Electronic Supplementary Material (ESI) for Analyst.


# Supplementary information

## Rapid screening of colistin-resistant *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* by use of Raman spectroscopy and hierarchical cluster analysis

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**Table S1 The antibiogram of the clinical isolates of *E. coli* (μg/ml)**

| isolates | specimen | ampicillin /sulbactam | ampicillin | amikacin | gentamicin | tobramycin | aztreonam | cefazolin | ciprofloxacin | levofloxacin | ceftriaxone | ceftazidime | cefepime | cefotetan | trimethoprim /sulfamethoxazole |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E1** | **Sputum** | 4 | 8 | 2 | 1 | 1 | 1 | 4 | 4 | 8 | 1 | 1 | 1 | 4 | 20 |
| **E2** | **Wound secretion** | 32 | 32 | 2 | 1 | 1 | 64 | 64 | 4 | 8 | 64 | 16 | 64 | 4 | 320 |
| **E3** | **Urine** | 32 | 32 | 2 | 1 | 1 | 64 | 64 | 4 | 8 | 64 | 64 | 64 | 4 | 320 |
| **E4** | **Blood** | 32 | 32 | 4 | 16 | 8 | 16 | 64 | 4 | 8 | 64 | 64 | 4 | 4 | 320 |
| **E5** | **Urine** | 2 | 2 | 2 | 1 | 1 | 1 | 4 | 0.25 | 0.25 | 1 | 1 | 1 | 4 | 20 |
| **E6** | **Ascitic fluid** | 16 | 32 | 2 | 16 | 16 | 64 | 64 | 4 | 8 | 64 | 16 | 64 | 4 | 320 |
| **E7** | **Urine** | 32 | 32 | 2 | 16 | 8 | 64 | 64 | 4 | 8 | 64 | 64 | 64 | 64 | 320 |
| **E8** | **Urine** | 32 | 32 | 2 | 1 | 1 | 64 | 64 | 4 | 8 | 64 | 64 | 64 | 4 | 320 |
| **E9** | **Urine** | 32 | 32 | 2 | 1 | 1 | 64 | 64 | 4 | 8 | 64 | 32 | 32 | 4 | 320 |
| **E10** | **Urine** | 8 | 32 | 4 | 1 | 1 | 1 | 64 | 0.25 | 1 | 64 | 1 | 1 | 1 | 20 |
| **E11** | **Urine** | 4 | 8 | 2 | 1 | 1 | 1 | 4 | 0.25 | 0.25 | 1 | 1 | 1 | 4 | 20 |
| **E12** | **Urine** | 32 | 32 | 2 | 16 | 8 | 1 | 4 | 0.5 | 1 | 1 | 1 | 1 | 4 | 320 |
| **E13** | **Blood** | 16 | 32 | 2 | 16 | 8 | 1 | 4 | 4 | 8 | 1 | 1 | 1 | 4 | 320 |
| **E14** | **Sputum** | 32 | 32 | 2 | 1 | 1 | 16 | 64 | 4 | 8 | 16 | 64 | 1 | 32 | 320 |
| **E15** | **Sputum** | 32 | 32 | 2 | 1 | 1 | 4 | 64 | 0.25 | 1 | 64 | 1 | 2 | 4 | 20 |
| **E16** | **Urine** | 16 | 32 | 2 | 1 | 1 | 1 | 4 | 0.25 | 0.25 | 1 | 1 | 1 | 4 | 20 |
| **E17** | **Ascitic fluid** | 2 | 2 | 2 | 1 | 1 | 1 | 4 | 0.25 | 1 | 1 | 1 | 1 | 4 | 20 |
| **E18** | **Bile** | 2 | 4 | 2 | 1 | 1 | 1 | 4 | 0.25 | 0.25 | 1 | 1 | 1 | 4 | 20 |
| **E19** | **Blood** | 4 | 32 | 2 | 16 | 4 | 1 | 64 | 0.25 | 1 | 16 | 16 | 1 | 4 | 20 |
| **E20** | **Urine** | 16 | 32 | 2 | 16 | 8 | 1 | 4 | 0.25 | 1 | 1 | 1 | 1 | 4 | 320 |
| **E21** | **Bile** | 32 | 32 | 2 | 16 | 8 | 64 | 64 | 4 | 8 | 64 | 16 | 64 | 4 | 320 |
| **E22** | **Bile** | 16 | 16 | 2 | 1 | 1 | 1 | 8 | 4 | 4 | 1 | 1 | 1 | 4 | 20 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E23** | **Sputum** | 16 | 32 | 2 | 1 | 1 | 1 | 64 | 1 | 1 | 1 | 4 | 1 | 4 | 20 |
| **E24** | **Sputum** | 16 | 32 | 2 | 1 | 1 | 16 | 64 | 0.25 | 1 | 64 | 4 | 2 | 4 | 320 |
| **E25** | **Ascitic fluid** | 32 | 32 | 2 | 1 | 1 | 2 | 64 | 4 | 8 | 32 | 1 | 2 | 4 | 20 |
| **E26** | **Sputum** | 32 | 32 | 2 | 16 | 8 | 64 | 64 | 4 | 8 | 64 | 64 | 64 | 4 | 20 |
| **E27** | **Urine** | 32 | 32 | 2 | 16 | 8 | 1 | 4 | 4 | 8 | 1 | 1 | 1 | 4 | 20 |
| **E28** | **Blood** | 4 | 32 | 2 | 1 | 1 | 4 | 64 | 4 | 8 | 64 | 4 | 1 | 4 | 320 |
| **E29** | **Ascitic fluid** | 16 | 32 | 4 | 16 | 16 | 4 | 64 | 4 | 8 | 64 | 4 | 2 | 4 | 320 |
| **E30** | **Urine** | 32 | 32 | 2 | 1 | 1 | 1 | 64 | 4 | 8 | 64 | 1 | 1 | 4 | 320 |

