

Table S1 Information of recombinant plasmids

Targets	NCBI accession number	Sequence of synthesized plasmids
<i>E. coli</i>	AF28036	TATCTGACAACAGGCATGACGGTGGCGGAGCTGTCCGC GGCCGCCGTGCAATACAGTGATAACGCCGCCCAATTT GTTGCTGAAGGAGTTGGGCGGCCCGGGCTGACGG CCTTCATGCGCTCTATCGGCGATAACCAGTTCCGTCTGG ACCGCTGGGAGCTGGAGCTGAACTCCGCCATCCCAGGC GATGCGCGGATACCTCATCGCCGCGCGCCGTGACGGA AAGCTTACAAAACACTGACACTGGGCTCTGCACTGGCTGC GCCGCAGCGGCAGCAGTTTGTGATTGGCTAAAGGGAA ACACGACCGGCAACCACCGCATCCGCGCGGGGTGCCG GCAGACTGGGCAGTCGGAGACAAAACCGGAACCTGCGG AGTGTATGGCACGGCAAATG
<i>K. pneumoniae</i>	AF293352	ACCAGCAGACGAACCTCCTGCTCGGTGTTATTGAGAAA AGTGTGGCAGATACCGGTACCCGCGGGAAAACCCACGC TGTCGCCCGGCTCCAGCTTCCAGAGATAGCCGTTTATCC ACACTTCCGGATAGCCCTCCAGCACGTAGATGAACTCTT CCTCATCGCTCTCCGCGTGCGGGTACGAGGTGCGCCGCC CGGGCGGCAGTCGCTCGTGGTGGATCCCCAGCCGGTTG AGACGTAAACCGCGCCCCAGCGGCGCCAATCGAAAA ACGCTCCGGGCTGTCGGGATAAGTGGCATCGTCGGGCC CTTCCAGTTCGCGCCAGTGGCGAATGCAATCAGGTCGTT TCATCGTTTTCTCCCGAAAATGAGACACTTCAGATA
<i>E. faecium</i>	CP053704.1	ACTTCGCTAACTTATAAGATAGACAAACGTACAAGTGTA AATGATAGACATGTACATTTAAAAGCGGGGAAGCAATC GCGCTTCTCCGCTTTTTTTGATAATAAGTTTATTCAGGA GGAAATAAAAATGGCTAAGAAAACAGTGAAAGATATCGA TTTAAAAGACAAAAAAGTTCTTGTCCGTGTTGACTTCAA TGTTCCTTTGAAAGAGGCGTGATCACTGACGACACTCGT ATCAAAGCAGCATTGCCAACAATCAACTACGTTTTAGAA CAAGGCGGAAAAGCAATCCTTTTCTCTCACCTTGGACGT GTAAAAACAGAAGAAGATAAAGAAGGAAAATCTTTAGC TCCAGTTGCAAAAACGTCTAGGCGAATTATTAGGT
<i>E. faecalis</i>	NC_004668	TACAGGAACACGTTGAGCAGCGCCATCTAATTTACCGTT CAATTCTGGGATTACTAAGCCGATTGCTTTAGCAGCACC AGTTGAGTTAGGTACGATGTTTGCAGCAGCAGCGCGCG CACGACGGAAGTCGTGTCATTTGGTCACCTGTGTAAGCG

Table S1 (Continued)

