Facile fabrication of superior antibacterial cotton fabric based on ZnO nanoparticles/quaternary ammonium salts hybrid composites and mechanism study

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Supplementary material

Experimental

1 Materials and reagents

All regents and chemicals were used without further purification. Cotton fabric and Live/Dead BacLight Bacterial Viability Kit (L7005) were acquired from Fluorochem Company and Thermo Fisher Scientific Company in the United States, respectively. Zinc oxide (ZnO, (30±10) nm, 99.9% metals basis), 60 wt.% dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride in methanol solution ($C_{26}H_{58}ClNO_3Si$, DMOAP), sodium dodecyl sulfonate ($C_{12}H_{25}SO_3Na$), L-histidine, neutral silica sol, zinc chloride ($ZnCl_2$) and sodium hydroxide (NaOH) were purchased from Aladdin. Reagent Co. (shanghai, China). L-glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were obtained from Shanghai McLean Biochemical Technology Co., Ltd. Sodium chloride (NaCl) and isopropyl alcohol (IPA) were acquired from Hangzhou Gaojing Fine Chemical Co., Ltd. (Hangzhou, China).

2 Preparation of ZnO/QAS antibacterial cotton fabrics

A homogeneous antibacterial padding suspension of ZnO/QAS antibacterial agent was prepared by ultrasonically dispersing ZnO NPs, QAS in 20 mL 2% neutral silica sol deionized water (DI) for 5 min. The ratio of ZnO to QAS, 30 $g \cdot L^{-1}$: X (X = 5, 10, 15, 20 $g \cdot L^{-1}$), was adjusted to explore the effects of QAS concentration on antibacterial cotton properties. ZnO or QAS suspensions were prepared using the same preparation procedure as described above.

The antibacterial agent was loaded on the clean cotton fabric by the dipping–padding–drying method. Firstly, the raw cotton fabric (20 cm × 6 cm) was ultrasonically soaked in 2% sodium dodecyl sulfonate solution for 30 min. The cotton was washed several times with DI water and anhydrous ethanol, and then dried at 60 °C in an oven. After adding the washed cotton in the padding solution, the mixture was ultra-sounded for 5 min and then soaked for 15 min. After that,

the washed cotton was immersed in the antibacterial padding suspension and then processed by a padding machine (the pressure between the two rollers was 0.2 MPa, the speed of the rollers was 15 $r \cdot min^{-1}$). The cotton fabric maintains 100% moisture absorption and the whole process was repeated for three times. The antibacterial cotton fabric was dried at 60 °C to remove moisture, cured at 140 °C. Finally, the ultimate antibacterial cotton was washed several times with DI water to remove weakly adherent antimicrobial agents from the cotton surface and then dried at 60 °C.

3 Characterization of ZnO/QAS antibacterial cotton fabrics

X-ray powder diffraction (XRD) measurements was performed on D8 Discover two-dimensional wide-angle X-ray diffractometer (made in Bruker, Germany) operated at 40 kV and 40 mA using a Ni filtered Cu Kα radiation with the wavelength of 1.5408 Å in the wide angle-region from 5° to 75° on 2θ scale. Element analysis with CHNS mode was performed to measure N content in QAS. Zn element content was detected by Agilent 720ES inductively coupled plasma-atomic emission spectrometry (ICP-OES). Fourier transform infrared spectroscopy (FTIR) of different cotton fabrics were recorded using a Nicolet 5700 Fourier transform infrared spectrometer (made by Thermo, USA), employing acquisition methods of infrared spectrum transmission and attenuated total reflection. X-ray photoelectron spectra were recorded on a Thermo Scientific K-Alpha X-ray photoelectron spectrometer (XPS). The surface morphologies of the modified cotton and the elemental distribution of various elements on the treated cotton fabrics were also determined by field-emission scanning electron microscope (FESEM, Gemini 500, made by Carl Zeiss, Germany) combined with energy-dispersive X-ray spectroscopy (EDS). The static contact angle on the surface of the antibacterial fiber was measured by the contact angle meter (JY-82B, Japan). Ultraviolet–visible diffuse reflectance spectroscopy (UV–vis-DRS) was performed on UV–vis-NIR spectrophotometer (UH4150, Hitachi, Japan). Thermogravimetric analysis (TGA) of the dried cotton fabrics was performed under nitrogen atmosphere through thermogravimetric analyzer from Mettler Toledo. Zeta potential of tested surfaces was evaluated on SurPASS Solid Surface ZETA Potential Tester under the condition of pH = 7 (Anton Paar, Austria). All the samples were measured four times at constant pH (pH = 7.0) with a relative error lower than 5%. The concentration of Zn^{2+} ions was detected by Agilent 7700 inductively coupled plasma-mass spectrometry (ICP-MS).

