Rapid identification of bacterial mixtures in urine using MALDI-TOF MS-based algorithm profiling coupled with magnetic enrichment

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33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *S. aureus* with 33% *K. pneumoniae*, (f) 67% *P. aeruginosa* with 33% *K. pneumoniae*.

Fig. S10. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in urine. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumonia*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*.

Fig. S11. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in urine. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumoniae*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*.

Fig. S12. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in urine. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *P. aeruginosa* with 33% *K. pneumoniae*.

Fig. S13. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in urine. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *S. aureus* with 33% *K. pneumoniae*, (f) 67% *P. aeruginosa* with 33% *K. pneumoniae*.

Table S1. Identification scores for bacterial mixtures in urine enriched by Fc-MBL@Fe3O4.

MALDI-TOF MS identification results details:

The blue, yellow, and red bars in identification spectra represent high, medium, and low similarity between the sample and reference spectra, respectively. Cutoff values were defined as the relative error of *m/z* between the sample and reference peaks using the unit parts per million (ppm). The cutoff values for high, medium, and low similarity between a peak pair were <1000 ppm, 1000-1500 ppm, and >1500 ppm, respectively. The bacterial concentrations after enrichment were about 10^8 CFU·mL^{-1}.

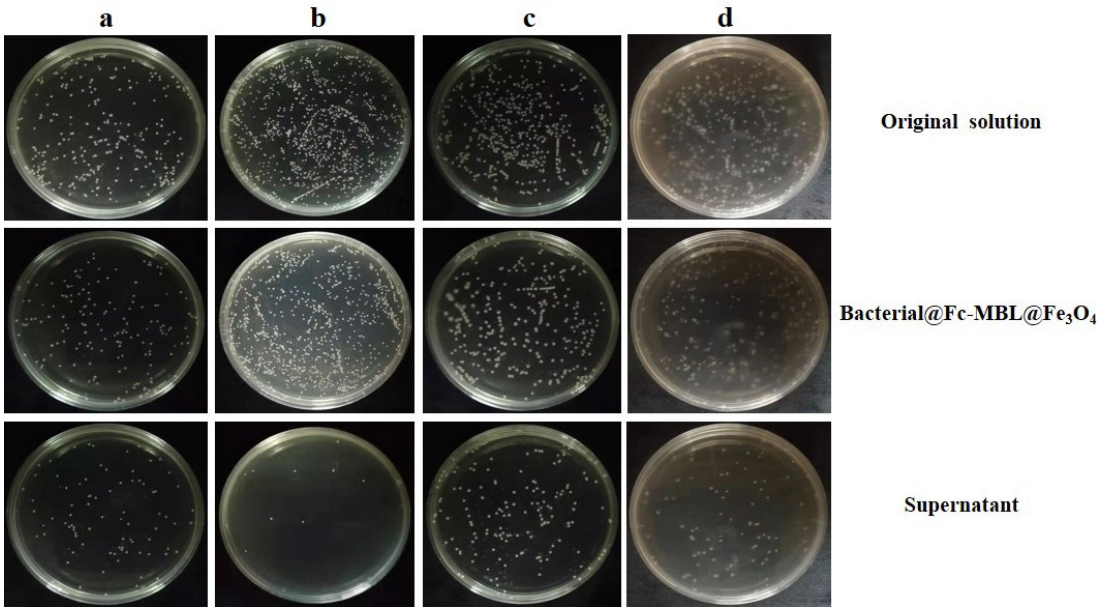


Fig. S1. Photograph of Fc-MBL@Fe_3O_4 enrichment efficiency for select bacteria in urine. Bacterial culture from the original solution is shown in the first row. The enriched bacteria were cultured and shown in the second row, and the third row shows the culture of the remaining bacteria (i.e., non-enriched) left in the supernatant. (a) *E. coli*, (b) *S. aureus*, (c) *K. pneumoniae*, and (d) *P. aeruginosa*.

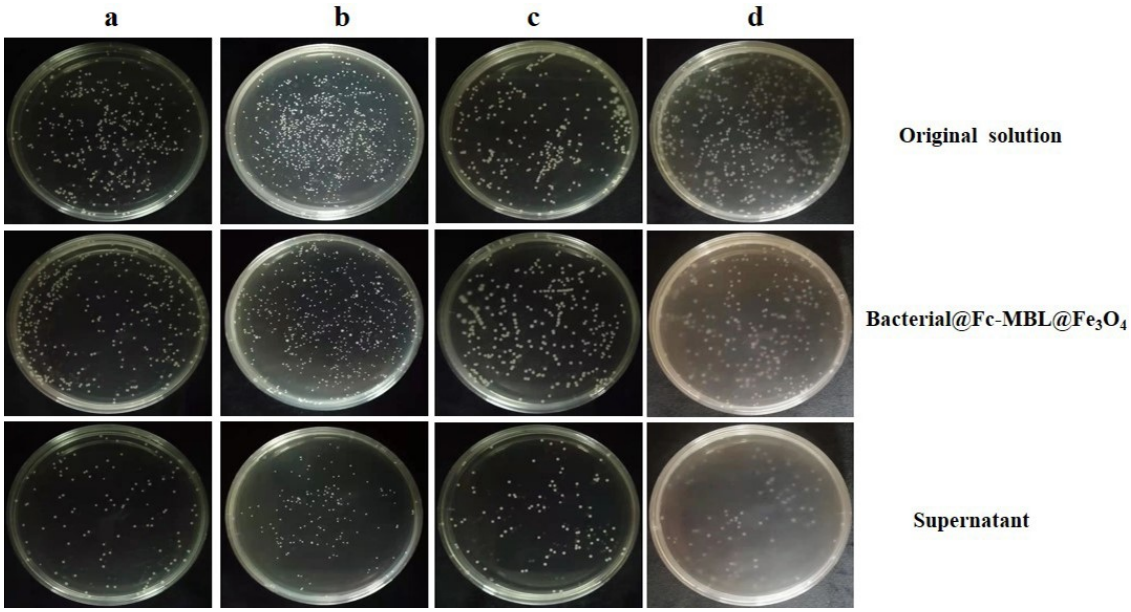


Fig. S2. Photograph of Fc-MBL@Fe_3O_4 enrichment efficiency for select bacteria in aqueous solution. Bacterial culture from the original solution is shown in the first row. The enriched bacteria were cultured and shown in the second row, and the third row shows the culture of the remaining bacteria (i.e., non-enriched) left in the supernatant. (a) *E. coli*, (b) *S. aureus*, (c) *K. pneumoniae*, and (d) *P. aeruginosa*.

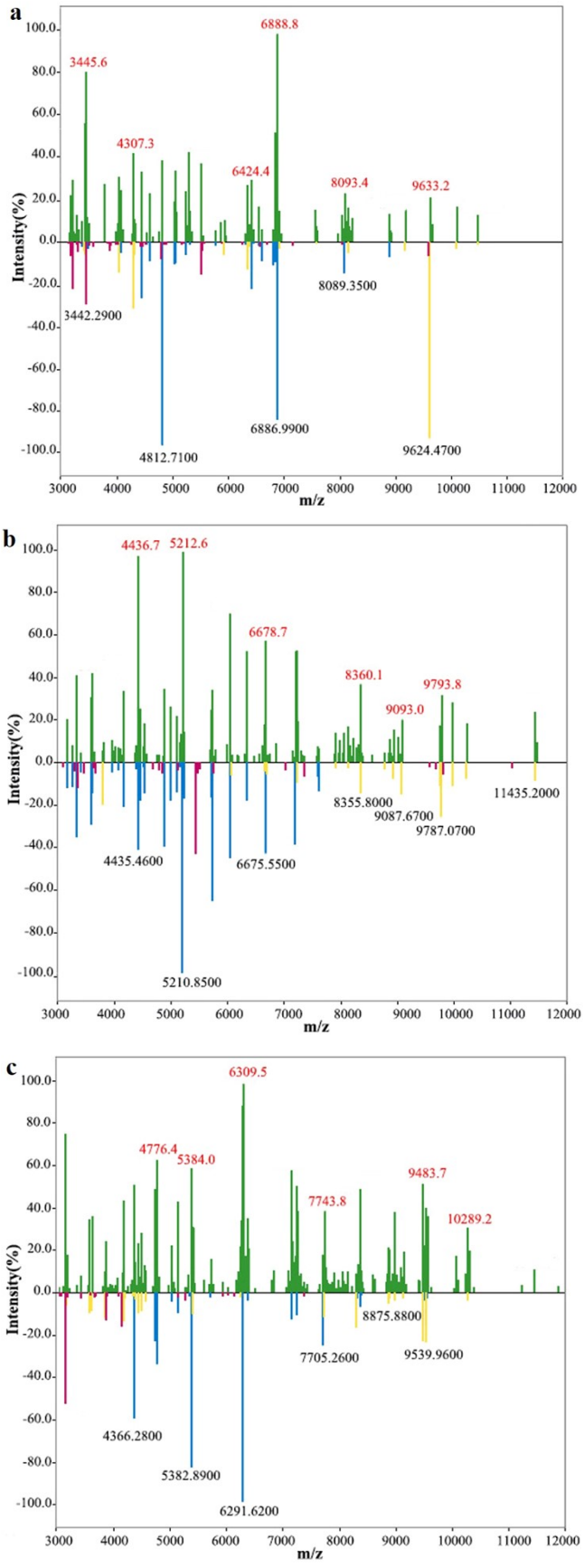


Fig. S3. MALDI-TOF MS of a single bacterial strain enriched by Fc-MBL@Fe_3O_4 from urine. (a) *S. aureus*, (b) *P. aeruginosa*, and (c) *K. pneumoniae*.

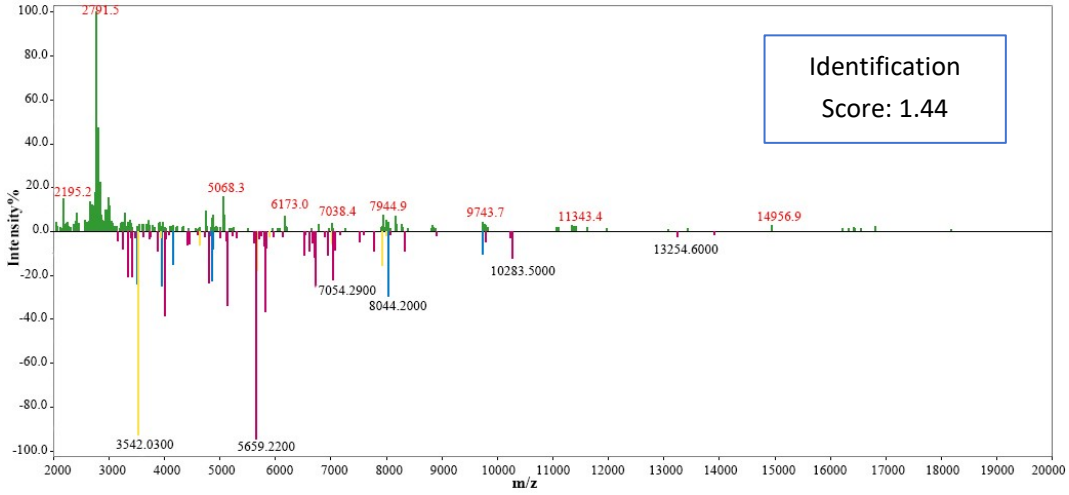


Fig. S4. MALDI-TOF MS identification of a urine sample from a healthy person with identification score 1.44.

