Table S1. qPCR HRM settings for the different species.

Species	pPCR HRM settings								
	Hold	Hold	Cycles 1		Cycles 2		Hold	Hold	Range for HRMA
Enterococcus faecium	72°C 7min	95°C 5min	10x	**78**°C 60s	15x	**82**°C 60s	72°C 5min	50°C 30s	76-84°C
				72°C 120s		72°C120s			
Staphylococcus aureus	72°C 7min	95°C 5min	10x	**76**°C 60s	20x	**80**°C 60s	72°C 5min	50°C 30s	75-81°C
				72°C 120s		72°C 120s			
Klebsiella pneumoniae	72°C 7min	95°C 5min	10x	**80**°C 60s	15x	**84**°C 60s	72°C 5min	50°C 30s	78-86°C
				72°C 120s		72°C 120s			
Acinetobacter baumannii	72°C 7min	95°C 5min	8x	**76**°C 60s	18x	**80**°C 60s	72°C 5min	50°C 30s	74-82°C
				72°C 120s		72°C 120s			
Pseudomonas aeruginosa	72°C 7min	95°C 5min	22x	**87.2**°C 60s			72°C 5min	50°C 30s	83-90°C
				72°C 120s					
Enterobacter cloacae	72°C 7min	95°C 5min	10x	**80**°C 60s	18x	**84**°C 60s	72°C 5min	50°C 30s	76-86°C
				72°C 120s		72°C 120s			

Table legend. The first hold is to release unligated HINDLIG oligonucleotides (7) and to fill in the single-stranded ends and create templates. The second hold is for initial denaturation and enzyme activation followed by cycling optimized for each species. After the last cycle prior to HRMA, samples were incubated at 50° C for 30 s to allow single-stranded DNA to become double-stranded. Melting-curve analysis was performed by using high resolution melt analysis at different temperature-spans depending on species as indicated and denaturation temperature (T_D) in the cycles (indicated with bold style), with stepwise increases of 0.05°C.

Figure S1. LMqPCR HRMA results for *E. cloacae*, all graphs included. In A complete melting curves for all isolates (in duplicates) are visualized plotting df/dt against temperature (°C). These melting curves were evaluated using the algorithm (1) and by subjective evaluation using visual inspection of the whole curves. In B normalized fluorescence is plotted against temperature for all isolates (°C) (in duplicates). These curves were used to subjectively study shifts between isolates. In C-L isolate EnC01-EnC10 respectively, is set as reference, plotting the difference to all other isolates enabling clustering as ±U-value. Isolates with U-values ≤3 (for *E. cloacae*) was considered belonging to the same cluster as the one set as reference. Analysis of these three different kind of curves was considered together resulting in a final decision regarding clustering for each isolate.