Stress–strain tests were carried out at room temperature using INSTRON 34 TM-30 in accordance with GB/T 3923-1997. In order to maintain the accuracy of the experiment, the certain sample parameters to ensure more accurate results were formulated. Parameters for each sample are as follows: (i) a certain size of the cotton fabric (100 mm × 15 mm); (ii) the clamping distance (30 mm); (iii) the thickness of four layers (7.4 mm); (iv) the initial applied load (1.2 N); (v) the stretching rate (10 $mm \cdot min^{-1}$). Different cotton samples with the size of 5 cm × 5 cm were soaked into the DI water, separately, taken out after full soaking, and hung on the applicator for 10 min, weighing out the masses of the fibers before and after the water absorption, and calculating the water absorption rate of the fibers. The water absorption rate is calculated by the following equation:

$$W/\% = \frac{W_a - W_b}{W_b} \times 100 \quad (1)$$

where W is the water absorption rate (%), W_b is the mass (g) of the fiber sample before water absorption, and W_a is the mass (g) of the fiber sample after water absorption. Different fiber samples

were sealed at the mouth of the sample bottle with the same amount of DI water, and the mass change of each sample bottle liquid before and after water absorption was weighed after 24 h. The vapor transmission rate (T, unit: g·m^{-2}) is then calculated by the following equation:

$$T = \frac{4\times10^4}{\pi d^2}\times\left(m_b - m_a\right) \tag{2}$$

where m_b is the mass (g) of DI water in vial before 24 h, m_a is the mass (g) of DI in vial after 24 h, and d is the diameter (cm) of the vial mouth. The limpness test was to fold each sample of 20 cm × 6 cm in half naturally, and then used a vernier caliper to measure the maximum loop height of each sample. Three groups of parallel experiments were carried out on the water absorbability, vapor transmission rate, and limpness of different antibacterial samples.

4 Antibacterial activity assay

The antibacterial activities of the samples were assessed by the inactivation of the Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*, ATCC6538) and the Gram-negative bacteria *Escherichia coli* (*E. coli*, ATCC8739) according to the GB/T 20944.3-2008 Textiles - Evaluation of antibacterial properties - Part 3: Oscillation method established in Chinese National Standards. Before each bacterial experiment, all equipment's and materials except microorganisms were aseptically processed in an autoclave. The test bacteria were incubated in a shaking incubator (shaking incubator model BSD-150, Shanghai Boxun Industrial Co., Ltd., the shaking speed: 360 r·min^{-1}) at 37 °C for 18 h in Letheen broth fluid nutrient medium (containing 5 g·L^{-1} yeast extract powder, 10 g·L^{-1} tryptone, 10 g·L^{-1} NaCl). Then, 200 μL of activated *E. coli* or *S. aureus* was added in 3.8 mL Phosphoric acid buffer solution (PBS) for dilution in the sterile tubes, and dilute again after being shaken at room temperature for 5 s. Cotton, ZnO/cotton, QAS/cotton, and ZnO/QAS/cotton with 53.6 mg (an approximately size of 2.18 cm × 2.18 cm) were sterilized by the UV light for 30 min and placed in sterile tubes, respectively. 360 μL of the diluted bacterial suspension (for *E. coli*, from 3×10^6 to 6×10^6 CFU·mL^{-1}; for *S. aureus*, from 3×10^6 to 6×10^6 CFU·mL^{-1}) and 5 mL PBS were added into sterile tubes containing antibacterial fabrics, and then cultured under dark condition at 37 °C for 8 h in a constant temperature shaking incubator. From the above sterile tube, 100 μL of bacterial suspension was drew for the observation of bacterial proliferation, and the bacterial proliferation was assessed by agar plate counting after a given interval of time of dark conditions at 37 °C for 18 h. The Colony count method was used to determine the number of survival bacteria. The reduction rate of bacteria is calculated according to the following formula by AATCC100-2004 standard method:

