Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR


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Salmonella infections continue to cause gastrointestinal and systemic disease throughout the world. *Salmonella typhimurium* DT104 further poses a major health concern due to its acquisition of resistance to multiple antibiotics. The rapid detection of multiresistant *S. typhimurium* DT104 would facilitate strategies aimed at controlling this pathogen. We developed a specific and sensitive polymerase chain reaction (PCR) assay that amplifies a segment of DNA that is conserved in multiresistant *S. typhimurium* DT104. To provide further specificity for this PCR-based diagnostic test, we amplified two other gene fragments that are present in *S. typhimurium* DT104. A multiplex PCR containing primers for targeted sequences resulted in the amplification of predicted size fragments from *S. typhimurium* DT104 exhibiting the ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamethoxazole and tetracycline) or ASSuT resistance phenotypes. A minor modification of the multiplex PCR enabled the detection of other related multiresistant *Salmonella* such as *S. typhimurium* U302. To augment the detection process, we also designed a fluorogenic PCR assay that can detect the DNA of multiresistant *S. typhimurium* DT104 in the presence of excess contaminating bacterial DNA. These results provide a method by which multiresistant *S. typhimurium* DT104, or potentially the next emerging multiresistant *Salmonella*, can be accurately detected in only 3–4 h.

**KEYWORDS:** PCR, *Salmonella typhimurium* DT104, multiplex, antibiotic resistance.

**INTRODUCTION**

*Salmonella* infections continue to be an important health concern in both developed and undeveloped countries. Treatment of this infectious disease is further complicated by its ability to acquire resistance to multiple antibiotics. Antibiotic resistance in *Salmonella* has been on the rise in the UK and in the US. The majority of the increased incidence of resistance can be attributed to *Salmonella typhimurium* phagetype DT104 (DT104). *Salmonella* infection caused by multiresistant DT104 has been reported in humans, cattle, swine, marine wildlife, cats and a variety of other species. Recent studies have identified the arrangement of antibiotic resistance genes in the chromosome of
The genetic arrangement is largely comprised of two integrons, mobile cassettes of DNA that can facilitate the transfer of resistance genes, that includes commonly encountered streptomycin, tetracycline and sulphonmethoxazol resistance genes (aadA2, tetR, tetA and sul1, respectively). The integrons also contain a less commonly encountered gene for beta-lactamase (PSE-1) and a chloramphenicol resistance gene, not usually found in Salmonella, with high identity to the cmlA gene.

While the resulting multiresistant phenotype is alarming, the existence of multiresistant resistance genes in S. typhimurium is not a new phenomenon. Due to its highly effective mechanisms for acquiring and maintaining resistance genes, DT104 may exhibit a higher prevalence of resistance to antibiotics than that observed in other Salmonella important in clinical or veterinary circles. While it is unclear if genetic differences in multiresistant DT104 have also led to a more virulent phenotype than that observed in other S. typhimurium phagetypes, the ability of DT104 to evade conventional pharmacotherapies is of concern. Crude DNA was isolated from 1 ml of a Lennox L broth (Life Technologies, Paisley, Scotland) culture (approximately 10⁹ cells). Cells were pelleted (14 000 rpm for 1 min, 25°C), resuspended in 200 l distilled water and boiled (10 min). Following boiling, lysates were centrifuged (14 000 rpm for 5 min, 4°C), and supernatants were diluted 1:1 with distilled water. Purified DNA was isolated from broth using the Gnome kit (Bio101, Vista, CA, USA). DNA concentration was determined using the spectrophotometric analysis of Kalb and Bernlohr.

In this work, we demonstrate that DNA from multiresistant DT104 and U302 organisms can be specifically detected with high sensitivity in crude DNA preparations using multiplex polymerase chain reaction (PCR). The multiplex PCR can be complemented with a fluorogenic PCR strategy capable of rapidly detecting DT104-specific sequences using one of the multiplex amplicons. Together, these methods allow the detection of currently prevailing as well as potentially emerging multiresistant Salmonella.