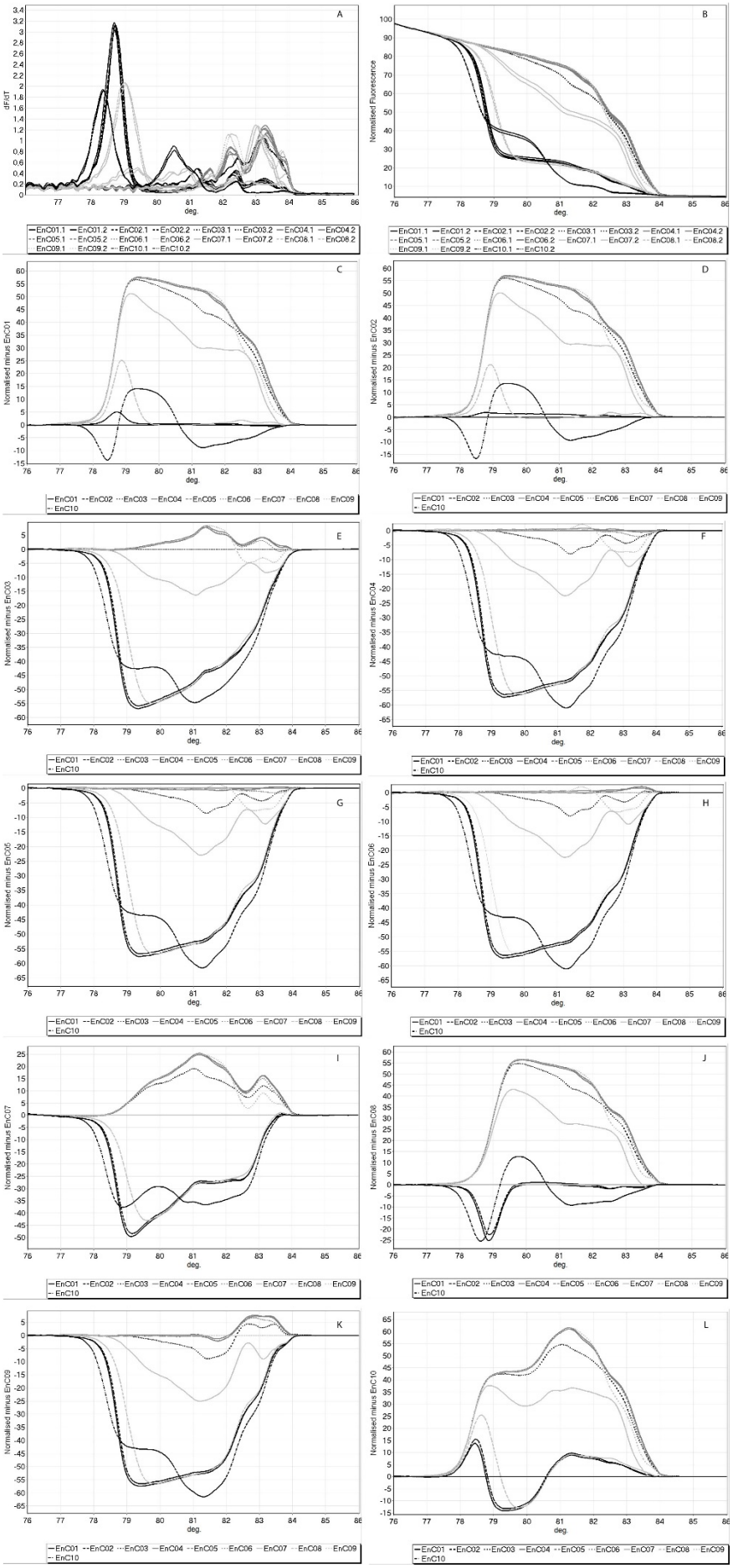
A
dF/dT
deg.
EnC01.1 EnC01.2 EnC02.1 EnC02.2 EnC03.1 EnC03.2 EnC04.1 EnC04.2
EnC05.1 EnC05.2 EnC06.1 EnC06.2 EnC07.1 EnC07.2 EnC08.1 EnC08.2
EnC09.1 EnC09.2 EnC10.1 EnC10.2
B
Normalised Fluorescence
deg.
EnC01.1 EnC01.2 EnC02.1 EnC02.2 EnC03.1 EnC03.2 EnC04.1 EnC04.2
EnC05.1 EnC05.2 EnC06.1 EnC06.2 EnC07.1 EnC07.2 EnC08.1 EnC08.2
EnC09.1 EnC09.2 EnC10.1 EnC10.2
C
Normalised minus EnC01
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
D
Normalised minus EnC02
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
E
Normalised minus EnC03
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
F
Normalised minus EnC04
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
G
Normalised minus EnC05
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
H
Normalised minus EnC06
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
I
Normalised minus EnC07
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
J
Normalised minus EnC08
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
K
Normalised minus EnC09
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10
L
Normalised minus EnC10
deg.
EnC01 EnC02 EnC03 EnC04 EnC05 EnC06 EnC07 EnC08 EnC09
EnC10

Figure S2. Results from agarose gel electrophoresis analysis of *E. cloacae*. For confirmation of HRMA cluster analysis, the results were compared to results from agarose gel electrophoresis. EnC01-02 cluster as do EnC04-06. EnC03, 07-10 are unique. M is the marker E-gel® Low Range Quantitative DNA ladder, 100-2000 bp (Invitrogen, Life Technologies). Analysis of gels was first done subjectively, then by GelClust using the Dice coefficient and UPGMA (data not shown).