$$R/\% = \frac{B-A}{B}\times100 \tag{3}$$

where R is the percent reduction (%), A is the number of bacteria recovered from the inoculated treated test specimen swatches in the sterile vials incubated over the desired contact period, and B is the number of bacteria recovered from the inoculated treated control swatches in the sterile vials incubated over the desired contact period. Each sample was measured at least three times, and the average was then taken.

The antibacterial activity was also performed according to the width standard of FZ/T 73023-2006 inhibition zone. For the qualitative evaluation, samples were cut into discs with a diameter of 1 cm and wetted with PBS, which placed onto agar plates and covered with agar seeded

with 10^6 CFU·mL^{-1} from overnight cultures of *E. coli* or *S. aureus*. Then plates were incubated at 37 °C for 18 h and the antibacterial activities were identified and estimated by clear zones of inhibition in the indicator lawn around the treated samples. Untreated sample was also tested as negative control.

5 Antibacterial mechanism assay

5.1 The bacteria morphology after antibacterial test

To study the interaction between bacteriostatic cotton and bacteria, the antibacterial activity of the bacteriostatic cotton fabric containing *E. coli* was evaluated by SEM. Briefly, bacterial cells and bacteriostatic cotton were harvested by centrifugation after 18 h of culture, and the isolated bacteriostatic cotton was fixed wit glutaraldehyde solution (2.5%) for 2 h in a refrigerator at 4 °C and washed 3 times with PBS (pH = 7.0). Then it was dehydrated with ethanol of various concentrations (30%, 50%, 70%, 90%, and 100%) for 15 min, centrifuged to obtain the antibacterial cotton at the bottom, and dried in air naturally. SEM was used to observe the bacteria morphology of the cotton fabric surface.

5.2 Live/dead bacterial viability assays

Live/dead bacterial viability assays were used to intuitively evaluate antibacterial properties of different antibacterial cotton fabrics against *E. coli*, which is a live/dead bacterial fluorescence stain method using the fluorescent dye mixture of a green SYTO9 (10 μg·mL^{-1}) and red propidium iodide (PI, 10 μg·mL^{-1}). First, 200 μL of bacteria suspension with *E. coli* at 10^6 CFU·mL^{-1} was applied in the obtained antibacterial cotton fabrics and further incubated for 18 h at 37 °C. Thereafter, 200 μL of fresh SYTO9/PI mixture was added on the surface of bacteria using a pipette and the test sample vial was wrapped with an appropriate amount of tinfoil to protect from light; the thorough staining of bacteria was allowed to occur at room temperature in darkness for 20 min. The excess dye mixture was washed by centrifugation at 6000 r·min^{-1}, and then the bacterial pellet was placed in a special dish for confocal laser scanning microscopy (CLSM). Afterward, the bacteria were observed under CLSM (FV1200, Japan) using green and red filters with excitation/emission wavelength 488/520 nm and 559/619 nm, respectively.

5.3 Reactive oxygen species (ROS) scavenging test

In order to determine the role of ROS in the antibacterial activity, active oxygen removal experiment was performed. GSH, CAT, IPA, SOD, and L-histidine were used for trapping all ROS, hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide radical anion ($•O_2^-$), singlet oxygen ($^1O_2$), respectively. Before the antibacterial test, one of the ROS trapping agents including GSH (100 μL, 5 mmol·L^{-1}), CAT (150 U·mL^{-1}), IPA (100 μL, 0.5 mmol·L^{-1}), SOD (150 U·mL^{-1}), or L-histidine (100 μL, 5 mmol·L^{-1}) was added to the bacterial solution. The solutions were placed in a shaker at a constant temperature of 37 °C for 8 h. The solutions were spread evenly at the surface of the medium and then placed in an incubator at constant temperature of 37 °C for 18 h.