Targets	NCBI accession number	Sequence of synthesized plasmids
<i>E. faecalis</i>	NC_004668	TGGATAGTTGTCATTAACCTTTAGGATGTGGTCCGTCT AACCTTCAACAACACCAAAGTTGTCATGTAAAGCTTTAG CCATTGGAGCTAAGCAGTTTGTAGTACAAGAAGCGCCT GAAATTACAGTTTCTTCTCCAGTTAATGTTTCATGGTTTG TGTTGTAAACGATTGTTGGTACATCGTTACCACCAGGAG CTGAAATAACTACACGTTTTGCACCAGCAGTTAAAT
<i>NDM</i>	AP023238.1	TGAATTCGCCCCATATTTTTGCTACAGTGAACCAAATTA AGATCATCTATTTACTAGGCCTCGCATTTCGCGGGTTTTT AATGCTGAATAAAAGGAAAACCTTGATGGAATTGCCCAA TATTATGCACCCGGTCGCGAAGCTGAGCACCGCATTAGC CGCTGCATTGATGCTGAGCGGGTGCATGCCCGGTGAAAT CCGCCCGACGATTGGCCAGCAAATGGAAACTGGCGACC AACGTTTTGGCGATCTGGTTTTCCGCCAGCTCGCACC GAATGTCTGGCAGCACACTTCCTATCTCGACATGCCGGG TTTCGGGGCAGTCGCTTCCAACGGTTTGATCGTCAGGGA TGGCGGCCGCGTGCTGGTGGTCGATACCGCCTGGACCG ATGACCAGACCGCCCAGATCCTC
<i>KPC</i>	MT463290.1	TATCTGACAACAGGCATGACGGTGGCGGAGCTGTCCGC GGCCGCCGTGCAATACAGTGATAACGCCGCCGCAATTT GTTGCTGAAGGAGTTGGGCGGCCCGGCCGGGCTGACGG CCTTCATGCGCTCTATCGGCGATAACCAGTTCCGTCTGG ACCGCTGGGAGCTGGAGCTGAACTCCGCCATCCCAGGC GATGCGCGGATACTCATCGCCGCGCGCCGTGACGGA AAGCTTACAAAACTGACACTGGGCTCTGCACTGGCTGC GCCGCAGCGGCAGCAGTTTGTGATTGGCTAAAGGGAA ACACGACCGGCAACCACCGCATCCGCGCGGGCGGTGCCG GCAGACTGGGCAGTCGGAGACAAAACCGGAACCTGCCG AGTGTATGGCACGGCAAATG

Table S2 Primers and probes tested in this study.

Name	Sequence (5'-3')
<i>E. coli</i> -F	ATATGGCGGTGAGTATTATCGTCAGGAACAACATC
<i>E. coli</i> -R	GAGATGACCATTTGTCCGCATCAACATCTTTGTAG
<i>K. pneumoniae</i> -F	TTATCCCGACAGCCCGGAGCGTTTTTCGATTGG
<i>K. pneumoniae</i> -R	CAGCTTCCAGAGATAGCCGTTTATCCACACTTCCG
<i>E. faecium</i> -F	GGAAGCAATCGCGCTTCTTCCGCTTTTTTTGA
<i>E. faecium</i> -R	GCTTTGATACGAGTGTCTGTCAGTGATCACGCC
<i>E. faecalis</i> -F	CACGTTGAGCAGCGCCATCTAATTTACCGTTC
<i>E. faecalis</i> -R	GCTTAGCTCCAATGGCTAAAGCTTTACATG
KPC-F (RPA)	AACGCCGCGCCAATTTGTTGCTGAAGGAG
KPC-R (RPA)	TTCCCTTTAGCCAATCAACAAACTGCTGCCG
NDM-F (RPA)	AATATTATGCACCCGGTTCGCGAAGCTGAGC
NDM-R (RPA)	GCCCCGAAACCCGGCATGTCTGAGATAGGAA
<i>E. coli</i> -UP1	GATTACCCTGCTTGGCGGAT
<i>E. coli</i> -DP1	ACGGACGCGGAGACCCGCTATGACAGT
<i>E. coli</i> -UP2	ATTACCCTGCTTGGCGGAAT
<i>E. coli</i> -DP2	ACGGACGCGGAGCCCGCTATGACAGTTATC
<i>E. coli</i> -UP3	GGCGCGACGACGGGT
<i>E. coli</i> -DP3	ACGGACGCGGAGCTTCCCGCAAGCAA
<i>K. pneumoniae</i> -UP1	TGCCGCCCCGGGCT
<i>K. pneumoniae</i> -DP1	ACGGACGCGGAGGGCGCACCTCGTA
<i>K. pneumoniae</i> -UP2	CAGTCGCTCGTGGTGGAC
<i>K. pneumoniae</i> -DP2	ACGGACGCGGAGTCCCCAGCCGG
<i>K. pneumoniae</i> -UP3	CCCGGGCGGGCGCT
<i>K. pneumoniae</i> -DP3	ACGGACGCGGAGACCTCGTACCCGC
<i>E. faecium</i> -UP1	TCGATATCTTTCCTGTTTTCTTAGCCAC
<i>E. faecium</i> -DP1	ACGGACGCGGAGTTTTATTTCTCCTGAATAAACTT
<i>E. faecium</i> -UP2	AATAAGTTTATTCAGGAGGAAATAAAATGGCTT
<i>E. faecium</i> -DP2	ACGGACGCGGAGAAGAAAACAGTGAAAGATATCG
<i>E. faecium</i> -UP3	TTTATTCAGGAGGAAATAAAATGGCTAAGAAAAT
<i>E. faecium</i> -DP3	ACGGACGCGGAGCAGTGAAAGATATCGATTTAAAAG
<i>E. faecalis</i> -UP1	AGCGCGCGCACGAT
<i>E. faecalis</i> -DP1	ACGGACGCGGAGCGGAAGTCGTGTCATT
<i>E. faecalis</i> -UP2	AGTTTAAATGACAACCTATCCACGCTTT
<i>E. faecalis</i> -DP2	ACGGACGCGGAGACACAGGTGACCAAATG
<i>E. faecalis</i> -UP3	CATTTGGTCACCTGTGTAAGCGC
<i>E. faecalis</i> -DP3	ACGGACGCGGAGTGGATAGTTGTCATTAAACCT
KPC-UP1	CGGCCTTCATGCGCTCTATT
KPC-DP1	ACGGACGCGGAGCGGGCGATAACCACG
KPC-UP2	TAGAGCGCATGAAGGCCGC
KPC-DP2	ACGGACGCGGAGTCAGCCCCGCC