30 clinical isolates of *E. coli* were collected from October, 2017 to December, 2017 in Southwest Hospital (Chongqing, China). Antibiotic susceptibility tests were performed by the Vitek 2 system. All of the bacteria exhibit different antibiograms and none of the same isolate was obtained repeatedly.

**Table S2 The antibiogram of the clinical isolates of *P. aeruginosa* (μg/ml)**

| isolates | specimen | imipenem | meropenem | piperacillin /tazobactam | amikacin | gentamicin | tobramycin | piperacillin | aztreonam | ciprofloxacin | levofloxacin | ceftriaxone | ceftazidime | cefepime | cefotetan | trimethoprim/sulfamethoxazole |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P1** | **Sputum** | 2 | 1 | 4 | 2 | 1 | 1 | 4 | 8 | 0.25 | 1 | 64 | 4 | 2 | 64 | 160 |
| **P2** | **Sputum** | 16 | 16 | 16 | 2 | 1 | 1 | 8 | 32 | 0.5 | 1 | 64 | 8 | 8 | 64 | 320 |
| **P3** | **Sputum** | 16 | 0.25 | 8 | 2 | 1 | 1 | 4 | 32 | 1 | 1 | 64 | 8 | 8 | 64 | 320 |
| **P4** | **Sputum** | 2 | 0.25 | 4 | 2 | 1 | 1 | 4 | 32 | 0.25 | 1 | 32 | 4 | 2 | 64 | 80 |
| **P5** | **Sputum** | 1 | 1 | 64 | 2 | 1 | 1 | 32 | 32 | 1 | 2 | 64 | 4 | 8 | 64 | 320 |
| **P6** | **Sputum** | 2 | 0.25 | 4 | 2 | 1 | 1 | 4 | 32 | 0.25 | 0.5 | 64 | 4 | 2 | 64 | 80 |
| **P7** | **Sputum** | 4 | 1 | 8 | 8 | 4 | 1 | 8 | 32 | 0.5 | 1 | 64 | 4 | 2 | 64 | 160 |
| **P8** | **Sputum** | 1 | 0.25 | 4 | 16 | 1 | 1 | 4 | 32 | 0.5 | 0.25 | 64 | 1 | 2 | 64 | 80 |
| **P9** | **Sputum** | 4 | 1 | 4 | 2 | 1 | 1 | 4 | 32 | 0.25 | 0.5 | 64 | 2 | 1 | 64 | 80 |
| **P10** | **Fester** | 1 | 0.25 | 4 | 2 | 1 | 1 | 4 | 32 | 0.5 | 0.5 | 64 | 4 | 1 | 64 | 80 |
| **P11** | **Bile** | 2 | 0.25 | 16 | 2 | 1 | 1 | 32 | 32 | 0.25 | 0.5 | 64 | 16 | 4 | 64 | 80 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P12** | **Ascitic fluid** | 2 | 0.25 | 4 | 2 | 1 | 1 | 4 | 32 | 0.5 | 0.5 | 64 | 4 | 2 | 64 | 80 |
| **P13** | **Sputum** | 1 | 0.25 | 4 | 16 | 8 | 1 | 4 | 32 | 0.25 | 1 | 64 | 4 | 2 | 64 | 160 |
| **P14** | **Sputum** | 4 | 0.25 | 4 | 2 | 1 | 1 | 4 | 4 | 0.25 | 0.5 | 64 | 4 | 1 | 64 | 80 |
| **P15** | **Fester** | 2 | 0.25 | 64 | 32 | 16 | 16 | 16 | 32 | 4 | 8 | 64 | 4 | 8 | 64 | 320 |
| **P16** | **Sputum** | 2 | 0.5 | 4 | 2 | 1 | 1 | 4 | 4 | 0.25 | 1 | 16 | 2 | 2 | 64 | 80 |
| **P17** | **Sputum** | 16 | 8 | 4 | 2 | 1 | 1 | 4 | 8 | 0.25 | 1 | 64 | 4 | 2 | 64 | 160 |
| **P18** | **Sputum** | 2 | 0.25 | 32 | 2 | 1 | 1 | 4 | 64 | 1 | 1 | 64 | 16 | 16 | 64 | 320 |
| **P19** | **Sputum** | 2 | 0.25 | 4 | 8 | 4 | 1 | 4 | 64 | 0.5 | 1 | 64 | 4 | 8 | 64 | 80 |
| **P20** | **Fester** | 2 | 0.5 | 4 | 16 | 8 | 2 | 4 | 2 | 0.25 | 1 | 16 | 1 | 2 | 64 | 80 |
| **P21** | **Sputum** | 16 | 4 | 4 | 2 | 2 | 1 | 8 | 8 | 0.25 | 0.5 | 16 | 4 | 2 | 64 | 80 |
| **P22** | **Sputum** | 2 | 2 | 120 | 8 | 2 | 1 | 128 | 16 | 0.25 | 1 | 64 | 64 | 16 | 64 | 80 |
| **P23** | **Sputum** | 16 | 16 | 120 | 32 | 16 | 16 | 128 | 16 | 1 | 4 | 64 | 4 | 8 | 64 | 320 |
| **P24** | **Sputum** | 1 | 0.25 | 4 | 2 | 1 | 1 | 4 | 16 | 0.25 | 0.5 | 64 | 2 | 1 | 64 | 320 |
| **P25** | **Sputum** | 1 | 0.25 | 64 | 16 | 8 | 2 | 4 | 64 | 0.5 | 1 | 64 | 8 | 32 | 64 | 320 |
| **P26** | **Sputum** | 8 | 8 | 8 | 8 | 4 | 1 | 8 | 2 | 0.25 | 1 | 64 | 4 | 4 | 64 | 160 |
| **P27** | **Sputum** | 8 | 8 | 8 | 8 | 2 | 1 | 8 | 4 | 0.25 | 1 | 64 | 4 | 2 | 64 | 80 |
| **P28** | **Sputum** | 4 | 1 | 4 | 2 | 1 | 1 | 4 | 8 | 0.5 | 1 | 64 | 4 | 2 | 64 | 160 |
| **P29** | **Throat swab** | 16 | 4 | 8 | 8 | 1 | 1 | 4 | 4 | 0.25 | 0.5 | 64 | 4 | 2 | 64 | 80 |
| **P30** | **Ascitic fluid** | 16 | 4 | 4 | 2 | 1 | 1 | 4 | 8 | 0.25 | 1 | 64 | 4 | 2 | 64 | 80 |

