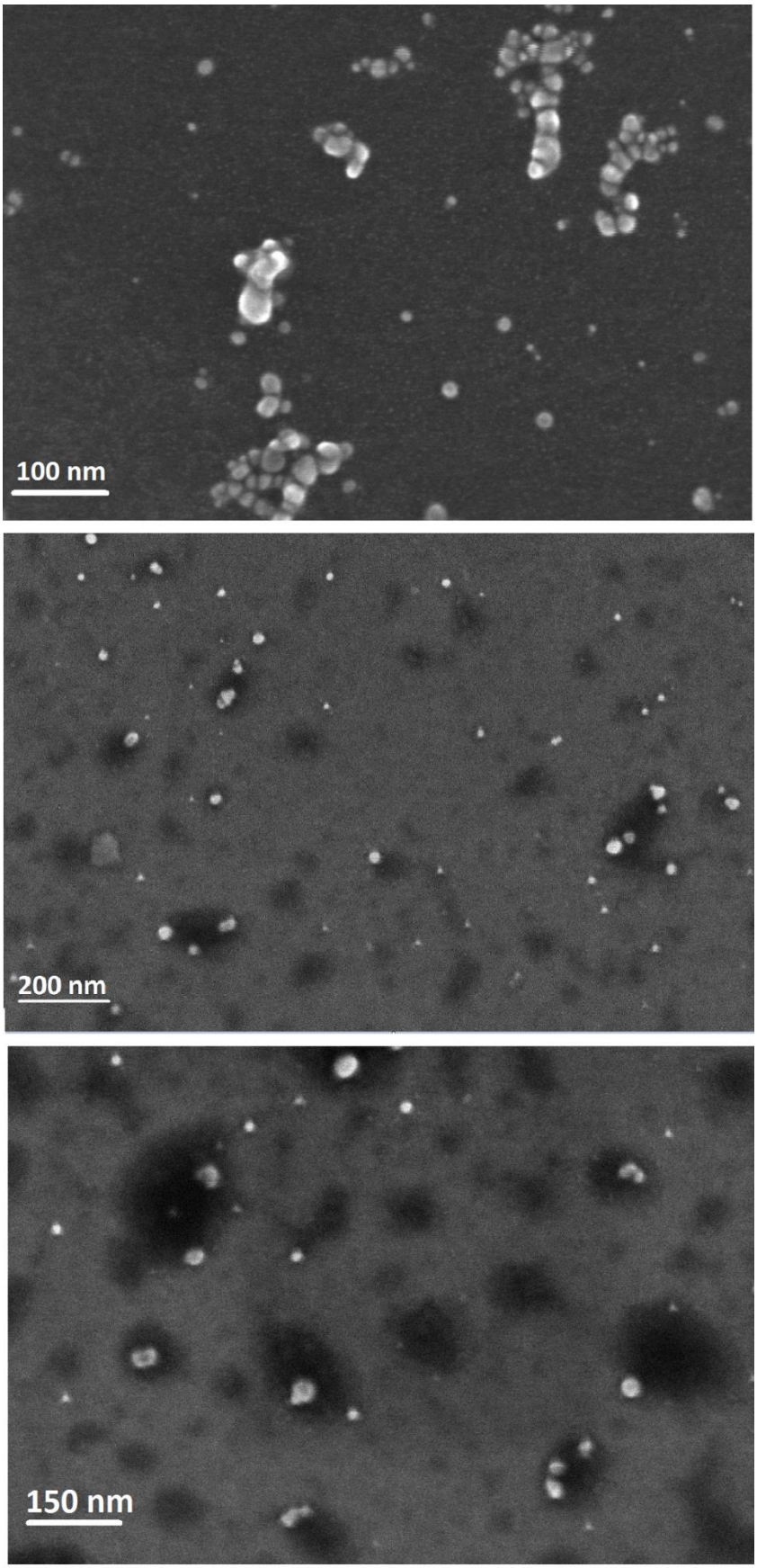

Figure S3. SEM micrographs of AgNP-Chit dried on silicon plates.

Table S1 shows that when the pH of the culture medium was above 5.0 and below 8.0 the growth of *E. coli* and *S. aureus* were not compromised.

Table S1. Impact of pH in the culture medium with three different acids

Acid	Concentration of acid solution / (% v/v)	pH of the culture medium after the adition of 0.15 mL of acid solution	*E. coli* (ATCC 11229)	*S. aureus* (ATCC 29213)
HCl	1.0	3-4	–	–
	0.1	5-6	+	+
HNO_3	1.0	3-4	–	–
	0.1	4-5	–	–
HAc	1.0	4-5	–	–
	0.1	5-6	+	+
Negative control		7-8	–	
Positive control for *E. coli*		7-8	+	
Positive controle for *S. aureus*		7-8		+

+: the growth of bacteria after incubation was present; –: the growth of bacteria after incubation was not present.