Table S2 (Continued)

Name	Sequence (5'-3')
<i>KPC-DP3</i>	ACGGACGCGGAGGAAGGCCGTCAGC
<i>NDM-UP1</i>	GCACCGCATTAGCCGCTT
<i>NDM-DP1</i>	ACGGACGCGGAGGCATTGATGCTGAGC
<i>NDM-UP2</i>	CAGCATCAATGCAGCGGCC
<i>KPC-F (PCR)</i>	CATTCAAGGGCTTTCTTGCTGC
<i>KPC-R (PCR)</i>	ACGACGGCATAGTCATTTGC
<i>NDM-F</i>	GGTTTGGCGATCTGGTTTTTC
<i>NDM-R</i>	CGGAATGGCTCATCACGATC
<i>FAM-HP</i>	FAM-TCTT (BHQ1) AGCCGGTTTTCCGGCTA AGACTCCGCGTCCGT-C6-NH2

Table S3 DNA concentration of 4 kinds of pathogens from 24 urinary samples quantified by qPCR

Pathogens	Ct	The linear regression equation	Concentration range (copies/ μ L)
<i>E. coli</i>	18.84	$y = -3.737x + 37.224$	9437.25~131061.13
	18.1		
	21.5		
	20.14		
	22.37		
	19.11		
<i>K. pneumoniae</i>	19.13	$y = -3.667x + 38.739$	4154.22~370093.94
	18.32		
	25.47		
	20.56		
	18.79		
	21.24		
<i>E. faecium</i>	25.22	$y = -3.513x + 40.946$	1658.57~131048.91
	28.17		
	22.53		
	27.48		
	24.39		
	28.84		
<i>E. faecalis</i>	24.43	$y = -3.304x + 40.398$	2792.94~174679.97
	29.76		
	24.14		
	28.92		
	28.57		
	28.49		

Note: Four pathogens (*E. coli*, *K. pneumoniae*, *E. faecium*, and *E. faecalis*) each from 6 different urinary samples were randomly lysed using Quick extraction solution to perform qPCR.