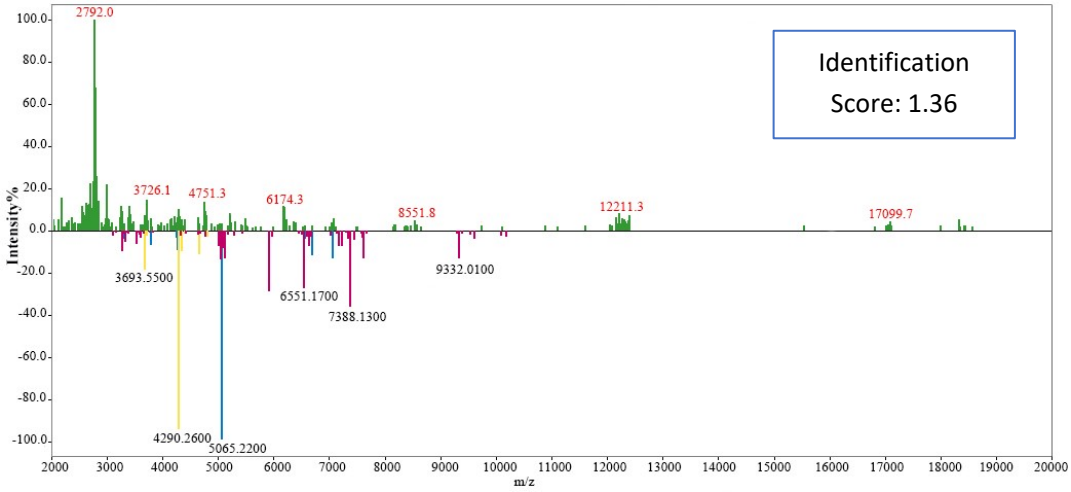
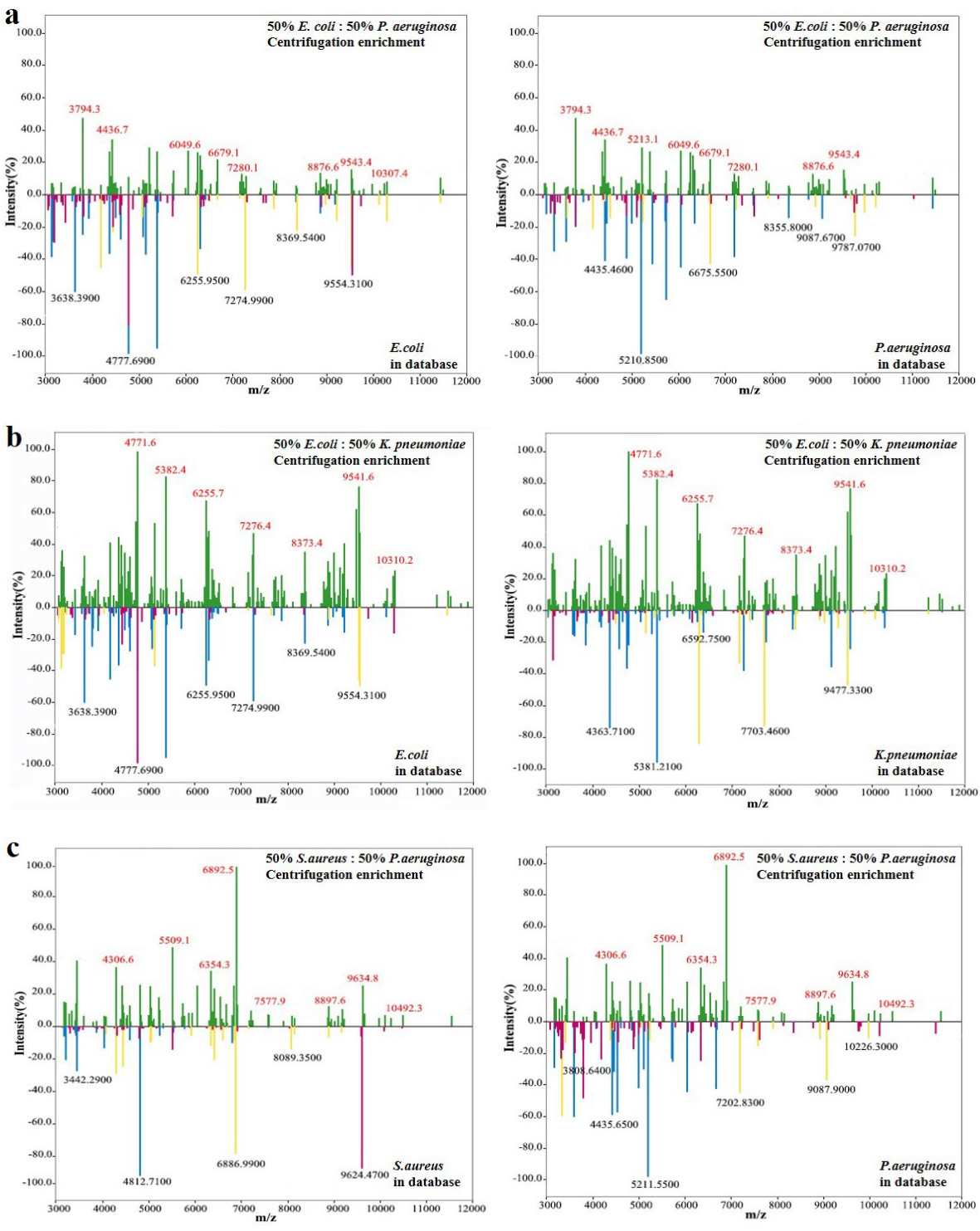


Fig. S5. MALDI-TOF MS identification of a bacteriuria sample without bacterial enrichment with identification score 1.36. The pathogen was *E. coli* and the colony counts were about 5.0×10^5 CFU·mL^{-1}.


a
50% E. coli : 50% P. aeruginosa
Centrifugation enrichment
3794.3
4436.7
6049.6
6679.1
7280.1
8876.6
9543.4
10307.4
8369.5400
6255.9500
9554.3100
7274.9900
3638.3900
4777.6900
E.coli
in database
50% E.coli : 50% P. aeruginosa
Centrifugation enrichment
3794.3
4436.7
5213.1
6049.6
6679.1
7280.1
8876.6
9543.4
8355.8000
9087.6700
9787.0700
4435.4600
6675.5500
5210.8500
P.aeruginosa
in database
b
50% E.coli : 50% K. pneumoniae
Centrifugation enrichment
4771.6
5382.4
6255.7
7276.4
8373.4
9541.6
10310.2
8369.5400
6255.9500
7274.9900
9554.3100
3638.3900
4777.6900
E.coli
in database
50% E.coli : 50% K. pneumoniae
Centrifugation enrichment
4771.6
5382.4
6255.7
7276.4
8373.4
9541.6
10310.2
6592.7500
9477.3300
4363.7100
7703.4600
5381.2100
K.pneumoniae
in database
c
50% S.aureus : 50% P.aeruginosa
Centrifugation enrichment
6892.5
5509.1
4306.6
6354.3
9634.8
7577.9
8897.6
10492.3
8089.3500
3442.2900
6886.9900
4812.7100
9624.4700
S.aureus
in database
50% S.aureus : 50% P.aeruginosa
Centrifugation enrichment
6892.5
5509.1
4306.6
6354.3
9634.8
7577.9
8897.6
10492.3
10226.3000
3808.6400
9087.9000
7202.8300
4435.6500
5211.5500
P.aeruginosa
in database
Intensity(%)
m/z