Tables S2 and S3 show the MIC values of chitosan and AgNP-Chit tested against *E. coli* and *S. aureus*, which were similar for both investigated bacteria when using two different pH values (6.0 and 7.2) for the growth medium.

Table S2. MIC values of Chit and AgNP-Chit, tested against *E. coli* and *S. aureus*, by using a culture medium with pH at 6.0 (pH adjusted with HCl 1.0 mol L^{-1})

Tested agent	*E. coli* (ATCC 11229) / (μg mL^{-1})		*S. aureus* (ATCCC 29213) / (μg mL^{-1})	
	Bacteriostatic	Bactericide	Bacteriostatic	Bactericide
AgNP-Chit	32.0	32.0	32.0	64.0
Chit 2.2 g L^{-1}	176.0	352.0	176.0	352.0

Table S3. MIC values of Chit and AgNP-Chit, tested against *E. coli* and *S. aureus*, by using a culture medium with pH at 7.2 (standard preparation of the medium without any adjustment of pH)

Tested agent	*E. coli* (ATCC 11229) / (μg mL^{-1})		*S. aureus* (ATCCC 29213) / (μg mL^{-1})	
	Bacteriostatic	Bactericide	Bacteriostatic	Bactericide
AgNP-Chit	32.0	32.0	32.0	64.0
Chit 2.2 g L^{-1}	176.0	704.0	176.0	352.0

Figures S4 and S5 show TEM images of *K. pneumoniae* and *S. aureus*, respectively, in the absence and presence of AgNP-Chit. Changes in the cellular morphology and preferential interactions of AgNP-Chit with bacterial walls can be observed.