Table S4 Validation of *KPC* and *NDM* in 9 carbapenem-resistant urinary samples with qPCR assay

Carbapenem-resistant urinary samples	Ct	
	<i>KPC</i>	<i>NDM</i>
1	16.13	36.07
2	21.56	37.22
3	24.33	39.94
4	19.78	37.45
5	16.47	40.15
6	25.55	38.58
7	15.92	39.87
8	18.95	16.74
9	37.82	36.46

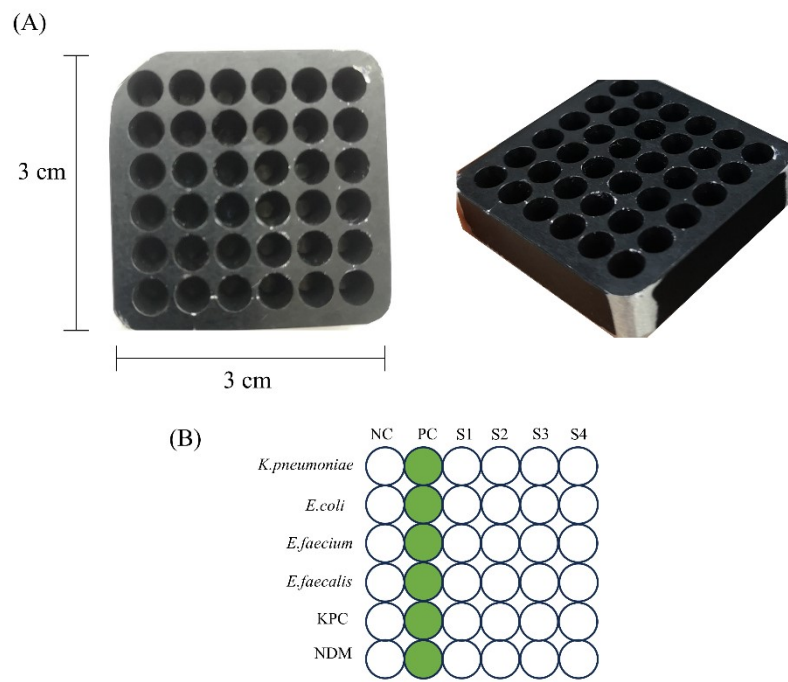


Fig. S1 (A) Images of Aluminum thermal module with 36 pores. (B) Design of each location in 36-tube array for detection of multiple targets and multiple samples.

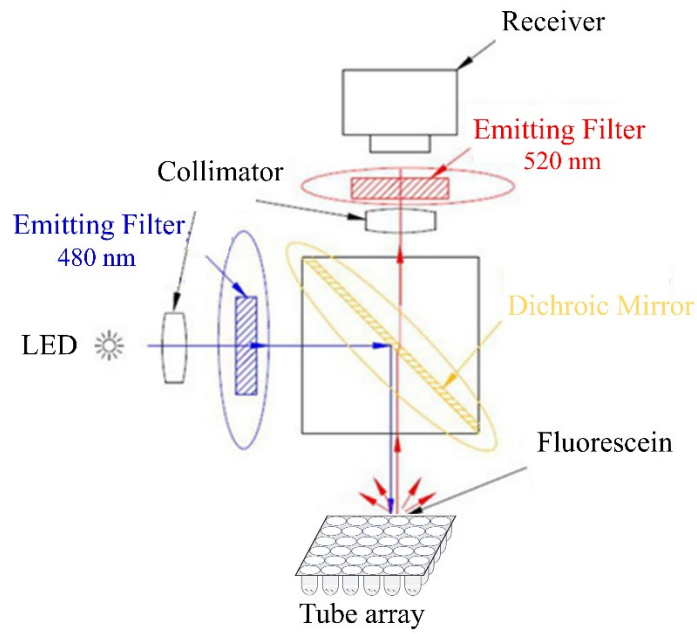


Fig. S2 Working principle of handheld analyser for detecting fluorescence signal.