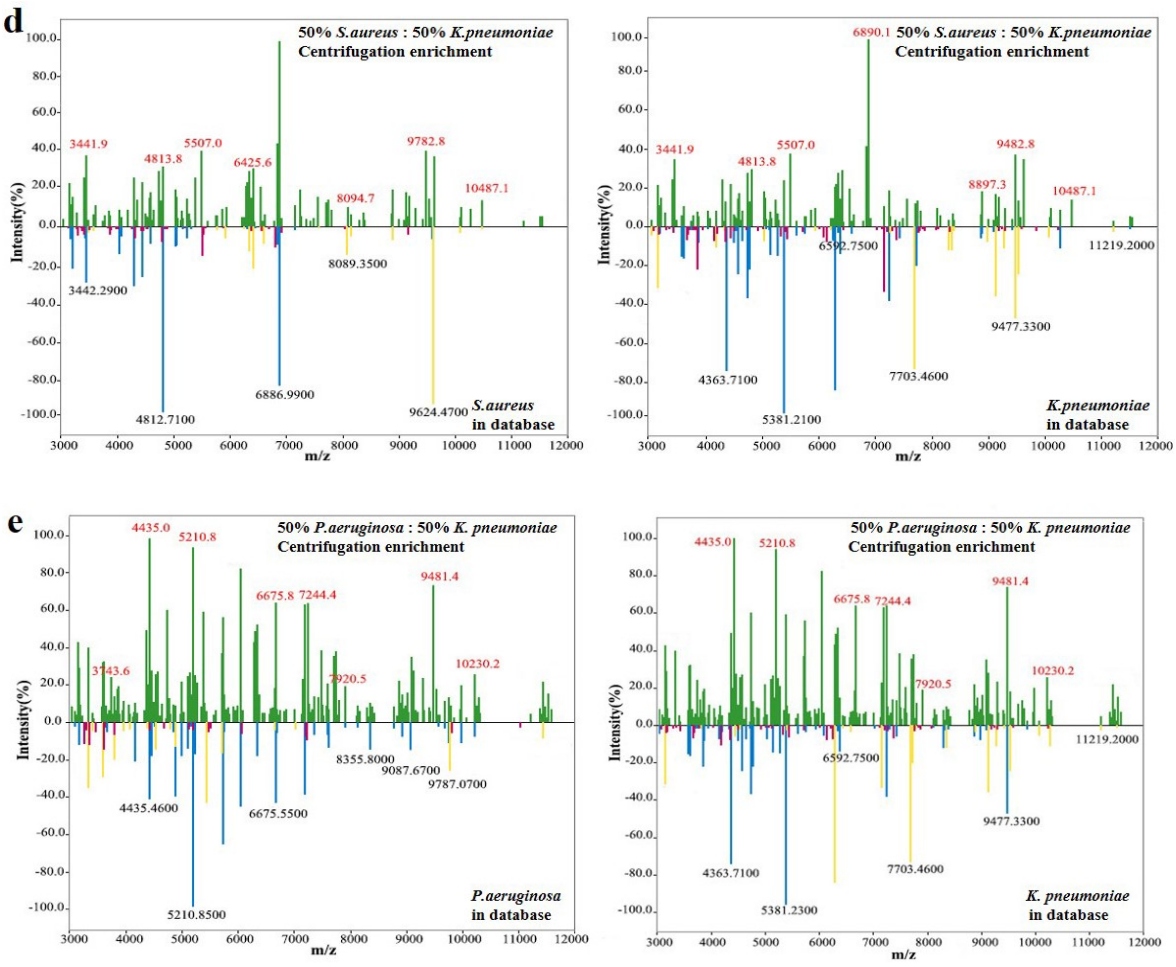
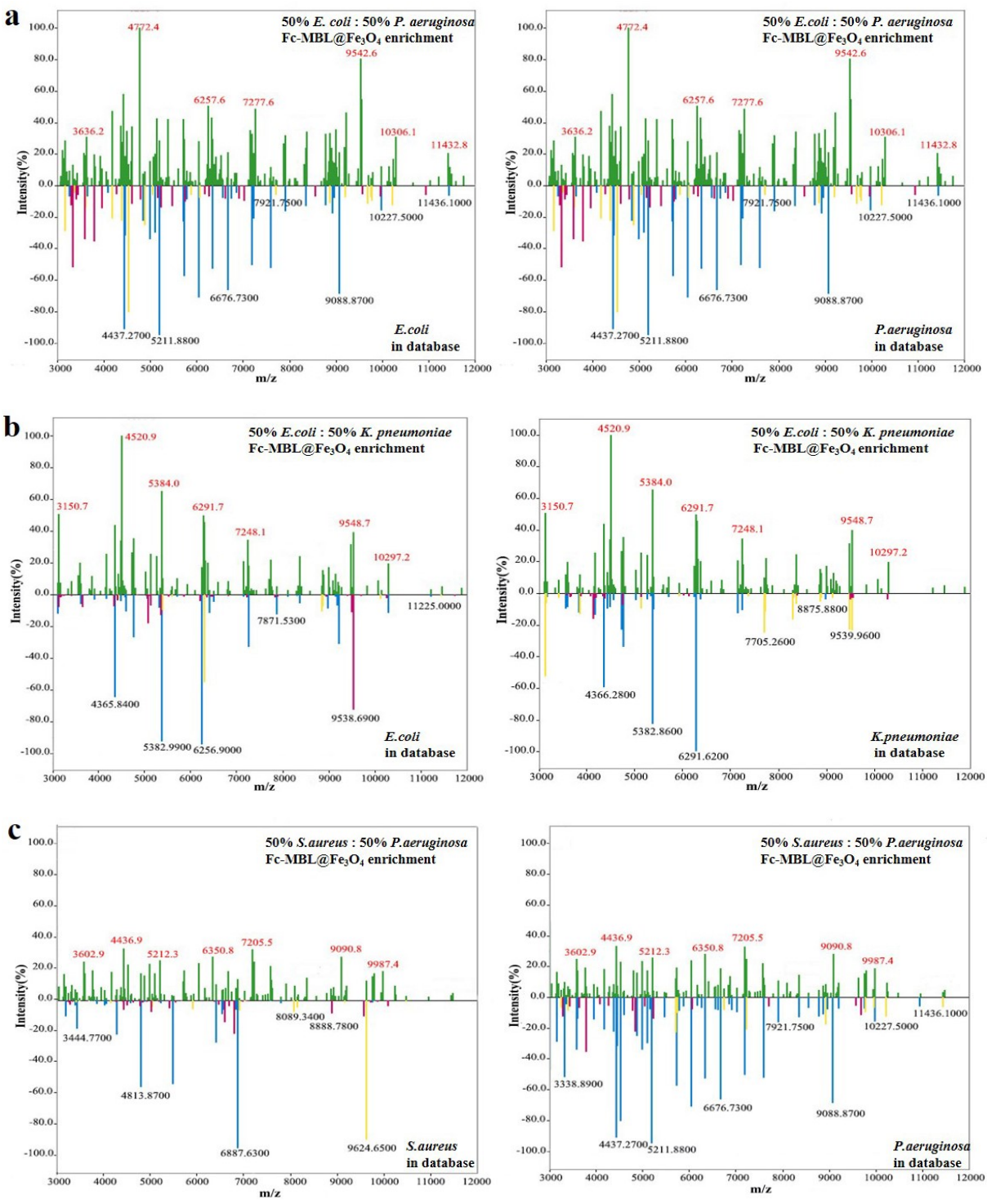


Fig. S6. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in aqueous solution. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumonia*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*.


a
50% E. coli : 50% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
4772.4
9542.6
6257.6
7277.6
3636.2
10306.1
11432.8
7921.7500
11436.1000
10227.5000
6676.7300
9088.8700
4437.2700
5211.8800
E.coli
in database
50% E.coli : 50% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
P.aeruginosa
in database
Intensity(%)
m/z
b
50% E.coli : 50% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
4520.9
5384.0
3150.7
6291.7
7248.1
9548.7
10297.2
11225.0000
7871.5300
4365.8400
5382.9900
6256.9000
9538.6900
E.coli
in database
4520.9
5384.0
3150.7
6291.7
7248.1
9548.7
10297.2
8875.8800
9539.9600
7705.2600
4366.2800
5382.8600
6291.6200
K.pneumoniae
in database
c
50% S.aureus : 50% P.aeruginosa
Fc-MBL@Fe3O4 enrichment
4436.9
3602.9
5212.3
6350.8
7205.5
9090.8
9987.4
8089.3400
8888.7800
3444.7700
4813.8700
6887.6300
9624.6500
S.aureus
in database
50% S.aureus : 50% P.aeruginosa
Fc-MBL@Fe3O4 enrichment
4436.9
3602.9
5212.3
6350.8
7205.5
9090.8
9987.4
11436.1000
7921.7500
10227.5000
3338.8900
6676.7300
9088.8700
4437.2700
5211.8800
P.aeruginosa
in database

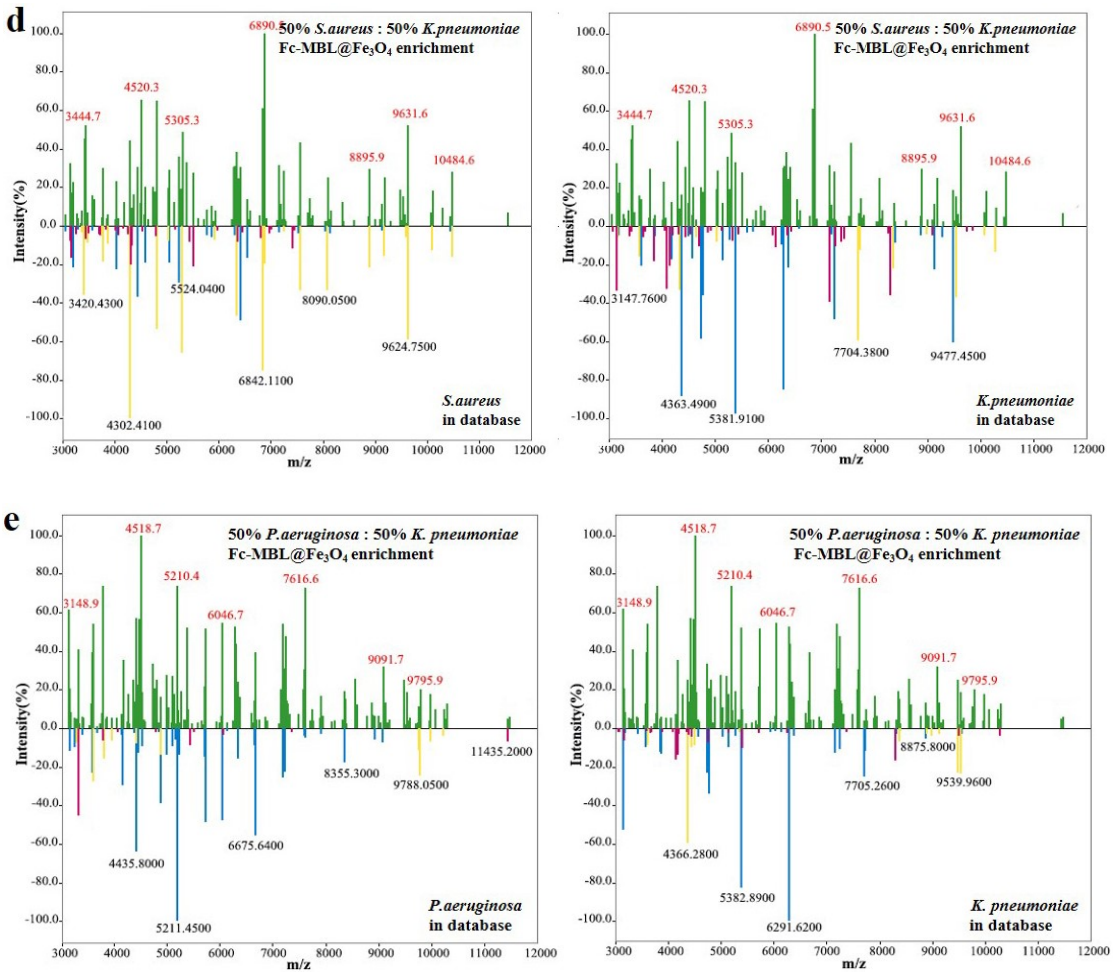
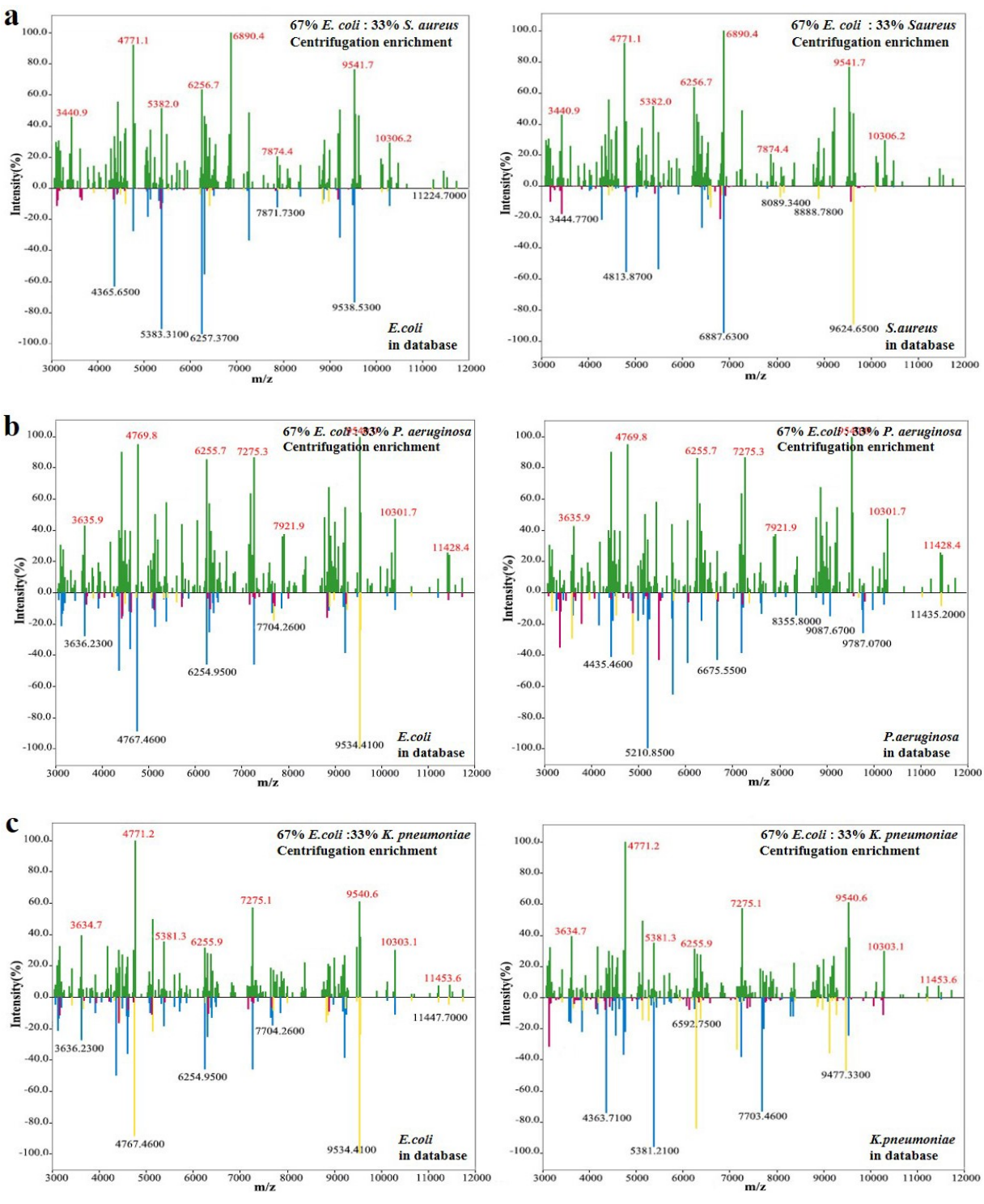


