Supplementary Text S1. Methods and materials

For the gram-negative bacteria, five to ten isolated colonies from blood agar medium were transferred to purified water in a 1.5 ml eppendorf tube and subsequently incubated for 10 min at 95 °C, and centrifuged briefly. DNA extraction was carried out with a Magna Pure Compact system (Roche Diagnostics Scandinavia AB, Bromma, Sweden) according to the manufacturer's instructions, and the DNA concentration was measured by use of a UV/Vis spectrophotometer (Techtum Lab AB, Sweden). The DNA concentration range was kept at 100 to 500 ng/µl. For the gram-positive bacteria, an agar plate with confluent growth of an isolate was flooded with 1 mL of PBS. This step was repeated two times. The eluate was centrifuged at 14 000 x g for 10 min. The supernantant was discarded and 800 µl MagNA Pure Bacteria Lysis Buffer (Roche Diagnostics Scandinavia AB, Bromma, Sweden) and 0.8 g of 0.5 mm zirconia/silica beads (BioSpec Products Inc, Bartlesville, Oklahoma, USA) was added. The samples were homogenized in a mini-beadbeater (BioSpec Products Inc, Bartlesville, Oklahoma, USA) for 30 s and centrifuged at 10 000 x g for 3 min after which 400 µl supernantant was transferred and DNA extraction was carried out with a Magna Pure Compact system (Roche Diagnostics Scandinavia AB, Bromma, Sweden) according to the manufacturer's instructions. The DNA concentration was measured by use of a UV/Vis spectrophotometer (Techtum Lab AB, Sweden) and the concentration range was kept at 15 to 30 ng/µl. DNA concentration was normalized for all samples and up to 1 µg DNA was used for the cleavage reaction as described previously (1). Subsequent PCR was carried out in a reaction mixture with 2 µl template i.e. ligation mix, as previously described (1). Real-time PCR with HRM (Rotor-Gene 6000; Corbett Research, Techtum Lab AB, Sweden) was optimized for each species as described in Table S1. Each isolate was analysed in duplicate on at least three different occasions. Following LMqPCR HRMA, the DNA products were analysed by agarose gel electrophoresis (E-gel EX, 2%; LifeTechnologies, Sweden) according

to the instructions provided by the manufacturer. DNA banding patterns were analysed by use of GelClust (www.bmsu.ac.ir/Services/Event/View.aspx?OId=1766). The Dice similarity coefficient and UPGMA were used for cluster analysis. For PFGE, bacterial isolates and control strains were analyzed using the restriction enzyme *Xba*I for *K. pneumoniae* and *E. cloacae* (2), *Spe*I for *P. aeruginosa* (3), *Apa*I for *Acinetobacter* spp. (4) and *Sma*I for *S. aureus* and *E. faecium* (5, 6). *Xba*I-digested DNA from *Salmonella enterica* serovar Braenderup H9812 (www.cdc.gov/pulsenet) was included as a normalization standard when analyzing *K. pneumoniae*, *E. cloacae* and *P. aeruginosa*, and *S. aureus* NCTC8325 for the gram-positive bacteria and *Acinetobacter* spp. DNA banding patterns were analysed by use of BioNumerics version 6.0 (Applied Maths NV, Belgium). The Dice similarity coefficient and UPGMA were used for cluster analysis; in general, isolates with >97 % similarity were defined as identical, isolates with 90-97 % similarity as being closely related, and isolates with <90 % similarity defined as unrelated.

Algorithm for evaluation of the HRMA analysis to discriminate between isolates

Several aspects were evaluated to determine if two or more HRM curves were different within the same run (please see example for *Enterobacter* spp. in Supplementary figure 1-2). First, to determine similarities objectively, an algorithm previously developed (1) was applied. Second, the whole HRM curves (df/dt plotted against temperature) were compared to each other by overlaying them and inspecting them visually. Third, the same approach was used to the normalized curves (normalized fluorescence plotted against temperature). Last, the curve representing the isolate in question was determined as different from another isolate if the difference curve (normalized fluorescence minus isolate of the control plotted against temperature) was beyond ±3.5 U for *E. faecium* (Fig 1C), ±5 U for *S. aureus*, ±3 U *K.*

pneumoniae, ±3 U for *A. baumannii* (Fig 1D), ±5 U *P. aeruginosa* and ±3 U E. *cloacae*. The definition of U is % difference between the normalized curves calculated by the RotorGene software. The cut-off for the ΔU were determined empirically in a pilot stage before the blinded analysis was initiated. For isolates within these limits, the curve shape was also considered in relation to the reference, i.e. how much the curve differed from the reference at each point along the entire X-axis. There were 11 isolates deviating from the set U-values (data not shown), mostly depending on shifts between identical curves resulting in similar curves lying outside these values, but also isolates with clearly different melting curves that fit within the ΔU cut-off. Such isolates were subjectively evaluated and not subjected to U-value analysis.