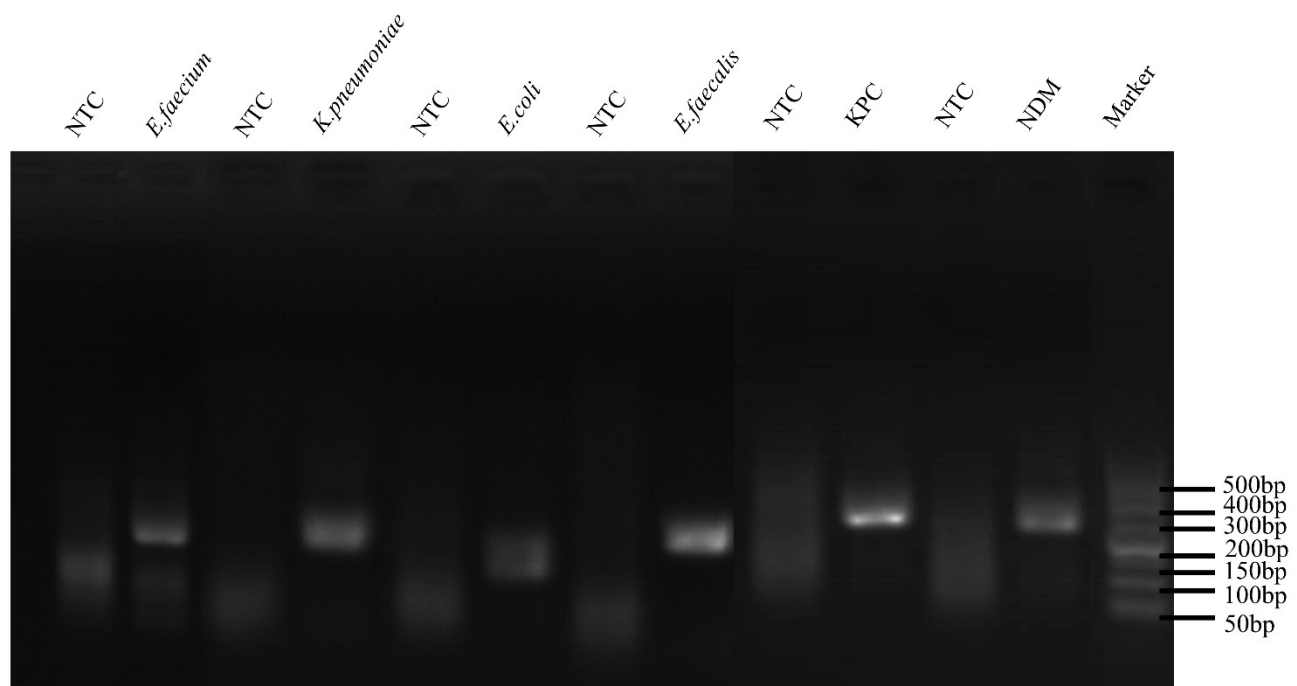


Fig. S3 Images of agarose gel electrophoresis (2%) for RPA primers validation of *E. faecium*, *K. pneumoniae*, *E. coli*, *E. faecalis*, *KPC*, and *NDM*. NTC is negative control without template. Lane Marker is DNA ladder.