30 clinical isolates of *P. aeruginosa* were collected from October, 2017 to December, 2017 in Southwest Hospital (Chongqing, China). Antibiotic susceptibility tests were performed by the Vitek 2 system. All of the bacteria exhibit different antibiograms and none of the same isolate was obtained repeatedly.

**Table S3 The antibiogram of the clinical isolates of *A. baumannii* (μg/ml)**

| isolates | specimen | macrodantin | imipenem | ampicillin /sulbactam | ampicillin | gentamicin | tobramycin | aztreonam | ciprofloxacin | levofloxacin | ceftriaxone | ceftazidime | cefepime | trimethoprim/sulfamethoxazole |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A1** | **Sputum** | 512 | 2 | 32 | 32 | 8 | 1 | 64 | 4 | 8 | 64 | 64 | 32 | 40 |
| **A2** | **Skin swab** | 512 | 1 | 2 | 8 | 1 | 1 | 16 | 0.25 | 0.25 | 8 | 4 | 2 | 20 |
| **A3** | **Throat swab** | 512 | 1 | 2 | 16 | 1 | 1 | 16 | 0.25 | 0.25 | 16 | 4 | 2 | 20 |
| **A4** | **Sputum** | 512 | 1 | 2 | 16 | 1 | 1 | 64 | 0.25 | 0.25 | 8 | 4 | 2 | 20 |
| **A5** | **Sputum** | 512 | 1 | 2 | 16 | 1 | 1 | 16 | 0.25 | 0.25 | 8 | 4 | 2 | 20 |
| **A6** | **Sputum** | 512 | 1 | 2 | 16 | 1 | 1 | 32 | 0.25 | 0.25 | 16 | 4 | 2 | 20 |
| **A7** | **Sputum** | 256 | 1 | 2 | 16 | 1 | 1 | 32 | 0.5 | 0.25 | 16 | 4 | 2 | 20 |
| **A8** | **Ascitic fluid** | 512 | 16 | 32 | 32 | 8 | 2 | 32 | 4R | 4 | 64 | 64 | 64 | 320 |
| **A9** | **Sputum** | 512 | 16 | 32 | 32 | 1 | 1 | 32 | 4R | 8 | 64 | 64 | 64 | 320 |
| **A10** | **Sputum** | 512 | 1 | 2 | 32 | 1 | 1 | 16 | 0.25 | 0.25 | 8 | 4 | 2 | 20 |
| **A11** | **Sputum** | 128 | 1 | 2 | 16 | 1 | 1 | 16 | 0.25 | 0.25 | 8 | 4 | 2 | 20 |
| **A12** | **Sputum** | 512 | 16 | 32 | 32 | 1 | 1 | 64 | 4 | 4 | 64 | 64 | 64 | 320 |
| **A13** | **Sputum** | 512 | 16 | 32 | 32 | 4 | 1 | 32 | 4 | 8 | 64 | 64 | 64 | 20 |
| **A14** | **Sputum** | 512 | 32 | 32 | 32 | 2 | 1 | 64 | 4 | 4 | 64 | 64 | 64 | 320 |
| **A15** | **Wound secretion** | 512 | 16 | 32 | 32 | 8 | 1 | 64 | 4 | 8 | 64 | 16 | 64 | 320 |
| **A16** | **Sputum** | 512 | 1 | 0.25 | 16 | 1 | 1 | 16 | 0.25 | 0.25 | 8 | 4 | 1 | 20 |
| **A17** | **Sputum** | 256 | 1 | 2 | 16 | 2 | 1 | 16 | 0.25 | 0.25 | 16 | 4 | 2 | 20 |
| **A18** | **Sputum** | 256 | 1 | 2 | 16 | 1 | 1 | 32 | 0.25 | 0.25 | 16 | 4 | 2 | 20 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A19** | **Sputum** | 512 | 1 | 2 | 32 | 1 | 1 | 16 | 0.25 | 0.25 | 16 | 16 | 8 | 20 |
| **A20** | **Sputum** | 512 | 1 | 2 | 32 | 1 | 1 | 32 | 0.5 | 0.25 | 32 | 8 | 4 | 20 |
| **A21** | **Bile** | 512 | 1 | 2 | 32 | 1 | 1 | 64 | 0.25 | 0.25 | 16 | 8 | 4 | 20 |
| **A22** | **Sputum** | 512 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 4 | 64 | 64 | 64 | 320 |
| **A23** | **Ascitic fluid** | 512 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 8 | 64 | 64 | 64 | 320 |
| **A24** | **Sputum** | 512 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 8 | 64 | 64 | 64 | 20 |
| **A25** | **Ascitic fluid** | 512 | 8 | 32 | 32 | 1 | 1 | 64 | 4 | 4 | 64 | 64 | 64 | 320 |
| **A26** | **Sputum** | 512 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 2 | 64 | 64 | 64 | 320 |
| **A27** | **Sputum** | 512 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 4 | 64 | 64 | 64 | 20 |
| **A28** | **Sputum** | 256 | 16 | 32 | 32 | 16 | 16 | 64 | 4 | 2 | 64 | 64 | 64 | 320 |
| **A29** | **Sputum** | 256 | 1 | 2 | 16 | 1 | 1 | 16 | 0.25 | 0.25 | 16 | 4 | 2 | 20 |
| **A30** | **Sputum** | 512 | 1 | 2 | 16 | 1 | 1 | 32 | 0.25 | 0.25 | 16 | 8 | 4 | 20 |

30 clinical isolates of *A. baumannii* were collected from October, 2017 to December, 2017 in Southwest Hospital (Chongqing, China). Antibiotic susceptibility tests were performed by the Vitek 2 system. All of the bacteria exhibit different antibiograms and none of the same isolate was obtained repeatedly.

