Methods

Antimicrobial susceptibility testing and pulsed field gel electrophoresis

Minimum inhibitory concentration (MIC) determination was performed using a commercially prepared dehydrated panel (Sensititre; TREK Diagnostic Systems Ltd., East Grinstead,England) and according to previously published methodology (8). The tested antimicrobials / classes in this study have been listed in Table 1. Clinical and Laboratory Standards Institute (CLSI) (3) clinical breakpoints interpretative criteria for resistance (R) were used except for the following antimicrobials; azithromycin, ceftiofur, colistin, florfenicol, neomycin, spectinomycin, and streptomycin, respectively where epidemiological cut-off values according to EUCAST recommendations was used (http://www.eucast.org). Apramycin was interpreted according to research results from DTU-Food. Quality control was performed by using reference strain *E. coli* ATCC 25922 according to CLSI guidelines (3,4).

Procedures for conducting PFGE of the two *S.* Typhi isolates included this study have previously been described and according to manufactures recommendation (8,13).

A XML file of the PFGE profiles was forwarded to US CDC where PFGE patterns were assigned based on the PulseNet USA database and the Global Typhi database.

Whole genome sequencing, multilocus sequence typing, antimicrobial resistance genes, plasmid replicons, plasmid multilocus sequence typing, and restriction-modification system

Chromosomal DNA of the two *S.* Typhi isolates and plasmid DNA of transformant #TY5359x MT102RN was extracted using an Invitrogen Easy-DNA™ Kit (Invitrogen, Carlsbad, CA, USA) and DNA concentrations were determined using the Qubit dsDNA BR assay kit (Invitrogen). The genomic DNA was prepared for Illumina pair-end sequencing

using the Illumina (Illumina, Inc., San Diego, CA) NexteraXT® Guide 150319425031942 following the protocol revision C (http://support.illumina.com/downloads/nextera_xt_sample_preparation_guide_15031942.html). A sample of the pooled NexteraXT libraries using the Nextera XT DNA sample preparation kit (Illumina, cat. No. FC-131-1024) was loaded onto a Illumina MiSeq reagent cartridge using MiSeq Reagent Kit v2 and 500 cycles with a Standard Flow Cell. The libraries were sequenced using an Illumina platform (Illumina, Inc., San Diego, CA) and MiSeq Control Software 2.3.0.3. Twenty previously published *S.* Typhi genomic sequences; the only ones containing full metadata profile were obtained from GenBank and Sanger Institute (accessed 20/12/2013). A total of 14 genomes from Asia were downloaded, of which 11 were from GenBank while the other three genomes were retrieved from Sanger bacterial genome database. (http://www.sanger.ac.uk/resources/downloads/bacteria/). Full genomic information is shown in Supplementary Table 1A.

Raw sequence data have been submitted to the European Nucleotide Archive http://www.ebi.ac.uk/ena/data/view/PRJEB6961 under accession no. ERS525820 and ERS525821. The raw reads were assembled using the pipeline available on the Center for Genomic Epidemiology (CGE) (www.genomicepidemiology.org) which is based on Velvet, algorithms for *de novo* short reads assembly (20). A complete list of genomic sequence data is available in the Supplementary Table 1A.

The *de novo* assembled sequences including the plasmid DNA of the transformant #TY3559 x MT102RN were analyzed using similar pipelines available on the CGE website. The web-servers; MLST version 1.7, ResFinder version 2.0, PlasmidFinder 1.1, and pMLST 1.2 (19) were used to identify the MLST sequence type for *Salmonella enterica*, the plasmid replicons, the plasmid multilocus sequence typing and acquired antimicrobial resistance

genes with a selected threshold equal to 85.00%. The identity and results of the Resfinder were compared with phenotypic antimicrobial susceptibility testing results.

The sequence similarity of the identified plasmids were assessed based on a BLAST analysis of the contigs related to the plasmids of the two *S.* Typhi isolates against the scaffold of a previously published incHI2/incHI2A plasmid (pEC-IMPQ. Genbank acc no. EU855788). Visual evaluation of the BLAST comparison was performed using the software BLAST Ring Image Generator (BRIG) v0.95 (1). In addition, a reference assembly against pEC-IMPQ of plasmid contigs from the two wild type strains was performed using the SNPtree webserver as described below.