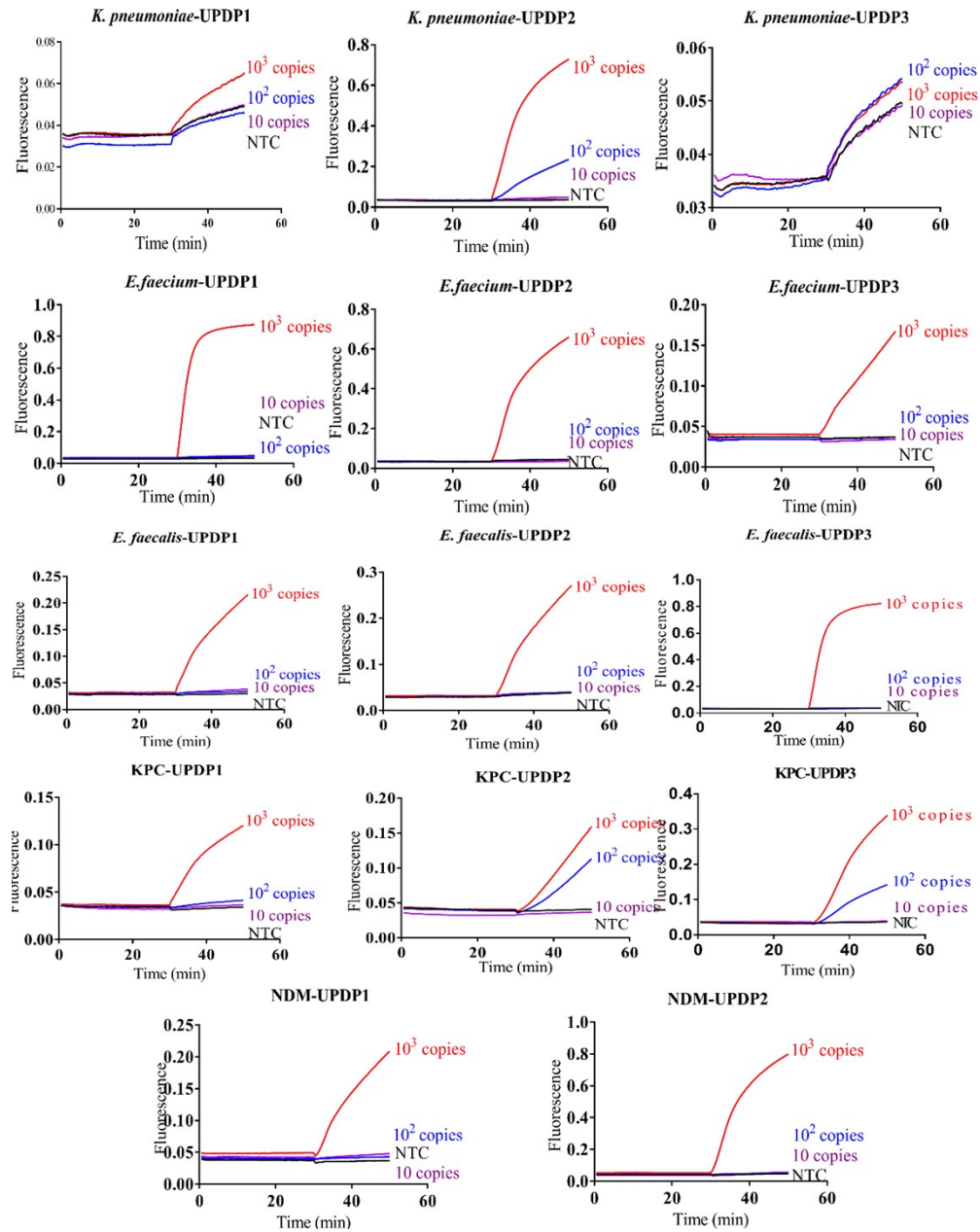


Fig. S4 Fluorescent profile of screening for optimum invasive probe. (*K. pneumoniae*, *E. faecium*, *E. faecalis*, *KPC*, *NDM*). We chose *E. faecium*-UP1 and DP1, *K. pneumoniae*-UP2 and DP2, *NDM*-UP and DP2, *E. faecalis*-UP3 and DP3, *KPC*-UP3 and DP3 as the invasive probes for subsequent experiments.