**Table S4 Detection of colistin resistance in *E. coli* by BMD method and the new method**

| Isolates | BMD method (μg/ml) | the new method (μg/ml) | Isolates | BMD method (μg/ml) | the new method (μg/ml) |
|---|---|---|---|---|---|
| E1 | 2 | Susceptible | E22 | ≤0.25 | Susceptible |
| E2 | 0.5 | Susceptible | E23 | 1 | Susceptible |
| E3 | ≤0.25 | Susceptible | E24 | 0.5 | Susceptible |
| E4 | ≤0.25 | Susceptible | E25 | 0.5 | Susceptible |
| E5 | ≤0.25 | Susceptible | E26 | 0.5 | Susceptible |
| E6 | 0.5 | Susceptible | E27 | 0.5 | Susceptible |
| E7 | ≤0.25 | Susceptible | E28 | 0.5 | Resistant (false positive) |
| E8 | ≤0.25 | Susceptible | E29 | ≤0.25 | Susceptible |
| E9 | ≤0.25 | Susceptible | E30 | ≤0.25 | Susceptible |
| E10 | ≤0.25 | Susceptible | E2R | 8 | Resistant |
| E11 | 1 | Susceptible | E3R | 4 | Resistant |
| E12 | 1 | Resistant (false positive) | E6R | ≥32 | Susceptible (false negative) |
| E13 | ≤0.25 | Susceptible | E8R | 4 | Resistant |
| E14 | ≤0.25 | Susceptible | E9R | 16 | Resistant |
| E15 | ≤0.25 | Susceptible | E10R | 4 | Resistant |
| E16 | 0.5 | Susceptible | E18R | 16 | Resistant |
| E17 | 0≤.25 | Susceptible | E20R | ≥32 | Resistant |
| E18 | ≤0.25 | Susceptible | E22R | 8 | Resistant |
| E19 | ≤0.25 | Susceptible | E24R | 8 | Resistant |
| E20 | ≤0.25 | Susceptible | E25R | 8 | Resistant |
| E21 | ≤0.25 | Susceptible | E28R | 8 | Resistant |

E2R is a resistant isolate induced from E2 with a serial passages exposed to colistin, so are the other resistant isolates. Of all the 30 susceptible (negative) tested by the new method, results of 28 susceptible corresponded to the standard BMD method, except 2 susceptible (E12, E28) were defined as resistant (false positive) ;Results of 11 resistant (positive) corresponded to the standard BMD method, only 1 resistant(E6R) was defined as susceptible (false negative). The detection specificity is 93.3% and the detection sensitivity is 91.7%.

**Table S5 Detection of colistin resistance in *P. aeruginosa* by BMD method and the new method**

| Isolates | BMD method (μg/ml) | the new method (μg/ml) | Isolates | BMD method (μg/ml) | the new method (μg/ml) |
|---|---|---|---|---|---|
| P1 | 2 | Susceptible | P22 | 1 | Susceptible |
| P2 | 1 | Susceptible | P23 | 2 | Susceptible |
| P3 | 1 | Susceptible | P24 | 2 | Susceptible |
| P4 | 2 | Susceptible | P25 | 2 | Susceptible |
| P5 | 2 | Susceptible | P26 | 2 | Susceptible |
| P6 | 1 | Susceptible | P27 | 2 | Susceptible |
| P7 | 1 | Susceptible | P28 | 1 | Susceptible |
| P8 | 1 | Susceptible | P29 | 0.5 | Susceptible |
| P9 | 0.5 | Susceptible | P30 | ≤0.25 | Susceptible |
| P10 | ≤0.25 | Resistant (false positive) | P1R | ≥32 | Susceptible (false negative) |
| P11 | 1 | Susceptible | P2R | 4 | Resistant |
| P12 | 2 | Susceptible | P7R | 4 | Resistant |
| P13 | 2 | Susceptible | P8R | 4 | Resistant |
| P14 | 1 | Susceptible | P11R | 8 | Resistant |
| P15 | 1 | Susceptible | P15R | 8 | Resistant |
| P16 | 1 | Susceptible | P17R | 8 | Resistant |
| P17 | 1 | Susceptible | P18R | 8 | Resistant |
| P18 | 1 | Susceptible | P21R | 16 | Resistant |
| P19 | 0.5 | Susceptible | P25R | ≥32 | Resistant |
| P20 | 0.5 | Resistant (false positive) | | | |
| P21 | 2 | Susceptible | | | |

P1R is a resistant isolate induced from P1 with a serial passages exposed to colistin, so are the other resistant isolates. Of all the 30 susceptible (negative) tested by the new method, results of 28 susceptible corresponded to the standard BMD method, except 2 susceptible (P10, E20) were defined as resistant (false positive) ;Results of 9 resistant (positive) corresponded to the standard BMD method, only 1 resistant (P1R) was defined as susceptible (false negative). The detection specificity is 93.3% and the detection sensitivity is 90.0%.