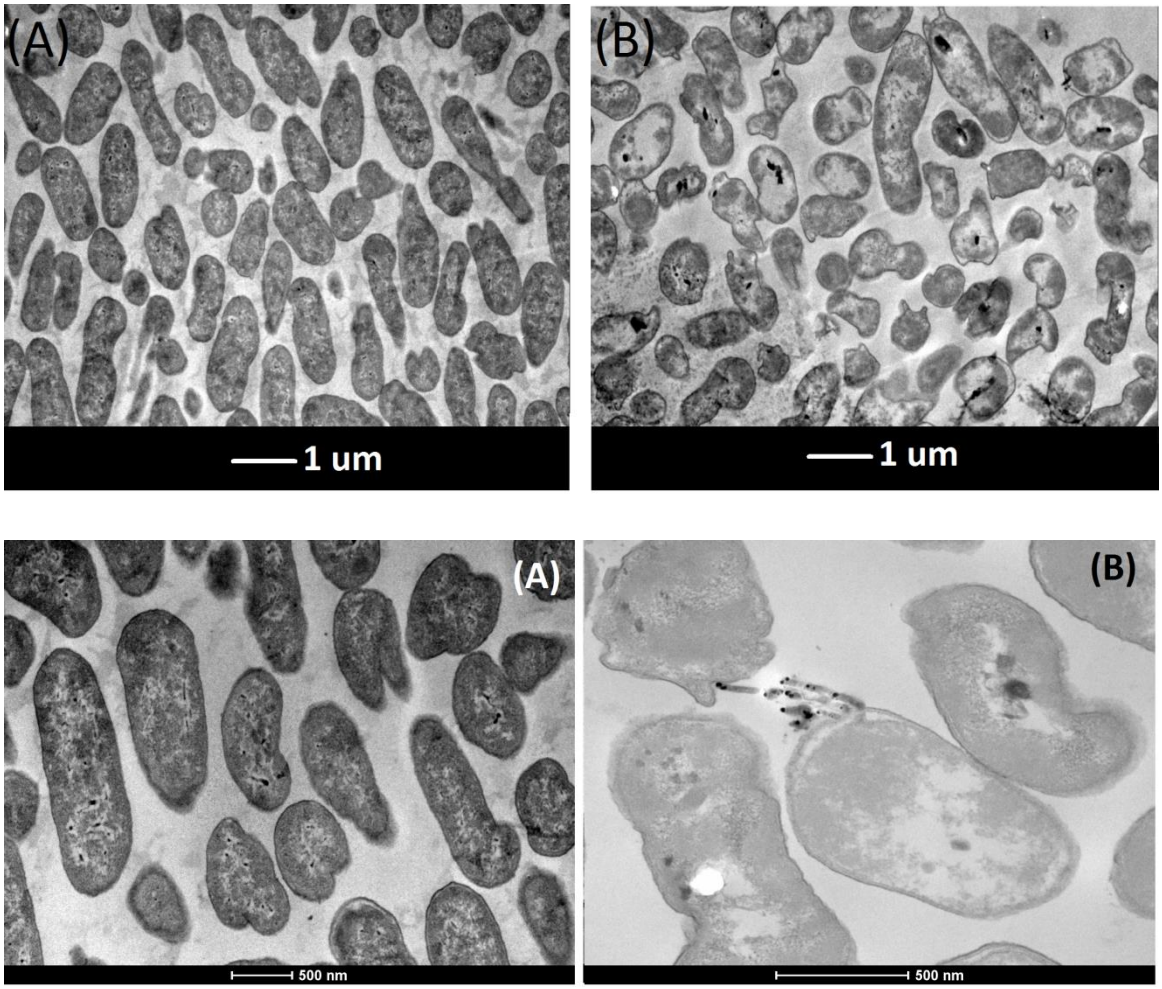

Figure S4. TEM images of *K. pneumoniae* in the (A) absence and (B) presence of AgNP-Chit on two different scales (1 µm and 500 nm).

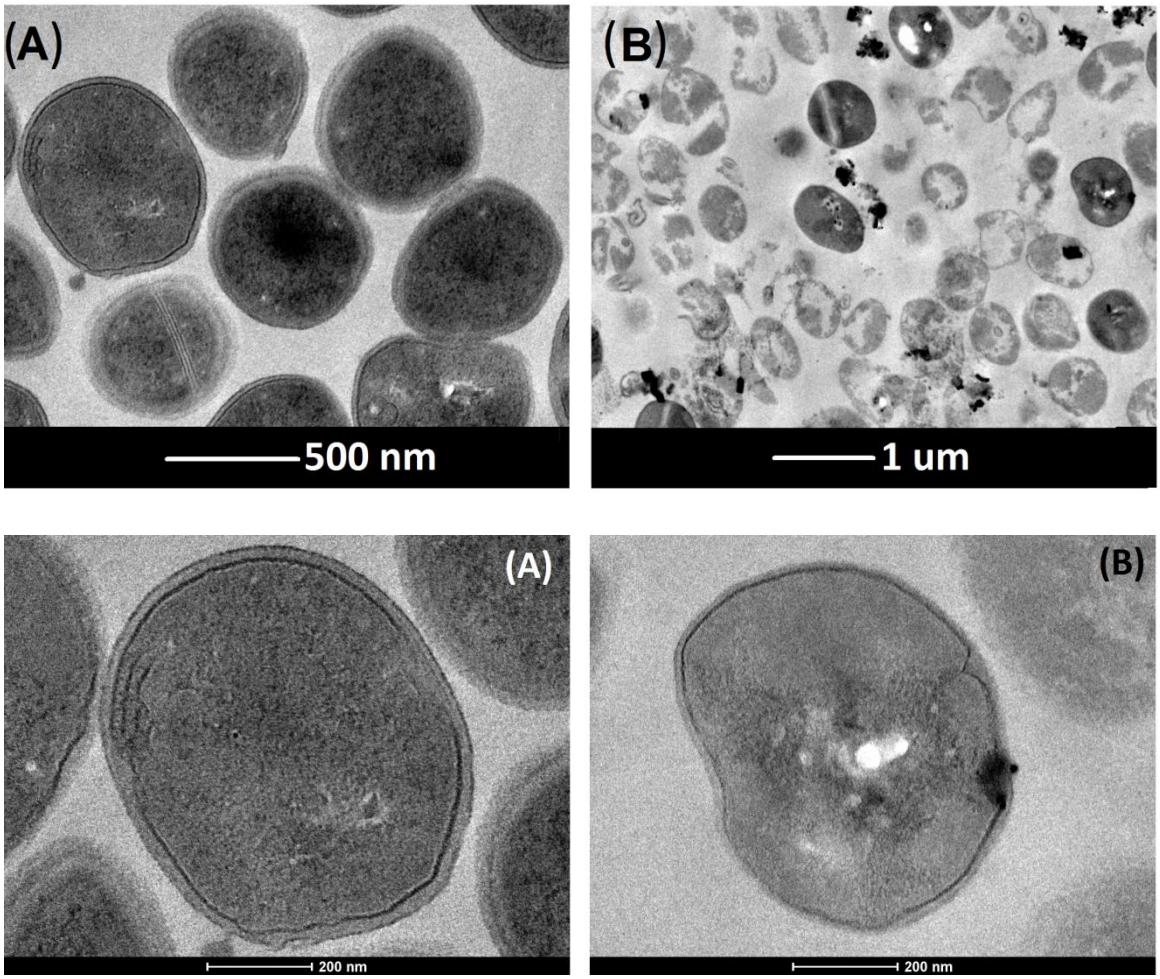

Figure S5. TEM images of *S. aureus* in the (A) absence and (B) presence of AgNP-Chit on two different scales (1 µm and 200 nm).