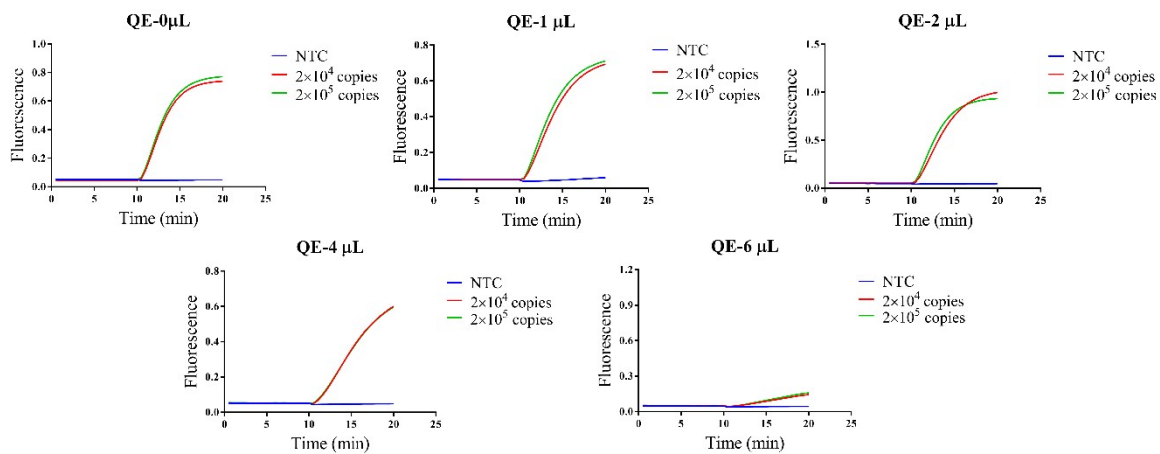


Fig. S5 The fluorescent profile of adding different volumes (0, 1, 2, 4, 6 μL) of QE extraction solution into the FARPA reaction system for detecting known concentration (2×10^4 , 2×10^5 copies/reaction) of the recombinant plasmid of *E. coli*.