**Table S6 Detection of colistin resistance in *A. baumannii* by BMD method and the new method**

| Isolates | BMD method (μg/ml) | the new method (μg/ml) | Isolates | BMD method (μg/ml) | the new method (μg/ml) |
|---|---|---|---|---|---|
| A1 | 1 | Susceptible | A22 | 0.5 | Susceptible |
| A2 | 1 | Susceptible | A23 | 0.5 | Susceptible |
| A3 | 1 | Resistant (false positive) | A24 | 1 | Resistant (false positive) |
| A4 | 0.5 | Susceptible | A25 | ≤0.25 | Susceptible |
| A5 | 1 | Susceptible | A26 | ≤0.25 | Susceptible |
| A6 | 0.5 | Susceptible | A27 | 1 | Susceptible |
| A7 | 0.5 | Resistant (false positive) | A28 | 1 | Susceptible |
| A8 | 0.5 | Susceptible | A29 | 0.5 | Susceptible |
| A9 | 0.5 | Susceptible | A30 | 0.5 | Susceptible |
| A10 | ≤0.25 | Susceptible | A1R | 8 | Susceptible(false negative) |
| A11 | 0.25 | Susceptible | A5R | 4 | Resistant |
| A12 | ≤0.25 | Susceptible | A6R | 4 | Resistant |
| A13 | 0.5 | Susceptible | A7R | 16 | Resistant |
| A14 | 0.5 | Susceptible | A9R | ≥32 | Resistant |
| A15 | 0.5 | Susceptible | A15R | 4 | Resistant |
| A16 | 2 | Susceptible | A17R | ≥32 | Resistant |
| A17 | 1 | Susceptible | A18R | 16 | Resistant |
| A18 | 1 | Susceptible | A20R | 8 | Resistant |
| A19 | 0.5 | Resistant (false positive) | A25R | 8 | Resistant |
| A20 | 0.5 | Susceptible | A28R | 8 | Resistant |
| A21 | 0.5 | Susceptible | | | |

A1R is a resistant isolate induced from A1 with a serial passages exposed to colistin, so are the other resistant isolates. Of all the 30 susceptible (negative) tested by the new method, results of 26 susceptible corresponded to the standard BMD method, except 4 susceptible (A3, A7, A19 andA24) were defined as resistant (false positive) ; Results of 10 resistant (positive) corresponded to the standard BMD method, only 1 resistant (A1R) was defined as susceptible (false negative). The detection specificity is 86.7% and the detection sensitivity is 90.9%.

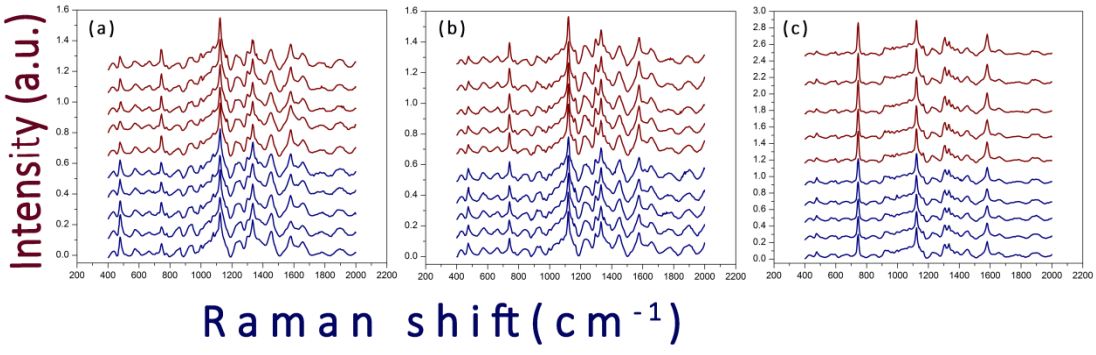



Figure S1. The 5 spectra of bacteria treated with colistin (blue) and without colistin (brown), (a) *E. coli*, (b) *A. baumannii*, (c) *P. aeruginosa.*

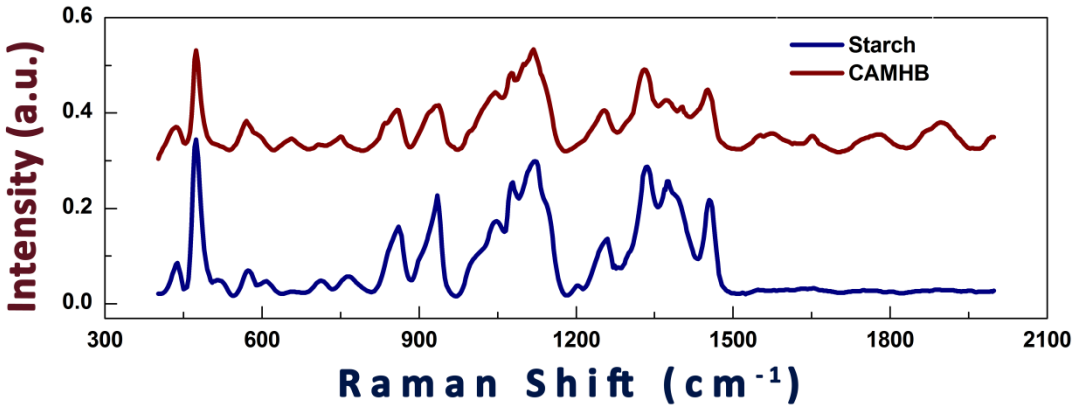



Figure S2. Raman spectra of the pure starch and the deposit of CAMHB. One spectrum is an average of five measurements.