Fig. S7. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in aqueous solution. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumoniae*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*.


a
67% E. coli : 33% S. aureus
Centrifugation enrichment
4771.1
6890.4
9541.7
6256.7
5382.0
3440.9
10306.2
7874.4
11224.7000
7871.7300
4365.6500
9538.5300
5383.3100
6257.3700
E.coli
in database
Intensity(%)
m/z
67% E. coli : 33% Saureus
Centrifugation enrichmen
4771.1
6890.4
9541.7
6256.7
5382.0
3440.9
10306.2
7874.4
8089.3400
8888.7800
3444.7700
4813.8700
6887.6300
9624.6500
S.aureus
in database
b
67% E. coli : 33% P. aeruginosa
Centrifugation enrichment
4769.8
6255.7
7275.3
3635.9
10301.7
7921.9
11428.4
3636.2300
7704.2600
6254.9500
4767.4600
9534.4100
E.coli
in database
67% E.coli : 33% P. aeruginosa
Centrifugation enrichment
4769.8
6255.7
7275.3
3635.9
10301.7
7921.9
11428.4
11435.2000
8355.8000
9087.6700
9787.0700
4435.4600
6675.5500
5210.8500
P.aeruginosa
in database
c
67% E.coli :33% K. pneumoniae
Centrifugation enrichment
4771.2
7275.1
9540.6
3634.7
5381.3
6255.9
10303.1
11453.6
11447.7000
7704.2600
3636.2300
6254.9500
4767.4600
9534.4100
E.coli
in database
67% E.coli : 33% K. pneumoniae
Centrifugation enrichment
4771.2
7275.1
9540.6
3634.7
5381.3
6255.9
10303.1
11453.6
6592.7500
9477.3300
4363.7100
7703.4600
5381.2100
K.pneumoniae
in database

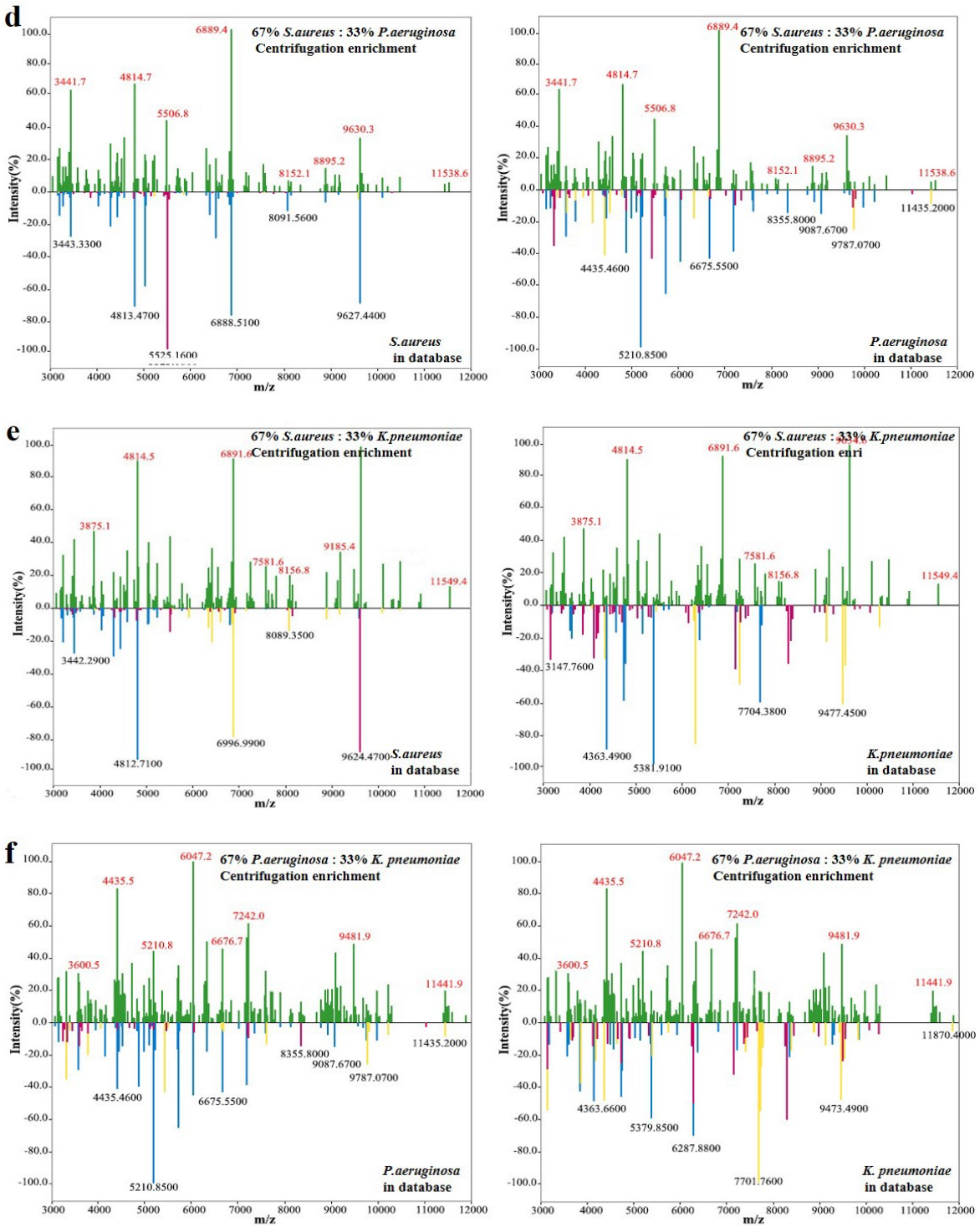
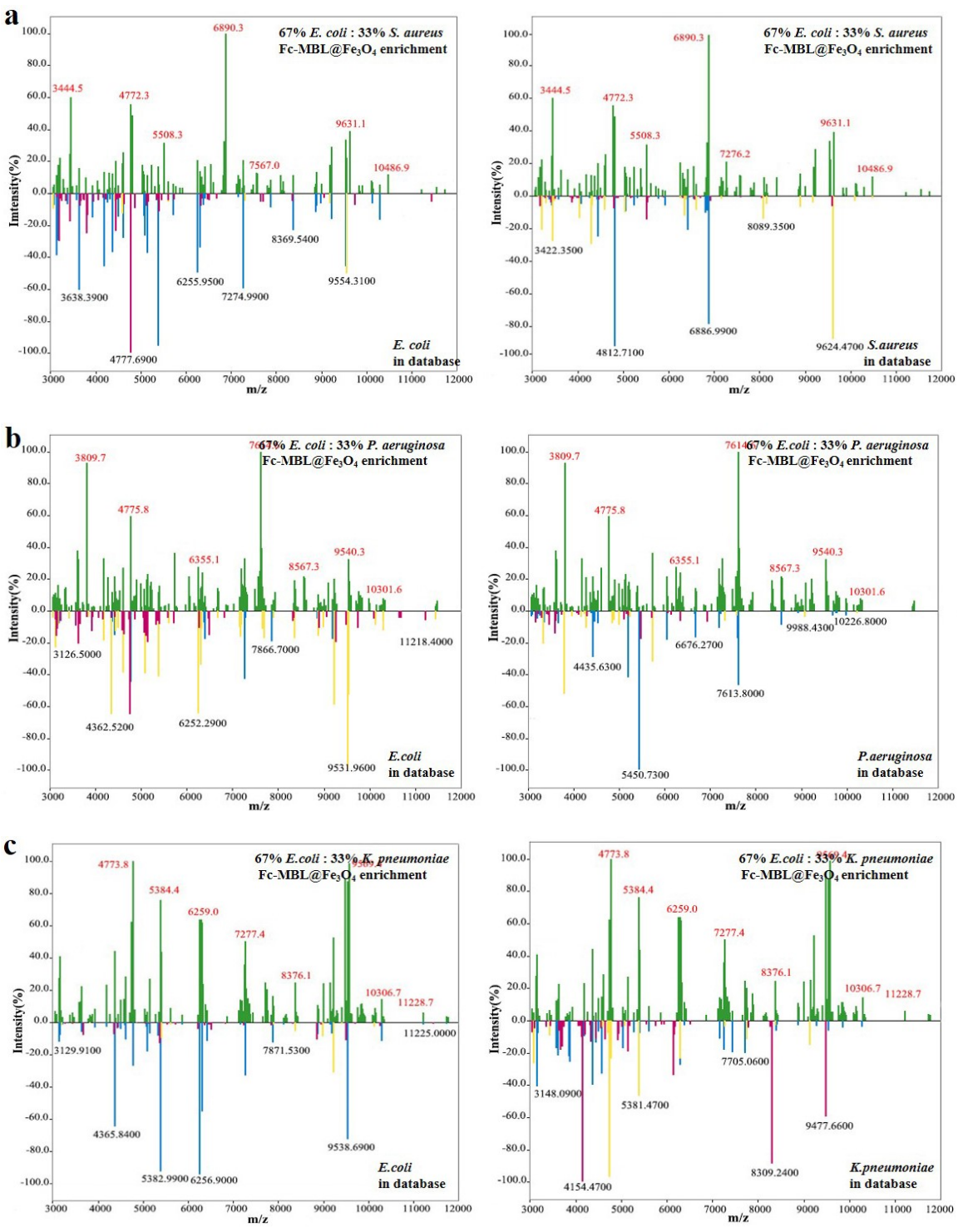