MATERIALS AND METHODS

Bacterial strains

Strains used in PCR were obtained from National Veterinary Services Laboratory (Ames, IA, USA; NVSL 98-12781, -16033, -12993, -12386, -11354, -12985, -6100, -9437, -12993, -12682, -12768, -12767, -12868, -12623 and -745; all DT104), Field Disease Investigation Unit (Pullman, WA, USA; includes commonly encountered streptomycin, tetracycline and sulphonmethoxazole resistance genes DT104 3464 and 3402, S. typhimurium 771 S3426 and S3444, S. typhimurium 208 S3447), UK Public Health Laboratory Service (DT104 TH34, 41, 16 and 10), US Centers for Disease Control (Atlanta, GA, USA; S. typhimurium U302 7601 and 8430), German Health Organization (Berlin, Germany, Salmonella dublin 9276) and frozen laboratory stocks (Salmonella enteritidis 5-1952, gallinarum, pullorum, cholerae-suis, typhi, typhimurium SL1344, Staphylococcus aureus, Shigella flexneri, Listeria monocytogenes, Yersinia pseudotuberculosis, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae. Proteus vulgaris, Pseudomonas aeruginosa and Citrobacter freundii). Strains used in colony blotting were obtained from the National Antimicrobial Resistance Monitoring System (Athens, GA, USA).

DNA isolation

Crude DNA was isolated from 1 ml of a Lennox L broth (Life Technologies, Paisley, Scotland) culture (approximately 10⁹ cells). Cells were pelleted (14 000 rpm for 1 min, 25°C), resuspended in 200 µl distilled water and boiled (10 min). Following boiling, lysates were centrifuged (14 000 rpm for 5 min, 4°C), and supernatants were diluted 1:1 with distilled water. Purified DNA was isolated from broth using the Gnome kit (Bio101, Vista, CA, USA; Cat. #2010-600) as per manufacturer’s protocol. DNA concentration was determined using the spectrophotometric analysis of Kalb and Bernlohr.

Multiplex PCR

Sequences and locations of oligonucleotide primers (Integrated DNA Technologies, Coralville, IA, USA) are described in Table 1. Multiplex PCR was performed in an automated thermocycler with (Hybaid, Teddington, UK) or without (Robocycler 40, Stratagene, LaJolla, CA, USA) a hot bonnet. Reactions performed in the absence of a hot bonnet were overlaid with 35 µl mineral oil (Sigma, St. Louis, MC, USA). Polymerase chain reactions were performed in 0.2 µl (Hybaid machine) or 0.5 µl (Robocycler) tubes with both machines yielding similar results. Reactions were performed in 20 µl containing 300 µM dATP, dTTP, dCTP and dGTP, 2.5 mM magnesium chloride, 4 pmoles of each of six primers, 10 mM Tris-HCl, 50 mM KCl, 0.5 units of AmpliTaq Gold (Perkin Elmer,
Table 1. Nucleotide sequences of oligonucleotide primers and the fluorogenic probe used in multiplex and fluorogenic polymerase chain reaction (PCR) assays

<table>
<thead>
<tr>
<th>Primer or probe name</th>
<th>Sequence (5’→3’)</th>
<th>Location a</th>
</tr>
</thead>
<tbody>
<tr>
<td>SipB/C (forward)</td>
<td>ACACCAAAATGCGGATGCTT</td>
<td>2305–2324</td>
</tr>
<tr>
<td>SipB/C (reverse)</td>
<td>GCGGCTCAGTTAGGACTC</td>
<td>2555–2536</td>
</tr>
<tr>
<td>cmlA/tetR (forward)</td>
<td>GCTCCTTTCGATCCCTG</td>
<td>2555–2536</td>
</tr>
<tr>
<td>cmlA/tetR (reverse)</td>
<td>TCTGACTTTCATCACAACAGAT</td>
<td>5890–5869</td>
</tr>
<tr>
<td>cmlA/tetR (probe) b</td>
<td>ATGCTGCCACCGAGAAGTCCGGCC</td>
<td>5628–5649</td>
</tr>
<tr>
<td>PSE-1 (forward)</td>
<td>TTCGCTCCGCGCTCTATCG</td>
<td>713–731</td>
</tr>
<tr>
<td>PSE-1 (reverse)</td>
<td>TACTCCGAGCACCAAATCCG</td>
<td>863–844</td>
</tr>
<tr>
<td>TEM (forward)</td>
<td>GCAGGATGAGGGTTACATCGA</td>
<td>118–137</td>
</tr>
<tr>
<td>TEM (reverse)</td>
<td>GGTCCCTCCGATCGTTGTCAG</td>
<td>428–408</td>
</tr>
</tbody>
</table>