5.4 Detection of Zn^{2+} ion dissolution

The bacteria and treated cotton fabric were cultured together for 8 h, and then centrifuged to obtain the bacterial solution containing Zn^{2+}. The release of Zn^{2+} ions from ZnO/cotton and ZnO/QAS/cotton in the liquid culture medium after the antibacterial test was measured by ICP-MS.

5.5 Mechanical damage of cell wall

In order to analyze the damage caused by mechanical damage to bacteria, two groups of antibacterial experiments were conducted. One of the two groups was centrifuged, and then the supernatant was collected for antibacterial experiments (Group B), while the other group was left unchanged (Group A). The Group A and Group B samples were placed in a shaker at 37 °C for 2 h. The solutions were spread evenly at the surface of the medium and then placed in an incubator at constant temperature of 37 °C for 18 h. Then, the antibacterial rate was calculated. The difference between the antibacterial activities in A and B groups is attributed to the mechanical sterilization.

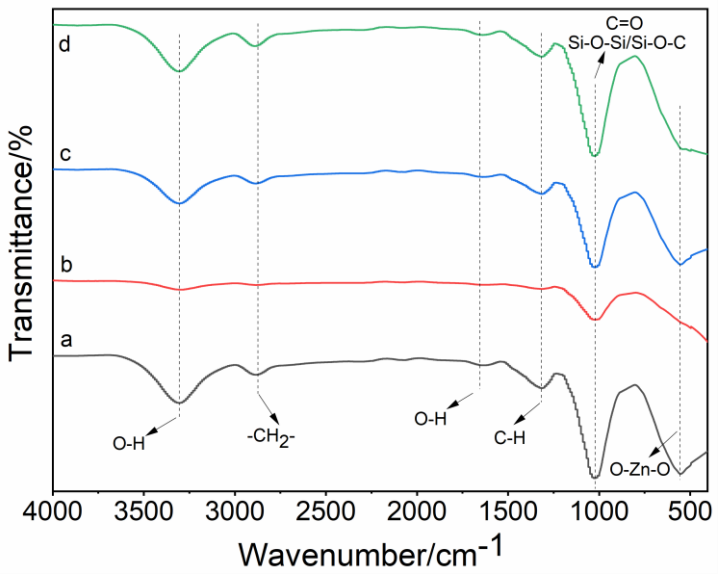


Fig. S1 FTIR spectra of cotton (a), ZnO/cotton (b), QAS/cotton (c), and ZnO/QAS/cotton (d).