Fig. S8. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in aqueous solution. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *S. aureus* with 33% *K. pneumoniae*, (f) 67% *P. aeruginosa* with 33% *K. pneumoniae*.


a
67% E. coli : 33% S. aureus
Fc-MBL@Fe3O4 enrichment
E. coli
in database
67% E. coli : 33% S. aureus
Fc-MBL@Fe3O4 enrichment
S.aureus
in database
b
67% E. coli : 33% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
E.coli
in database
67% E.coli : 33% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
P.aeruginosa
in database
c
67% E.coli : 33% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
E.coli
in database
67% E.coli : 33% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
K.pneumoniae
in database
Intensity(%)
m/z

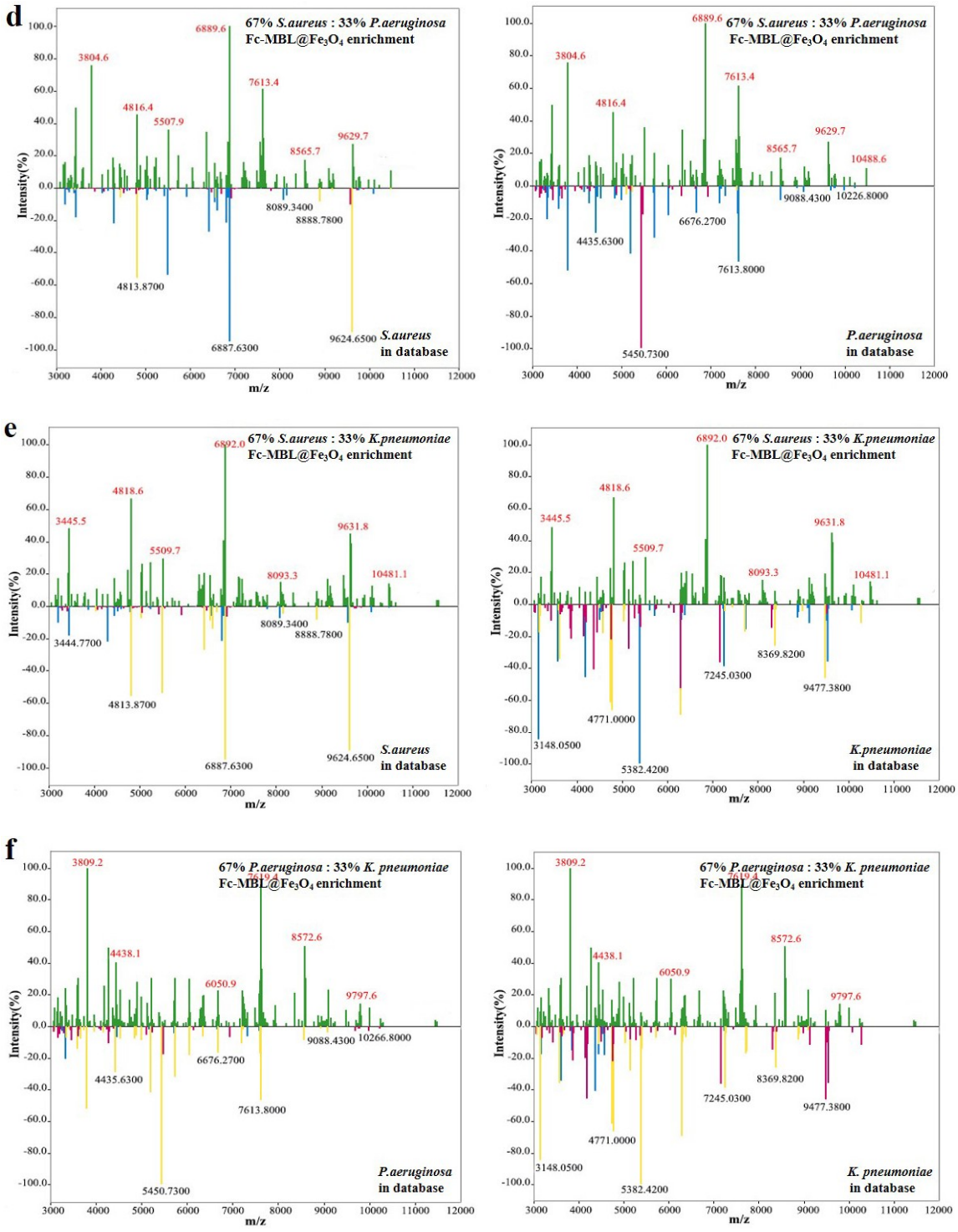
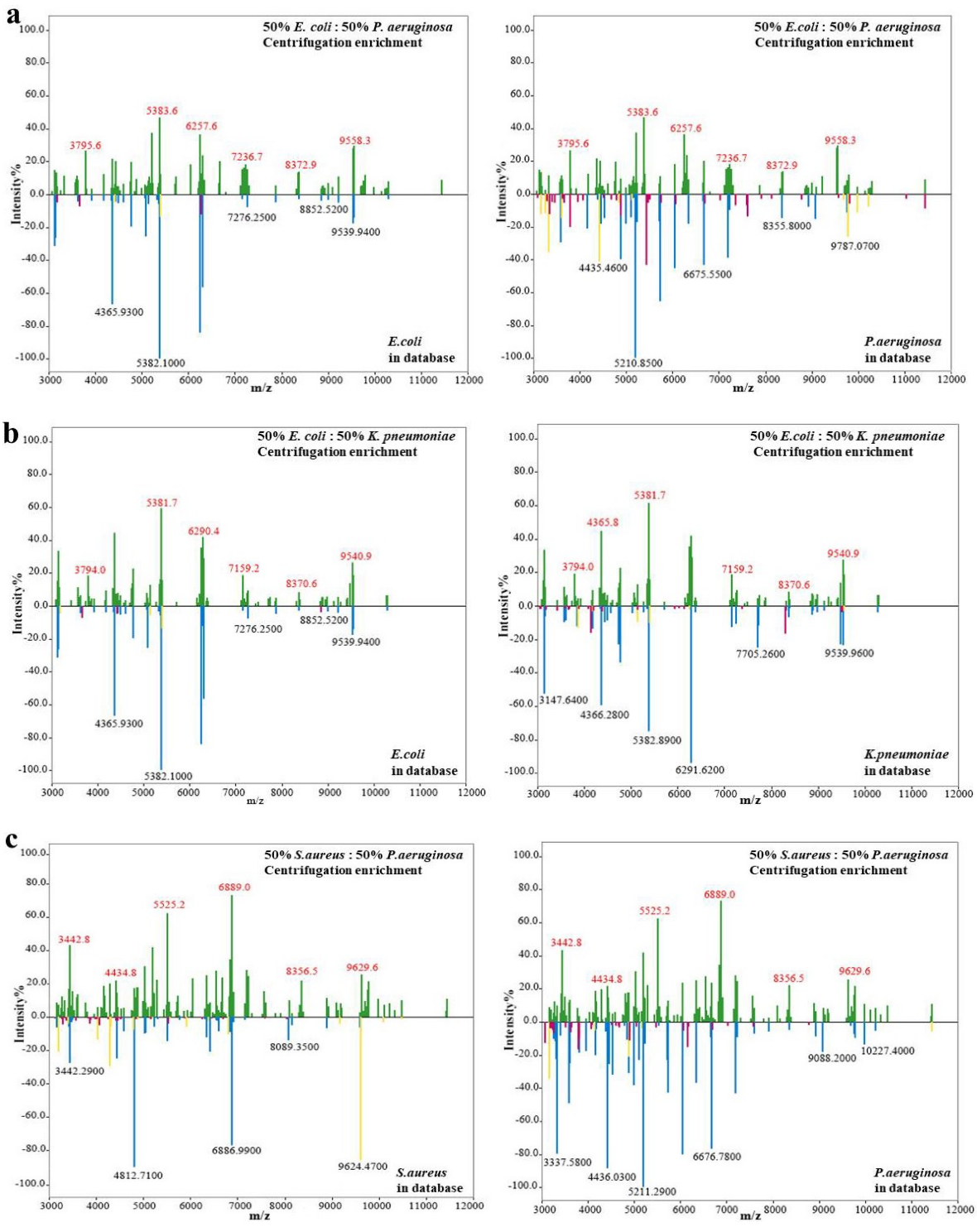


Fig. S9. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in aqueous solution. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *S. aureus* with 33% *K. pneumoniae*, (f) 67% *P. aeruginosa* with 33% *K. pneumoniae*.


a
50% E. coli : 50% P. aeruginosa
Centrifugation enrichment
3795.6
5383.6
6257.6
7236.7
8372.9
9558.3
4365.9300
5382.1000
7276.2500
8852.5200
9539.9400
E.coli
in database
50% E.coli : 50% P. aeruginosa
Centrifugation enrichment
3795.6
5383.6
6257.6
7236.7
8372.9
9558.3
4435.4600
5210.8500
6675.5500
8355.8000
9787.0700
P.aeruginosa
in database
b
50% E. coli : 50% K. pneumoniae
Centrifugation enrichment
3794.0
5381.7
6290.4
7159.2
8370.6
9540.9
4365.9300
5382.1000
7276.2500
8852.5200
9539.9400
E.coli
in database
50% E.coli : 50% K. pneumoniae
Centrifugation enrichment
3794.0
4365.8
5381.7
7159.2
8370.6
9540.9
3147.6400
4366.2800
5382.8900
6291.6200
7705.2600
9539.9600
K.pneumoniae
in database
c
50% S.aureus : 50% P.aeruginosa
Centrifugation enrichment
3442.8
4434.8
5525.2
6889.0
8356.5
9629.6
3442.2900
4812.7100
6886.9900
8089.3500
9624.4700
S.aureus
in database
50% S.aureus : 50% P.aeruginosa
Centrifugation enrichment
3442.8
4434.8
5525.2
6889.0
8356.5
9629.6
3337.5800
4436.0300
5211.2900
6676.7800
9088.2000
10227.4000
P.aeruginosa
in database
Intensity %
m/z