a Ascribed nucleotide locations are based on sequences obtained from the following accession numbers: U232561 for SipB/C; AF077555 for cmlA/tetR; M69058 for PSE-1; Y10281 for TEM.

Fluorogenic PCR

The sequence and location of the reporter/quencher fluorogenic probe (19; 5’-conjugated to 6-FAM, 3’-conjugated to TAMRA; Integrated DNA Technologies) is described in Table 1. Fluorogenic PCR was performed in 96-well plates (Perkin Elmer) with a reaction volume of 50 μl. Reactions were comprised of 300 μM dATP, dTTP, dCTP and dGTP, 2.5 mM magnesium chloride, 10 pmoles of each cmlA/tetR primer, 5 pmoles of fluorogenic probe, 10 mM Tris-HCl, 50 mM KCl, 1-25 units of AmpliTaq Gold, 20 μg of DT104 DNA and 200 μg ‘contaminating’ DNA. Thermocycling was performed in the Hybrid machine using the cycling conditions described above. Pre- and post-PCR fluorescence was assessed at 520 nm in a Perkin Elmer 7200 fluorimeter. Fluorescence
increase was calculated by subtracting pre-PCR relative fluorescent units from post-PCR relative fluorescent units at 520 nm. Assays were performed independently three times.

RESULTS

Detection of DT104 using multiplex PCR

Several groups have analysed the arrangement and identity of resistance genes in DT104.11-13 Using this information, we attempted to identify gene segments that could be useful for the detection of multiresistant, especially ACSSuT pentaresistant, DT104. Since sulphonamide, streptomycin and tetracycline resistance is overtly common in many Salmonella spp. (National Antimicrobial Susceptibility Monitoring Program- Veterinary Isolates, April, 1998; 20-22), we focused on the detection of ampicillin and chloramphenicol resistance genes. Resistance to these two antibiotics is commonly found in DT104, as determined by genomic mapping.11-13 and is conferred by the PSE-123 and cmlA-like24 genes, respectively. We also chose to detect tetR since this gene does not confer tetracycline resistance per se25 and is juxtaposed to the cmlA gene.13

Since PSE-1 and cmlA can be found in other bacteria, we deemed it necessary to also detect a Salmonella-specific gene segment. We chose the junction between SipB and SipC (SipB/C26) since most Salmonella spp. would be predicted to possess these two virulence genes based on the prevalence of a closely related Salmonella virulence gene (invA27), i.e. only certain environmental isolates of S. litchfield and senftenberg definitively lack these virulence genes.28 To detect specific resistance gene sequences, we chose the junction between cmlAtetR as the chloramphenicol resistance gene target and a fragment of the PSE-1 as the ampicillin resistance gene target. The cmlAtetR junction was chosen to prevent amplification of sequences from other bacteria, including Salmonella such as the older isolates described above, that may possess cmlA. Target sequences from these three genes were then simultaneously amplified in a multiplex PCR assay. As depicted in Fig. 1a, three amplicons (150, 250 and 280 bp) could be observed in DT104 isolates exhibiting the ACSSuT phenotype. The three amplicons could also be detected in two DT104 isolates exhibiting the ASSuT antibiogram (Fig. 1b). These ASSuT strains appear to differ from ACSSuT strains because of 5’ cmlA deletions that spare 3’ cmlA sites used for cmlAtetR amplification.13 The identity of the three amplicons was confirmed by nested PCR (data not shown).