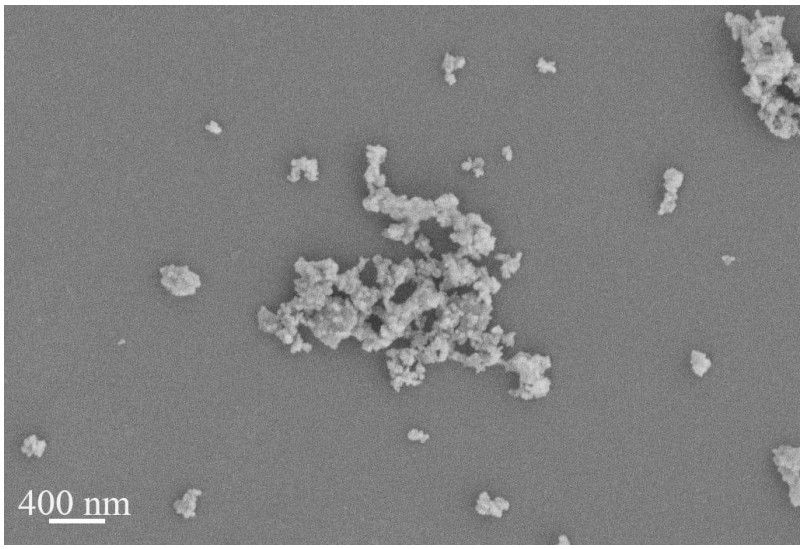
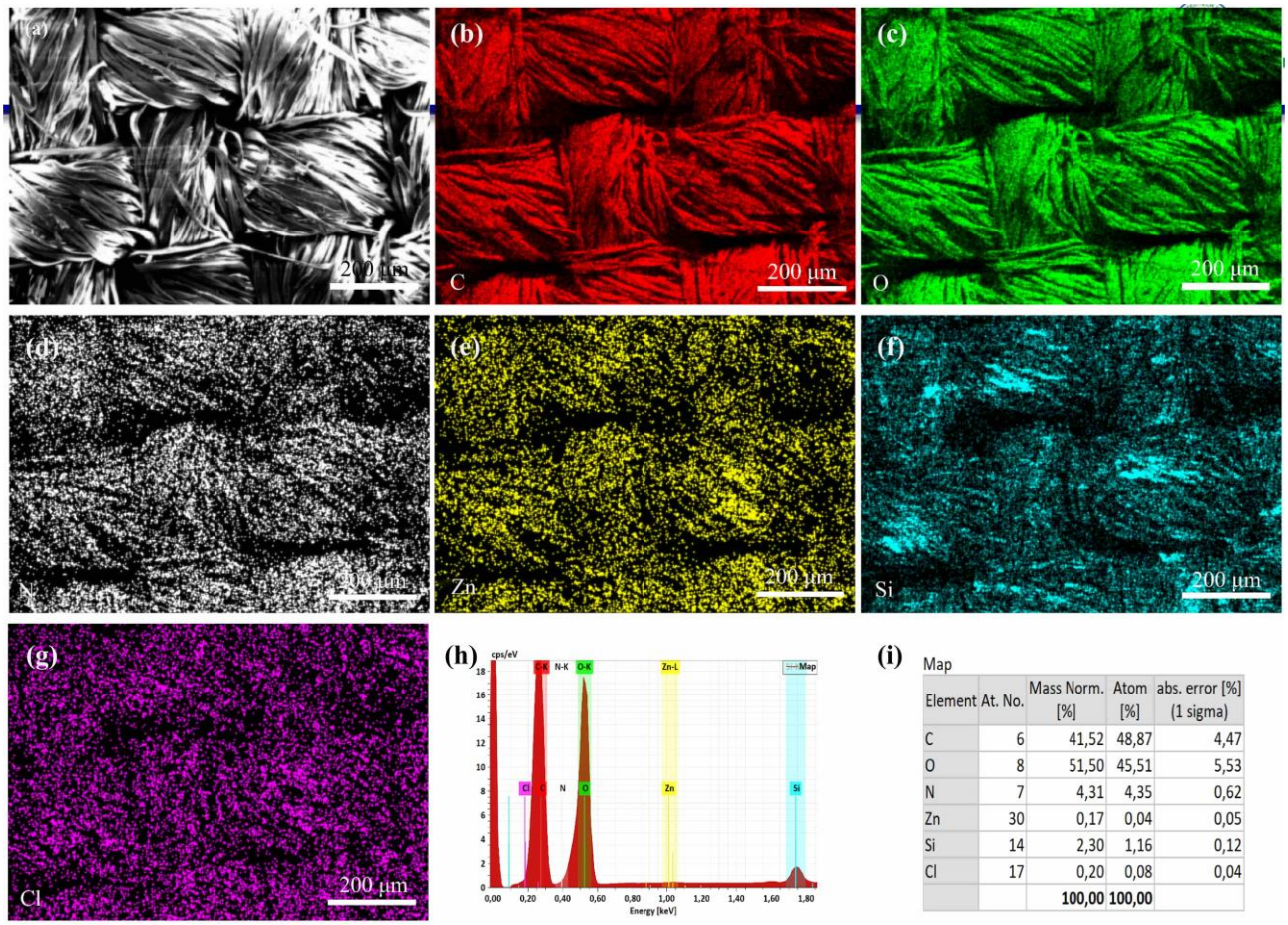


Fig. S2 SEM image of commercial ZnO NPs.