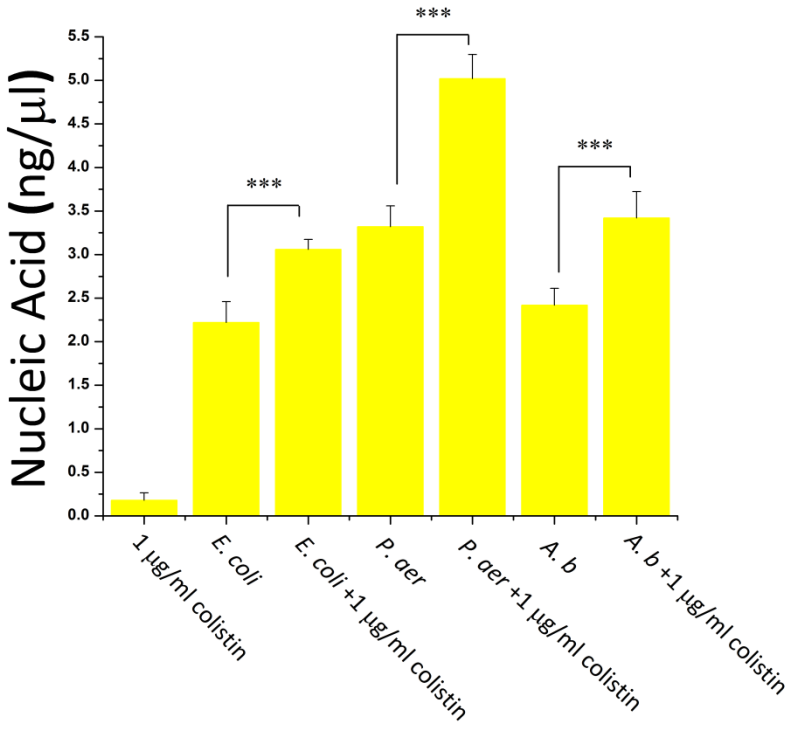



Figure S3. Nucleic acid concentrations of the supernatant of bacterial suspension after incubation with and without 1 µg/ml of colistin sulfate. *** $P<0.05$ is the result of student's test analysis and error bar is the standard deviation of mean for three samples.