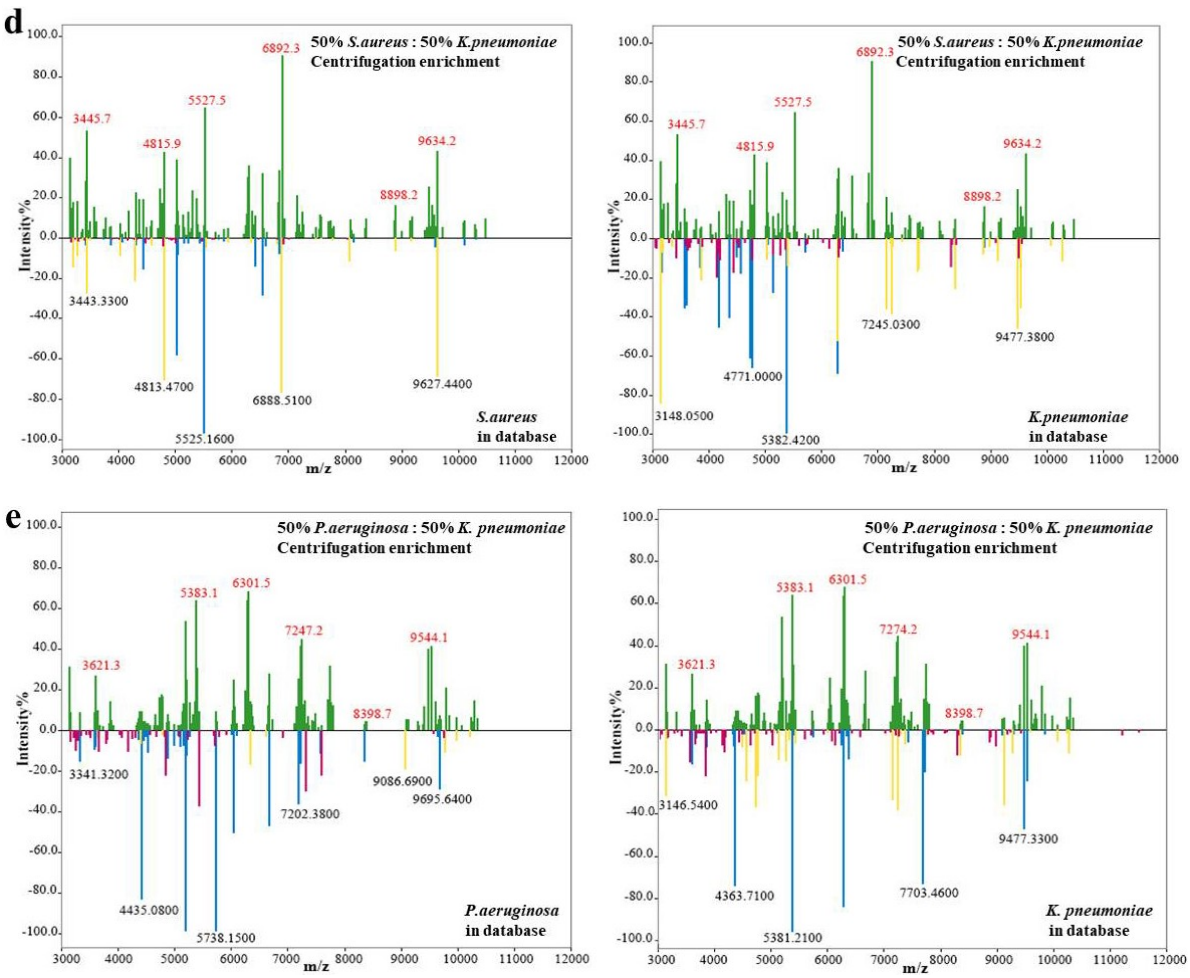
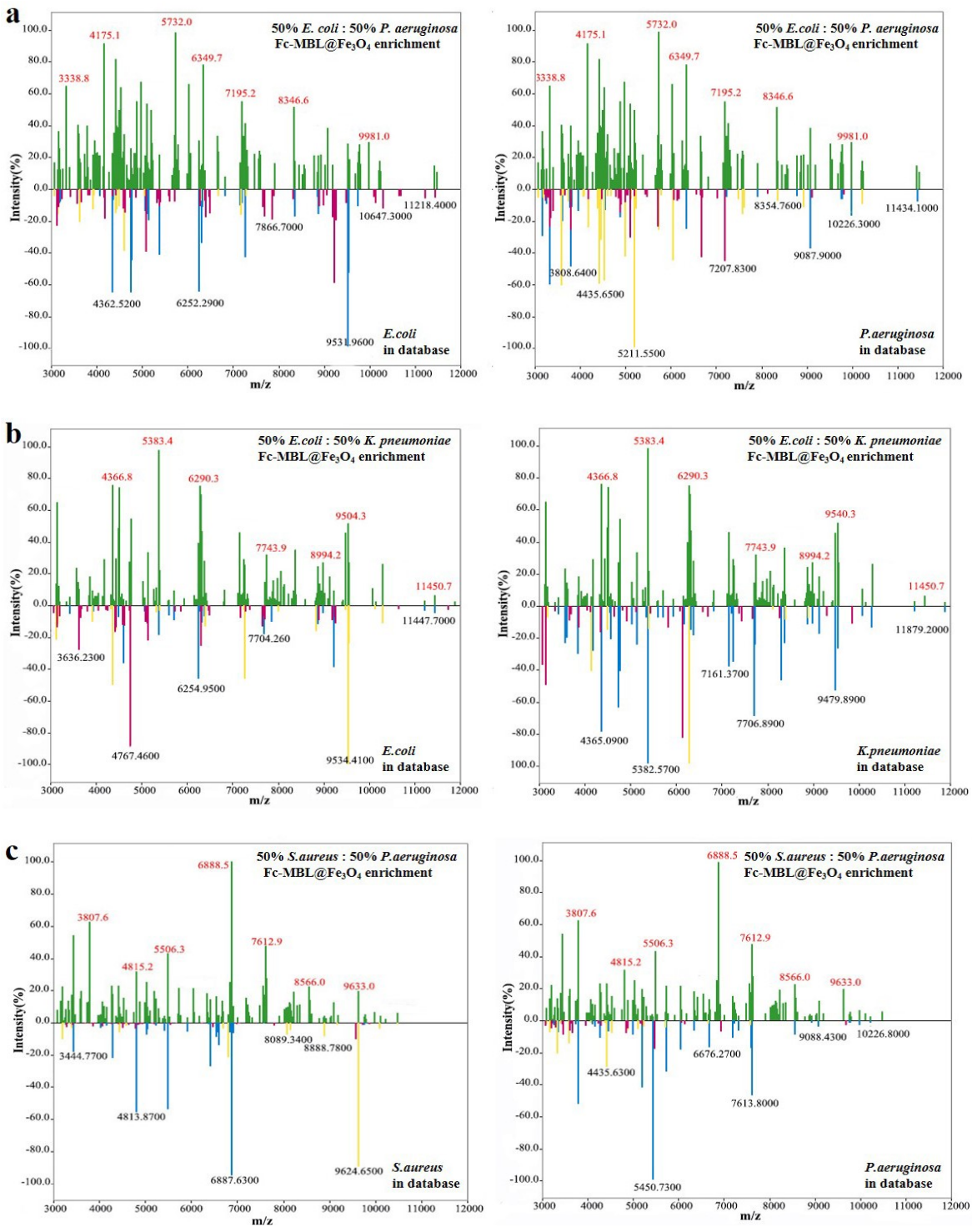


Fig. S10. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in urine. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumonia*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*


a
50% E. coli : 50% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
4175.1
5732.0
6349.7
3338.8
7195.2
8346.6
9981.0
11218.4000
10647.3000
7866.7000
4362.5200
6252.2900
9531.9600
E.coli
in database
50% E.coli : 50% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
4175.1
5732.0
6349.7
3338.8
7195.2
8346.6
9981.0
8354.7600
11434.1000
10226.3000
9087.9000
7207.8300
3808.6400
4435.6500
5211.5500
P.aeruginosa
in database
Intensity(%)
m/z
b
50% E.coli : 50% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
5383.4
4366.8
6290.3
9504.3
7743.9
8994.2
11450.7
11447.7000
7704.260
3636.2300
6254.9500
4767.4600
9534.4100
E.coli
in database
50% E.coli : 50% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
5383.4
4366.8
6290.3
9540.3
7743.9
8994.2
11450.7
11879.2000
7161.3700
9479.8900
7706.8900
4365.0900
5382.5700
K.pneumoniae
in database
c
50% S.aureus : 50% P.aeruginosa
Fc-MBL@Fe3O4 enrichment
6888.5
3807.6
7612.9
5506.3
4815.2
8566.0
9633.0
8089.3400
8888.7800
3444.7700
4813.8700
6887.6300
9624.6500
S.aureus
in database
6888.5
50% S.aureus : 50% P.aeruginosa
Fc-MBL@Fe3O4 enrichment
3807.6
7612.9
5506.3
4815.2
8566.0
9633.0
9088.4300
10226.8000
6676.2700
4435.6300
7613.8000
5450.7300
P.aeruginosa
in database
Intensity(%)
m/z