Integrity of the Restriction-Modification Systems (RMS) present in the two *S.* Typhi isolates were analyzed compared to 11 *S.* Typhi genomes of sufficient quality and full meta-data profile by using the web-server Restriction-ModificationFinder version 1.0 available from the CGE website (www.genomicepidemiology.org), with a threshold and minimum length equal to 80.00%. The webserver is based on the ResFinder software (19), and the database includes nucleotide sequences of all Type I-IV restriction- and modification enzymes with their cognate subunits recorded in REBASE (14).
The RM results were further explored by blasting the results against the Conserved Domain Database at NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), and compared with the reference genome of *S.* Typhi str. CT18.

Phylogenetic structure of S. Typhi using Single Nucleotide Polymorphisms, identification of S. Typhi haplotypes, and genomic deletions

Single nucleotide polymorphisms (SNPs) were determined using the pipeline available on the Center for Genomic Epidemiology (www.genomicepidemiology.org) (9) The pipeline contains various freely available SNP analysis software. Fundamentally, the paired-end reads from ESBL isolates were aligned against the reference genome, *S.* Typhi str. CT18 (National Center for Biotechnology Information, accession: AL513382, length of 4·809·037 bp), using Burrows-Wheeler Aligner (BWA)(10). SAMtools (11) 'mpileup' command and bedtools (12) [ref] were used to determine and filter SNPs.

Assembled genomes or contigs were aligned against the reference genome using the application "Nucmer" of MUMmer version 3.23 (5). SNPs were identified from the alignments using "Show-snps" (using option "-Cl1rT") from MUMmer. Subsequently, SNPs were selected when meeting the following criteria: 1) a minimum distance of 15 bps between each SNP, and 2) all indels were excluded. The qualified SNPs from each genome were concatenated to a single alignment corresponding to position of the reference genome using an in-house Perl script. In cases where SNPs were absent in the reference genome, they were interpreted as not being a variation and the relatively base from the reference genome was expected (9). The concatenated sequences were subjected to multiple alignments using MUSCLE from MEGA5 (18). The final phylogenetic SNP tree was computed by MEGA5 using the maximum likelihood method (17) of 1,000 bootstrap replicates (6). All SNPs identified between the two isolates and the included public available genomes are listed in the Supplementary Table 1B.

The haplotype of both genomes was assigned based on biallelic polymorphisms positions (BiP) previously described (15). Positions based on WGST are listed in the Supplementary Table 1C.

A BLAST atlas based on BLASTP (7) was used to detect putative deletions in a comparison of the genomes against the reference genome; CT18. Details of the genomic deletions detected among the isolates are listed in the Supplementary Table 1D.

Transferability of bla$_{SHV-12}$ genes by conjugation

Plate-mating experiments were performed with the two *S.* Typhi isolates as donors and plasmid-free, rifampicin and nalidixic acid resistant *E. coli* MT102RN as recipients (16). The strains were grown to both late exponential as well as stationary phase, mixed (1:1) and incubated on solid blood agar at both room temperature and at 37°C for 18 h.
Transconjugants were selected on BHI medium supplemented with 25 μg/ml rifampicin, 25 μg/ml nalidixic acid, and 4 μg/ml ceftriaxone.

Plasmid characterization

A transformant from the conjugation experiment described above was subjected to plasmid purification using Qiagen tip-100 as described by the manufacture (Qiagen, Hilden, Germany) followed by whole genome sequencing and sequence analysis.
The draft assembly of the plasmid was subsequently uploaded to the web-services as described above to confirm the presence of the *bla*$_{SHV-12}$ gene and origins or replication in the plasmid of the transformant #TY5359x MT102RN as well as presence of any non-ESC resistance determinants potentially co-transferred with the ESC plasmids.

S1 digestion for plasmid size determination

PFGE with S1 nuclease (Promega, Madison, Michigan, USA) digestion of whole genomic DNA performed as described below was used to estimate sizes of larger plasmids present in wild type isolates as well as transformants (2). Following pre-incubation for 10 min in 1:10

diluted S1 buffer, 2 mm slices of PFGE plugs made from cultures with an OD_{620} of 0.6 were digested with 5 U of S1 (Promega, Madison, Michigan, USA) for 45 min at 37°C. The slices were post-incubated on ice for 10 min in 200 µL of ice-cold TE-buffer (10:1), loaded on the gel and run on a CHEF-DR III device (Bio-Rad, Hercules, California, USA) with a pulse time of 6.8 s – 38.4 s at 6 V/cm for 19 h. The reference strain *S.* Braenderup H9812 digested with *Xba*I was used as size marker.

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