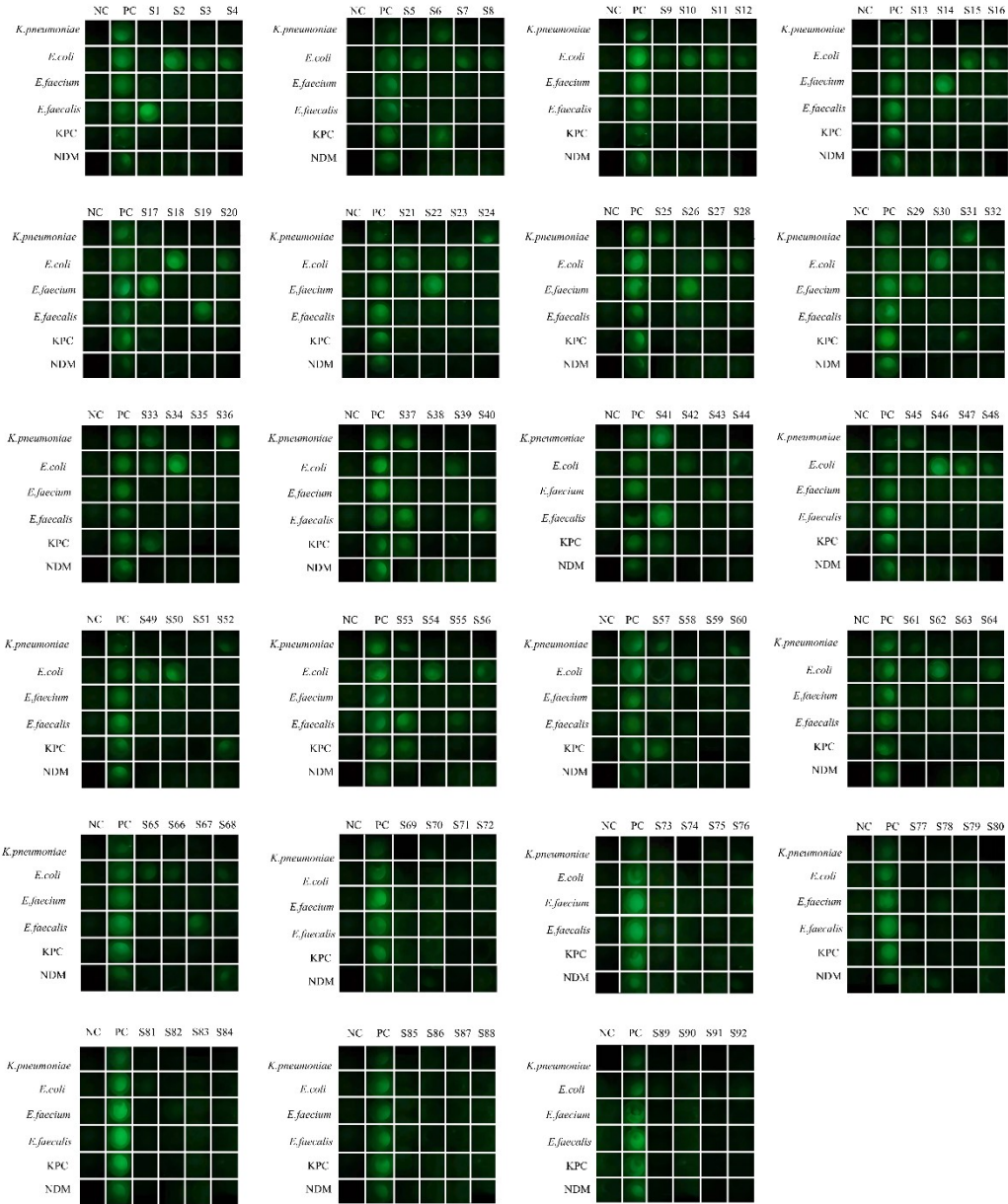


Fig. S6 Fluorescent images of FARPA-based tube array for detecting 92 clinical urinary samples. NC is the negative control without any target. PC is the positive control with 2×10^5 copies/reaction recombinant plasmids of each target.

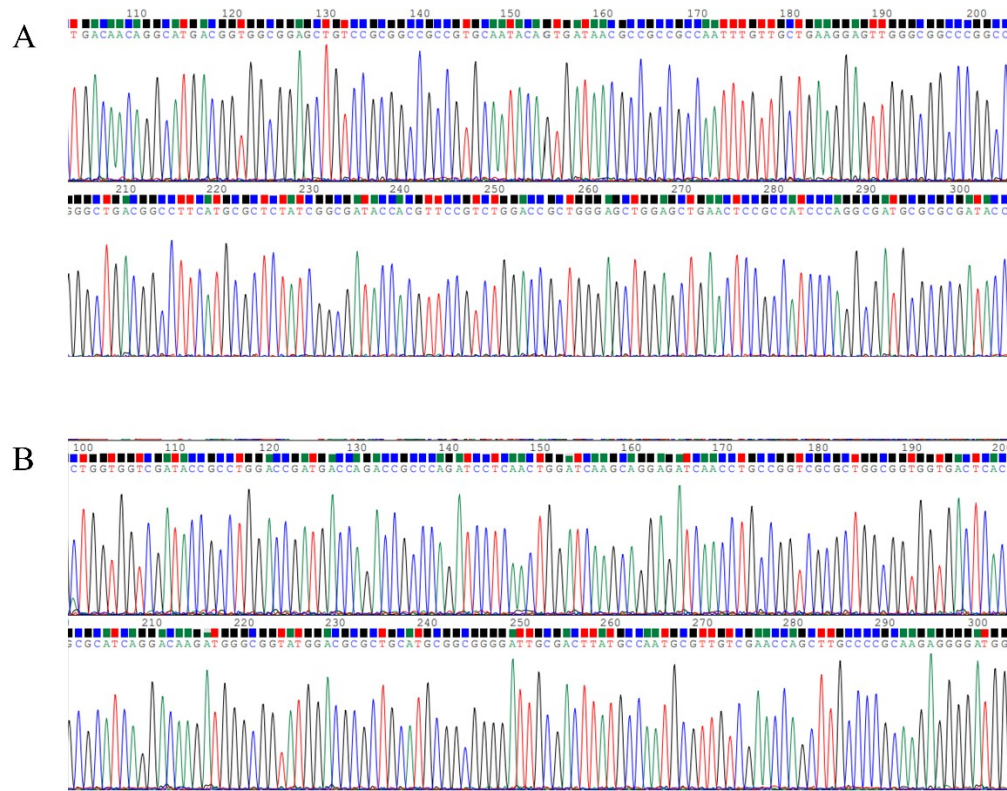


Fig. S7 The results of Sanger sequencing for 8 strains of *K. pneumoniae* carrying *KPC* resistant gene (A) and 1 strain of *E. coli* carrying *NDM* resistant gene (B).