Element	At. No.	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)
C	6	41,52	48,87	4,47
O	8	51,50	45,51	5,53
N	7	4,31	4,35	0,62
Zn	30	0,17	0,04	0,05
Si	14	2,30	1,16	0,12
Cl	17	0,20	0,08	0,04
		100,00	**100,00**	

Fig. S3 (a) Image of ZnO/QAS/cotton. Corresponding EDS elemental mapping images ZnO/QAS/cotton: **(b)** C; **(c)** O; **(d)** N; **(e)** Zn; **(f)** Si; **(g)** Cl. **(h)(i)** Analysis results on elemental contents.

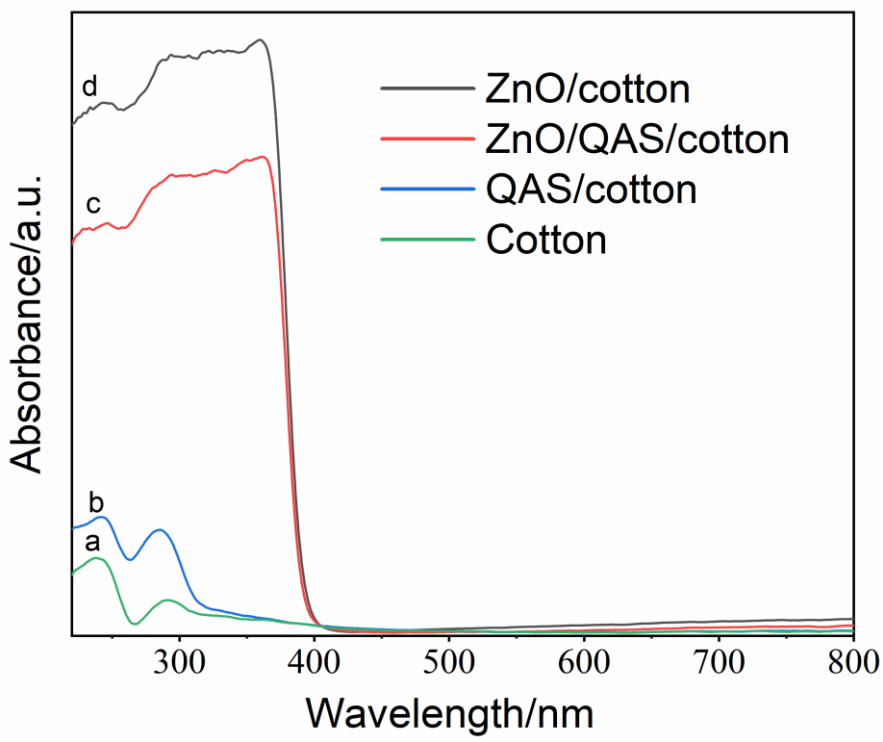


Fig. S4 UV–vis DRS spectra of cotton (a), QAS/cotton (b), ZnO/QAS/cotton (c), and ZnO/cotton (d).

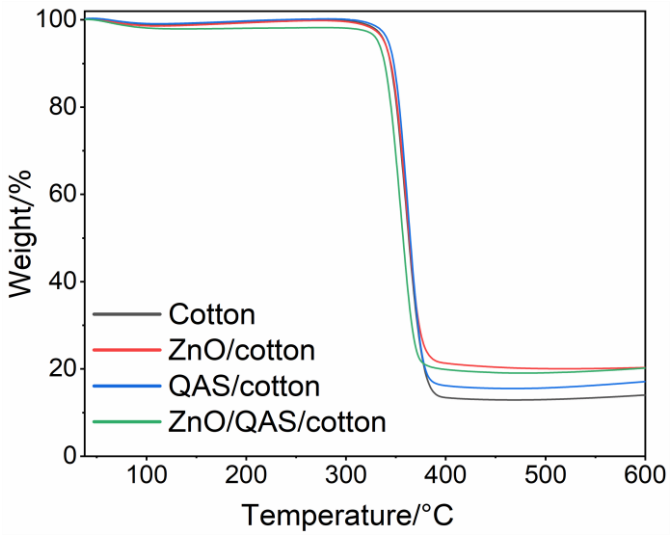


Fig. S5 TG curves of cotton, ZnO/cotton, QAS/cotton, and ZnO/QAS/cotton under the nitrogen atmosphere.