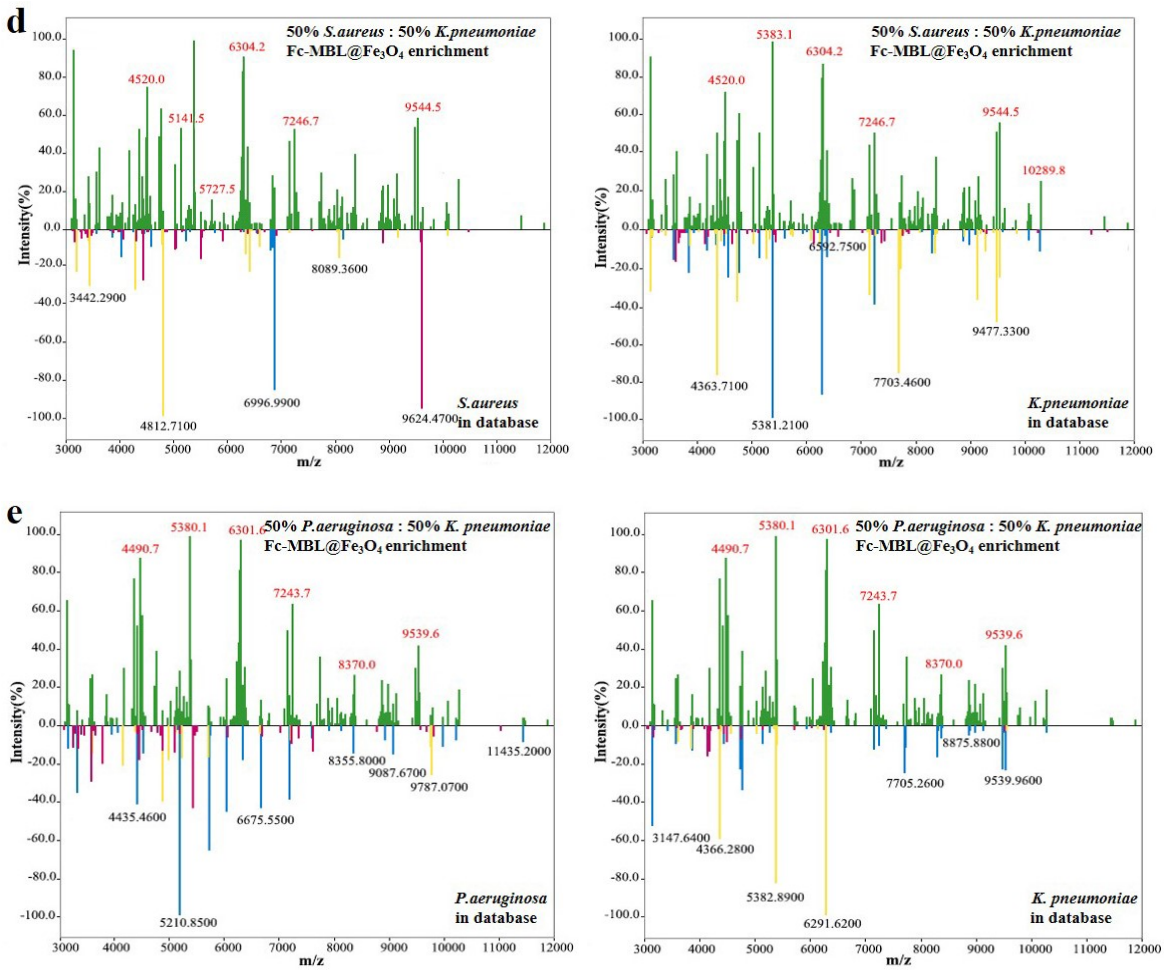
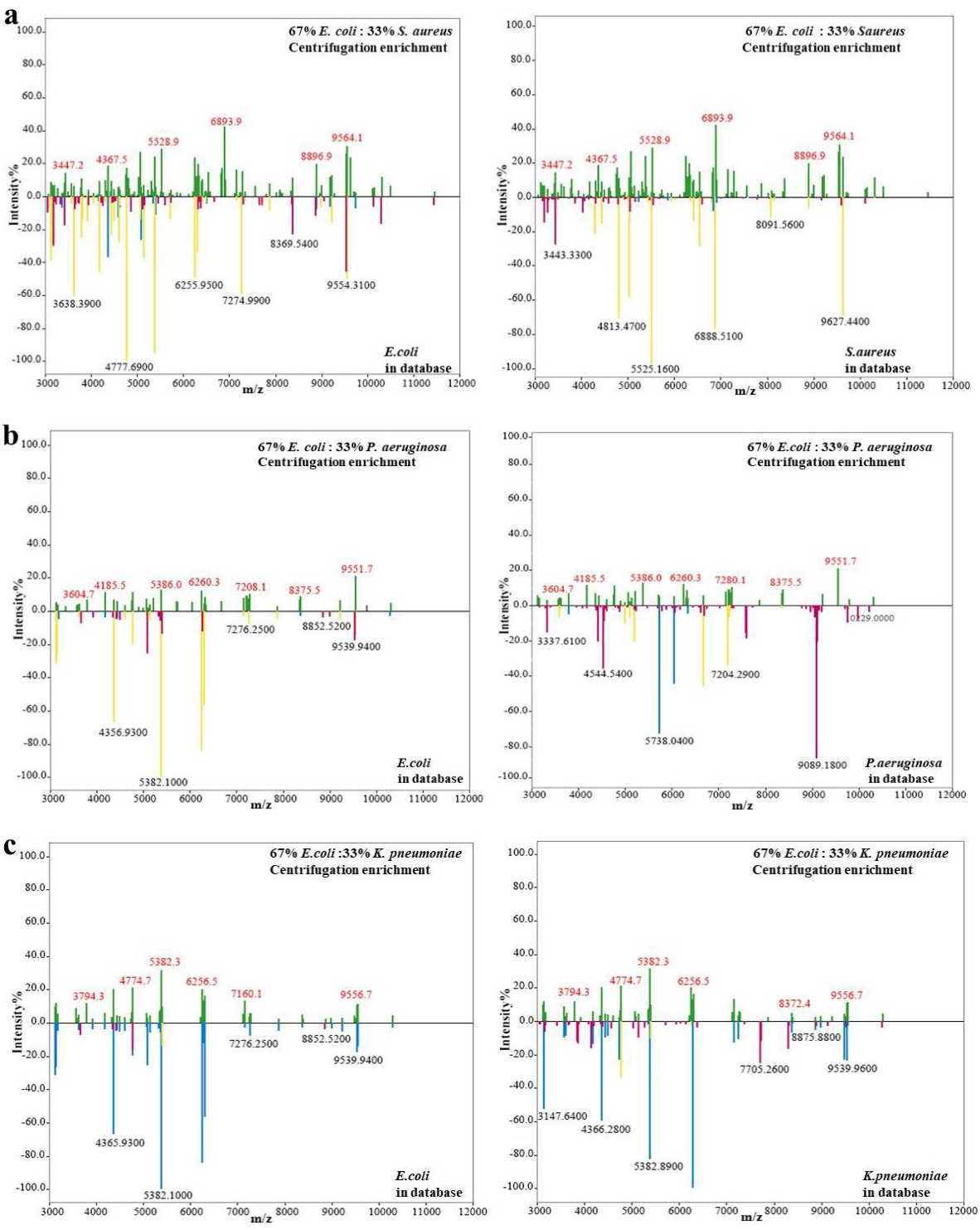


Fig. S11. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in urine. (a) 50% *E. coli* with 50% *P. aeruginosa*, (b) 50% *E. coli* with 50% *K. pneumoniae*, (c) 50% *S. aureus* with 50% *P. aeruginosa*, (d) 50% *S. aureus* with 50% *K. pneumoniae*, (e) 50% *P. aeruginosa* with 50% *K. pneumoniae*.


a
67% E. coli : 33% S. aureus
Centrifugation enrichment
3447.2
4367.5
5528.9
6893.9
8896.9
9564.1
8369.5400
6255.9500
7274.9900
9554.3100
3638.3900
4777.6900
E.coli
in database
67% E. coli : 33% Saureus
Centrifugation enrichment
3447.2
4367.5
5528.9
6893.9
8896.9
9564.1
8091.5600
3443.3300
4813.4700
6888.5100
9627.4400
5525.1600
S.aureus
in database
b
67% E. coli : 33% P. aeruginosa
Centrifugation enrichment
3604.7
4185.5
5386.0
6260.3
7208.1
8375.5
9551.7
7276.2500
8852.5200
9539.9400
4356.9300
5382.1000
E.coli
in database
67% E.coli : 33% P. aeruginosa
Centrifugation enrichment
3604.7
4185.5
5386.0
6260.3
7280.1
8375.5
9551.7
3337.6100
4544.5400
7204.2900
5738.0400
9089.1800
P.aeruginosa
in database
c
67% E.coli :33% K. pneumoniae
Centrifugation enrichment
3794.3
4774.7
5382.3
6256.5
7160.1
9556.7
7276.2500
8852.5200
9539.9400
4365.9300
5382.1000
E.coli
in database
67% E.coli : 33% K. pneumoniae
Centrifugation enrichment
3794.3
4774.7
5382.3
6256.5
8372.4
9556.7
8875.8800
7705.2600
9539.9600
3147.6400
4366.2800
5382.8900
K.pneumoniae
in database
Intensity %
m/z