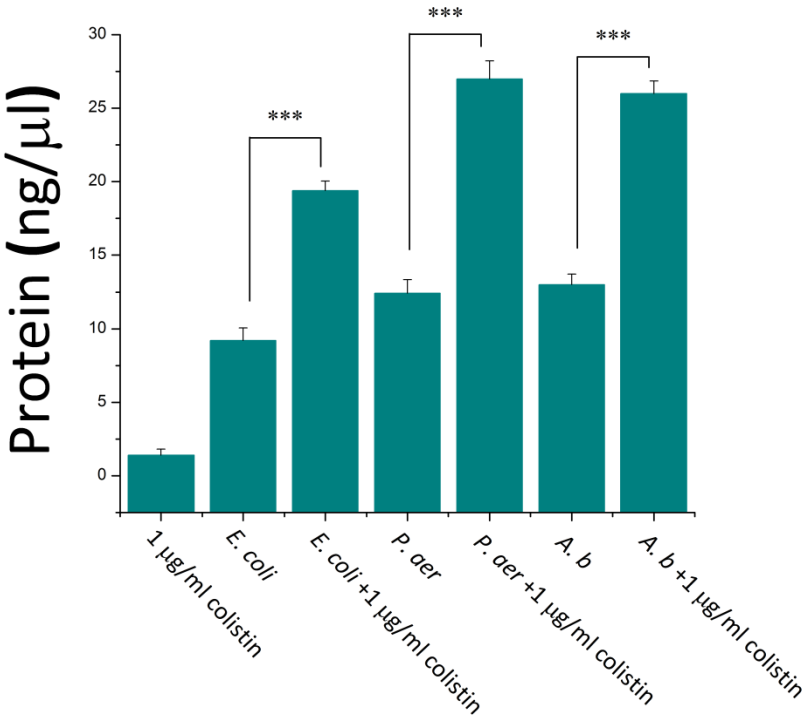
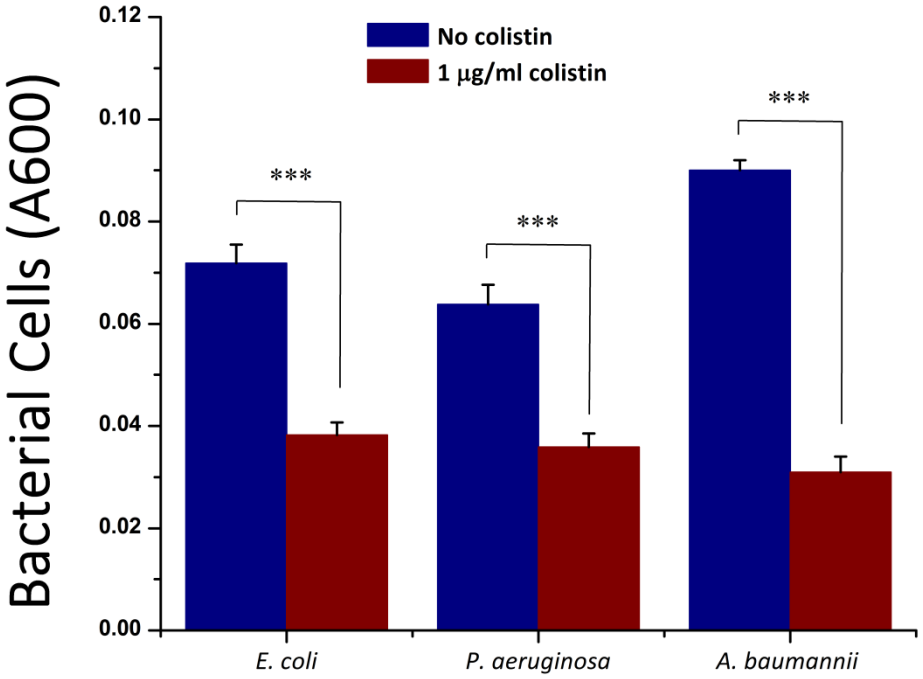



Figure S4. Protein concentrations of the supernatant of bacterial suspension after incubation with and and without 1 µg/ml of colistin sulfate. *** $P < 0.05$ is the result of student's test analysis and error bar is the standard deviation of mean for three samples.




Figures S5. The number of bacterial cells in the suspension of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606 treated with and without 1 µg/ml of colistin