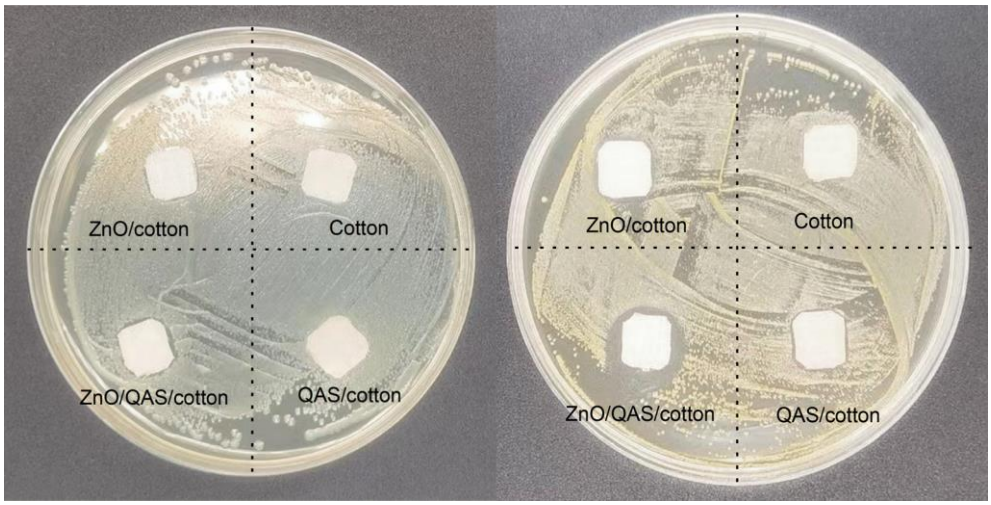


Fig. S6 The inhibition zone pictures of different antibacterial cotton fabrics: the Gram-negative bacteria *E. coli* (left); the Gram-positive bacteria *S. aureus* (right).

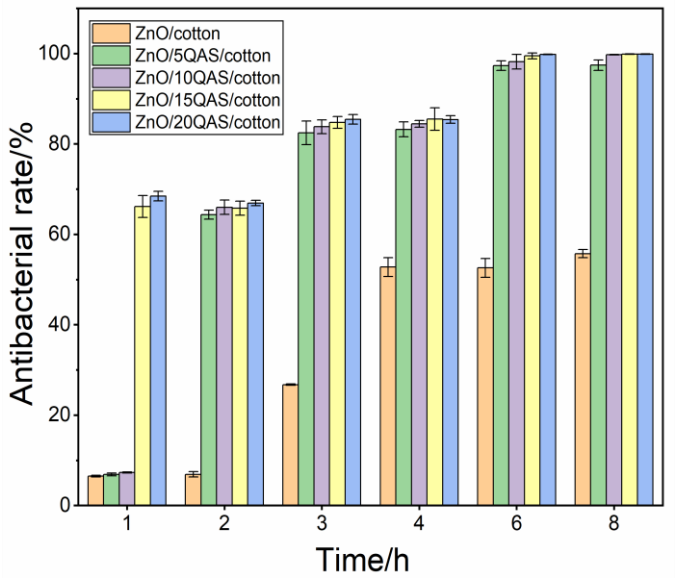


Fig. S7 Bacteriostatic properties of cotton fabrics at different QAS concentration levels.