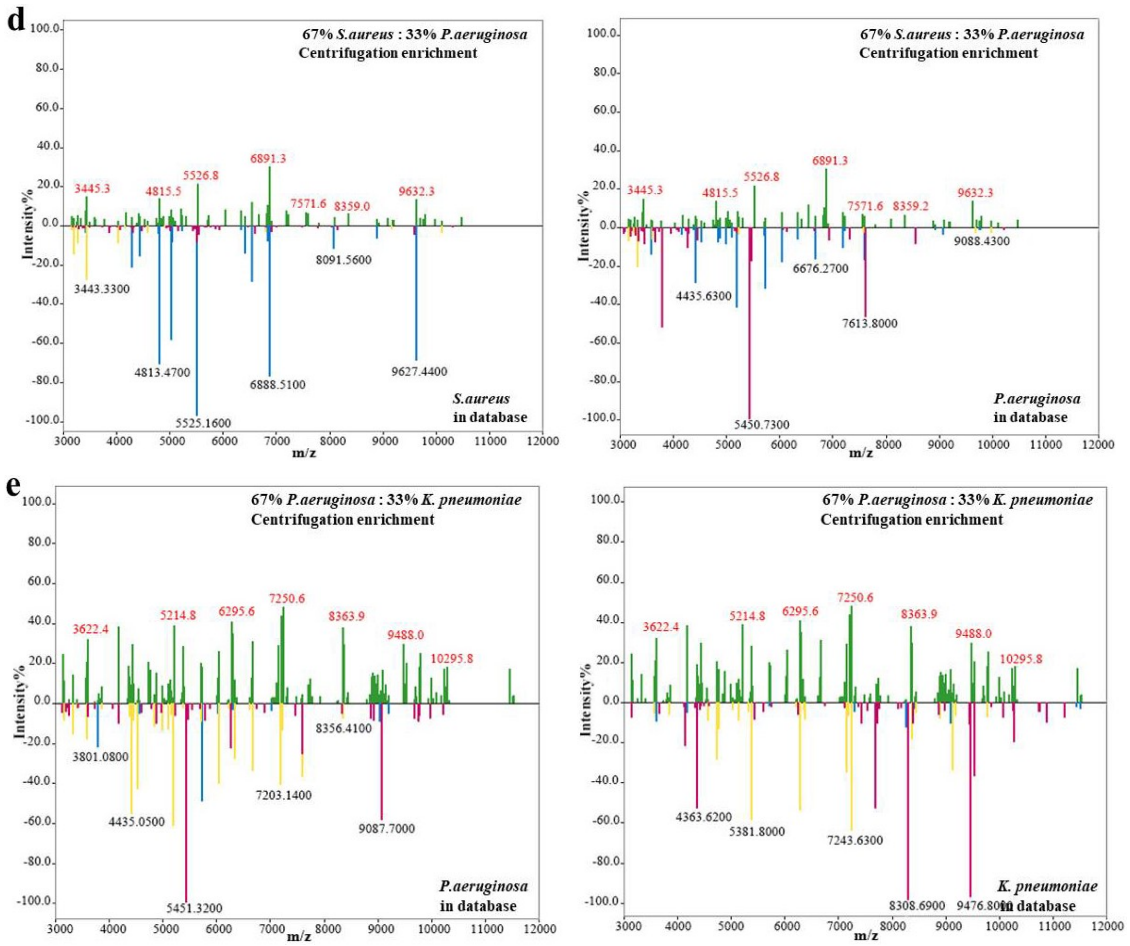
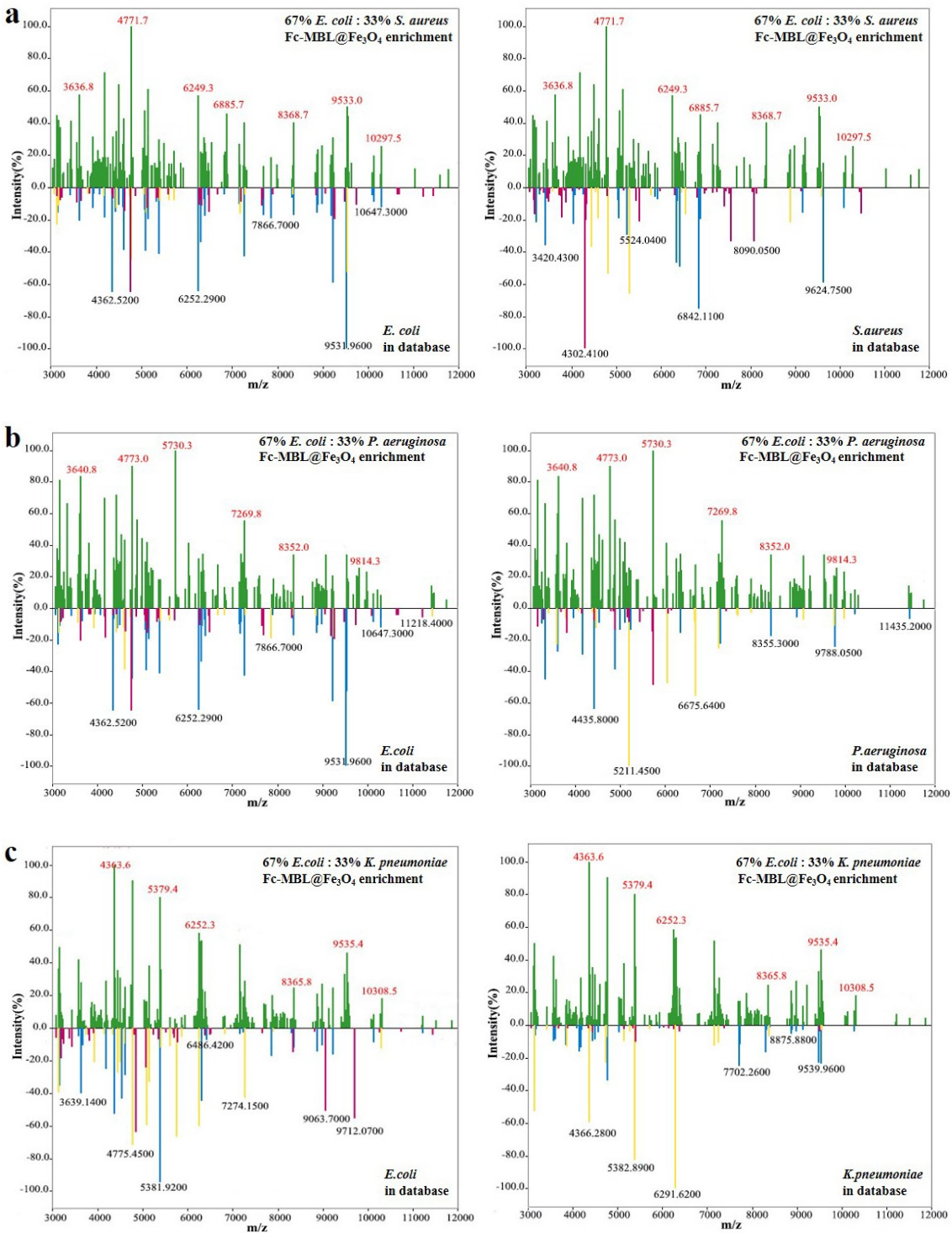


Fig. S12. MALDI-TOF MS of a bacterial mixture enriched by centrifugation in urine. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *P. aeruginosa* with 33% *K. pneumoniae*.


a
67% E. coli : 33% S. aureus
Fc-MBL@Fe3O4 enrichment
4771.7
3636.8
6249.3
6885.7
8368.7
9533.0
10297.5
10647.3000
7866.7000
4362.5200
6252.2900
9531.9600
E. coli
in database
67% E. coli : 33% S. aureus
Fc-MBL@Fe3O4 enrichment
4771.7
3636.8
6249.3
6885.7
8368.7
9533.0
10297.5
3420.4300
5524.0400
8090.0500
9624.7500
6842.1100
4302.4100
S.aureus
in database
b
67% E. coli : 33% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
5730.3
4773.0
3640.8
7269.8
8352.0
9814.3
11218.4000
10647.3000
7866.7000
4362.5200
6252.2900
9531.9600
E.coli
in database
67% E.coli : 33% P. aeruginosa
Fc-MBL@Fe3O4 enrichment
5730.3
4773.0
3640.8
7269.8
8352.0
9814.3
11435.2000
8355.3000
9788.0500
6675.6400
4435.8000
5211.4500
P.aeruginosa
in database
c
67% E.coli : 33% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
4363.6
5379.4
6252.3
9535.4
8365.8
10308.5
6486.4200
3639.1400
7274.1500
9063.7000
9712.0700
4775.4500
5381.9200
E.coli
in database
67% E.coli : 33% K. pneumoniae
Fc-MBL@Fe3O4 enrichment
4363.6
5379.4
6252.3
9535.4
8365.8
10308.5
8875.8800
7702.2600
9539.9600
4366.2800
5382.8900
6291.6200
K.pneumoniae
in database
Intensity(%)
m/z

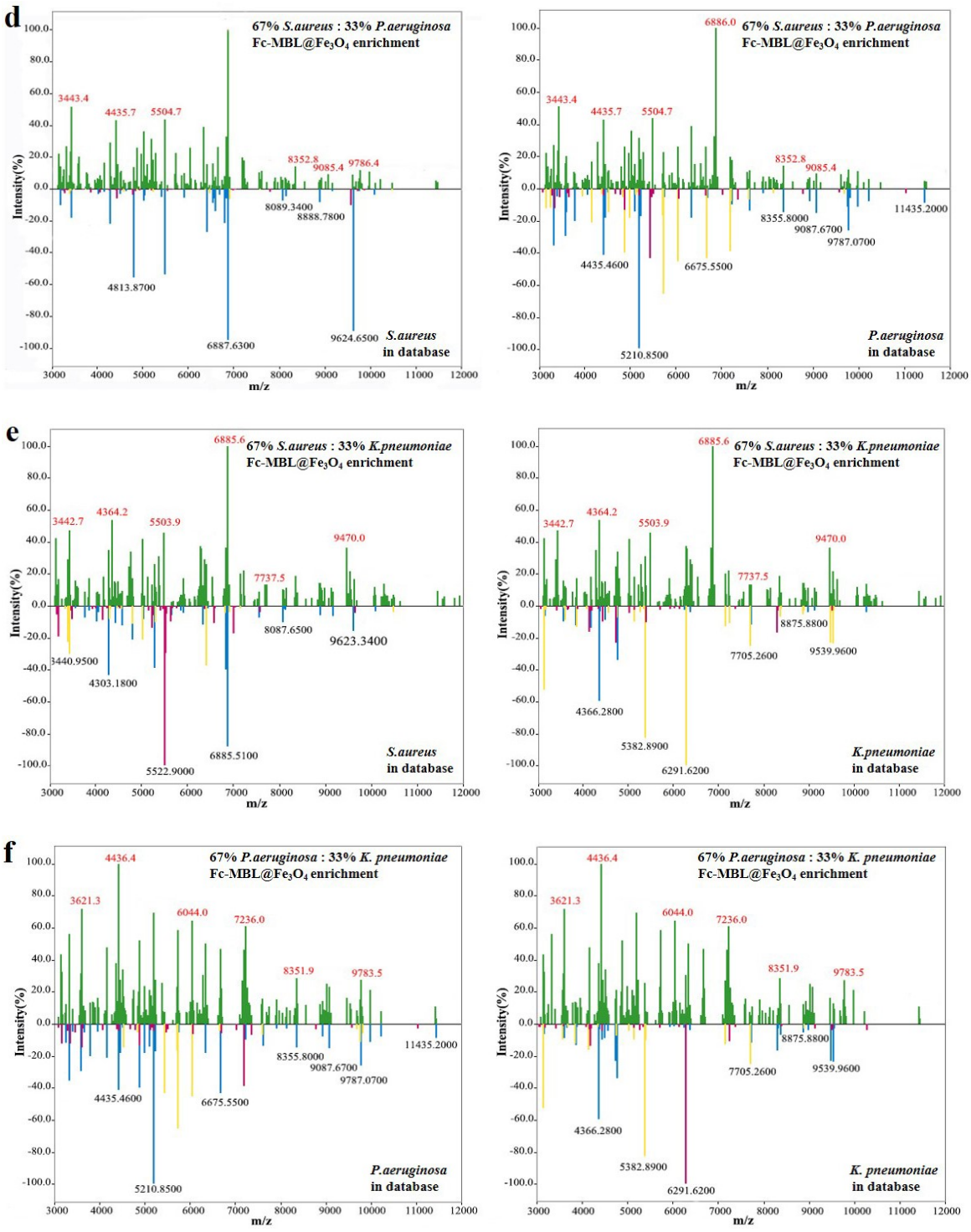


Fig. S13. MALDI-TOF MS of a bacterial mixture enriched by Fc-MBL@Fe_3O_4 in urine. (a) 67% *E. coli* with 33% *S. aureus*, (b) 67% *E. coli* with 33% *P. aeruginosa*, (c) 67% *E. coli* with 33% *K. pneumoniae*, (d) 67% *S. aureus* with 33% *P. aeruginosa*, (e) 67% *S. aureus* with 33% *K. pneumoniae*, (f) 67% *P. aeruginosa* with 33% *K. pneumoniae*.

Table S1. Identification scores for bacterial mixtures in urine enriched by Fc-MBL@Fe3O4.

Bacteria A / Bacteria B	Concentration ratio	Identification scores Bacteria A / Bacteria B
Ec / Sa	5/1	1.96/-
Ec / PA	5/1	2.02/-
Ec / KP	5/1	1.94/-
Sa / PA	5/1	2.10/-
Sa / KP	5/1	2.05/-
PA/KP	5/1	2.17/-