sulfate. *** $P < 0.05$ is the result of student's test analysis and error bar is the standard deviation of mean for three samples.

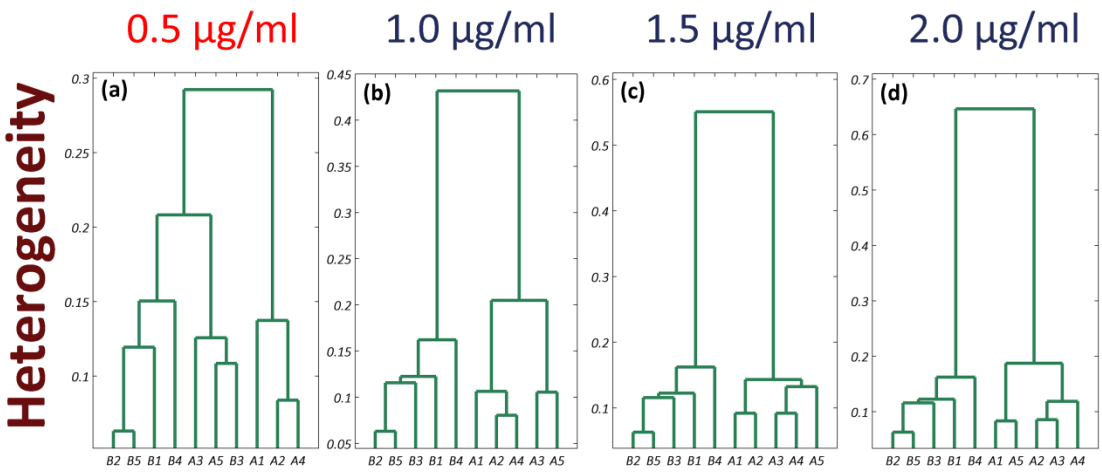



Figure S6. HCA dendrogram of the Raman spectra of *A. baumannii* ATCC 19606 treated with and without colistin, A1 to A5 are spectra of the bacteria exposed to the drug, B1 to B5 are spectra of unexposed bacteria, the concentration of colistin sulfate used in the test is 0.5 µg/ml (a), 1.0 µg/ml (b), 1.5 µg/ml (c) and 2.0 µg/ml (d) respectively.