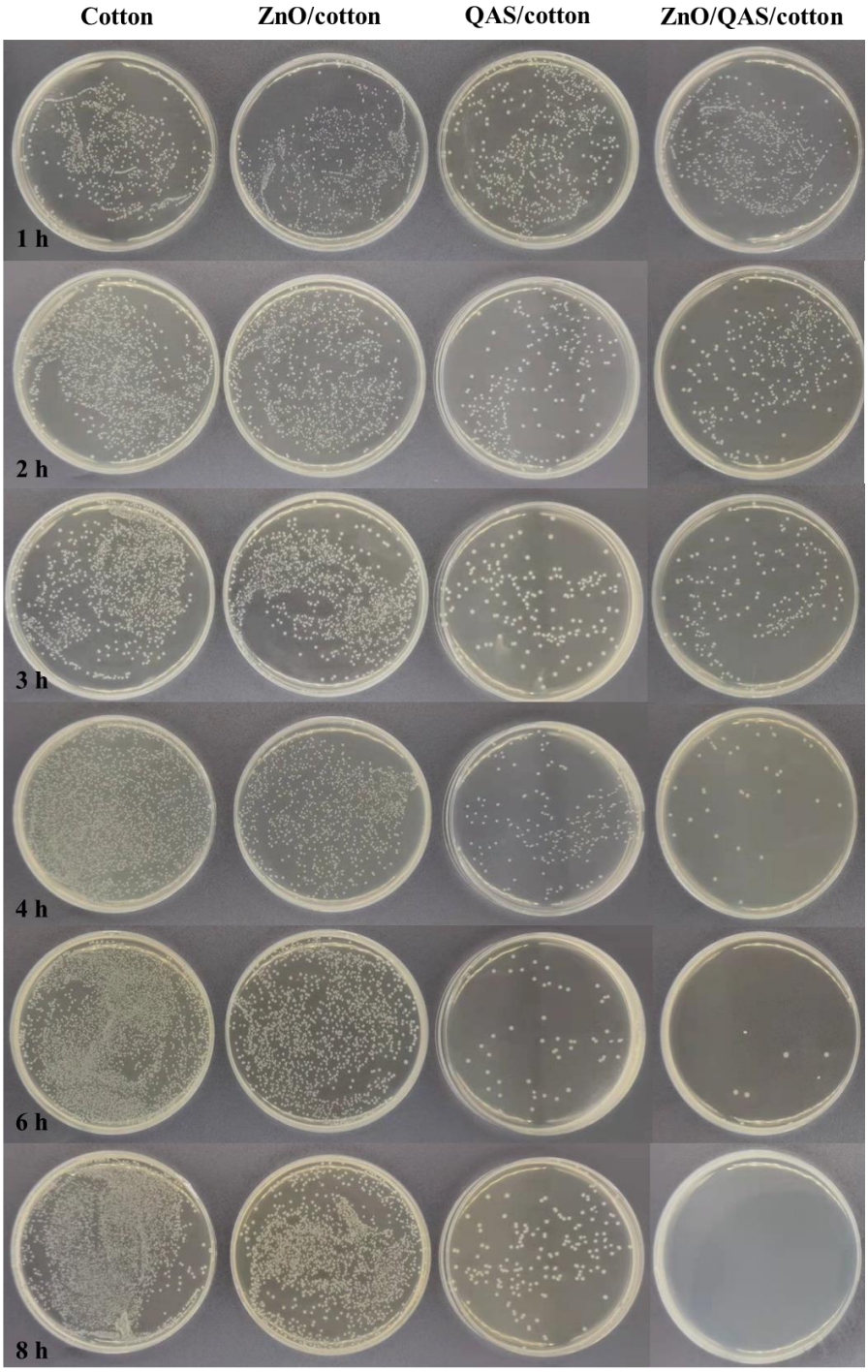


Fig. S8 Response time of *E. coli* treated with different antibacterial cotton fabrics.

Table S1 Summary of some experimental data for the different antibacterial cottons under different experiment conditions

Sample	Content/%						
	N [a]	C [a]	H [a]	QAS [b]	ZnO [c]	Zn^{2+} [d]	Residual solid [e]
Cotton	–	–	–	–	–	–	14.04
ZnO/cotton	–	–	–	–	4.22	2.38	20.34
QAS/cotton	0.15	41.96	6.07	5.32	–	–	17.12
ZnO/QAS/cotton	0.115	40.66	6.285	4.25	4.11	1.69	20.22

Note: The unit of the Zn^{2+} content is $mg \cdot L^{-1}$.

a) The C, N, and H contents were measured by the elemental analysis. b) The QAS content was calculated on the base of the N content in the modified cotton fabric. c) The amount of ZnO in samples was measured by ICP-OES. d) The amount of Zn^{2+} ions in the bacterial solution was measured by ICP-MS. e) The residual solid was calculated by TGA curves at 600 °C.