Sensitivity and specificity of multiplex PCR

All three multiplex primer pairs simultaneously amplified the corresponding regions of multiresistant DT104 DNA using as little as 40 pg crude DNA or 20 pg purified DNA (Fig. 1c). The multiplex PCR was specific for ACSSuT or ASSuT DT104 and ACSSuT S. typhimurium U302. Only the Salmonella-specific amplicon (SipB/C) was observed in other Salmonella such as S. dublin (ACSSuT), S. enteriditis (A), S. typhimurium 771 and 208 (ACSSuT and/or ASSuT), DT104 (SSu and non-resistant). The single SipB/C amplicon was also observed in sensitive host-adapted Salmonella such as S. pullorum, gallinarum.

Fig. 1. (a) Agarose gel electrophoresis of amplicons observed following multiplex polymerase chain reaction (PCR) for multiresistant DT104 genes of Salmonella typhimurium ACSSuT isolates. Lanes designated as M represent 50 bp molecular weight standards (GIBCO BRL, Gaithersburg, MD, USA). Strains were obtained from sources described in ‘Materials and Methods’. Lanes 1 through 11 represent amplicons derived from 11 different ACSSuT isolates of DT104 (lanes 1, 2, 5-11) or S. typhimurium U302 (lanes 3 and 4). Lane designations correspond to the following DNA templates: 1 (TH34), 2 (TH41), 3 (S. typhimurium U302 7601), 4 (S. typhimurium U302 8430), 5 (TH10), 6-11 (NVSL 98-12781, -16033, -12993, -12386, -11354 and -12985, respectively). Amplicons are the result of using 200 pg crude DNA as template. Lane 12 is a negative control. Specific molecular weight standards (bp) and amplicon designations are indicated on the right while molecular weights of amplicons are indicated on the left. (b) Comparison of agarose gel electrophoresis patterns observed following multiplex PCR using DNA from ACSSuT and ASSuT DT104 isolates. Lanes designated as M represent 50 bp molecular weight standards (GIBCO). Lanes 1 through 11 represent amplicons derived from eleven different ASSuT (lanes 1 and 2) or ACSSuT (lanes 3-11) DT104 isolates. Strains were obtained from sources described in ‘Materials and Methods’. Lane designations correspond to the following DNA templates: 1 and 2 (DT104 3464 and 3402, respectively); 3-11 (NVSL 98-6100, -9437, -12781, -12993, -12682, -12768, -12767, -12868, -12623 and -745, respectively). Amplicons are the result of using 200 pg crude DNA as template. Lane 12 is the negative control. Specific molecular weight standards (bp) and amplicon designations are indicated on the right while molecular weights of amplicons are indicated on the left. (c) Sensitivity of the multiplex PCR. Lanes designated as M represent 50 bp molecular weight standards (GIBCO). Lanes 1 through 6 represent amplicons derived from decreasing amounts of crude DNA from DT104 (NVSL 98-6100), Lanes 7 through 12 represent amplicons derived from decreasing amounts of purified DNA from DT104 (NVSL 98-6100).
Fig. 1. Caption on p 216.
Fig. 2. (a) Specificity of the multiplex polymerase chain reaction (PCR) using DNA from antibiotic resistant and host-adapted Salmonella spp. Lanes designated as lower case ‘m’ and upper case ‘M’ represent 50 and 100 bp molecular weight standards (GIBCO), respectively. Strains were obtained from sources described in ‘Materials and Methods’. Lane designations correspond to the following DNA templates: 1 (DT104 TH10); 2 (Salmonella typhimurium U302 8430); 3 and 4 (S. typhimurium 771 S3426 and S3444, respectively); 5 (DT104 TH16); 6 (S. typhimurium 208 S3447); 7 (S. dublin 9276); lanes 8–12; S. enteriditis 5-1952, S. gallinarum, pullorum, choleraesuis and typhi, respectively). Identical antibiograms are found in isolates used for: lanes 1, 2, 4 and 7 (ACSSuT); lanes 3 and 6 (ASSuT); lane 5 (SSu); lane 8 (A); lanes 9–12 (sensitive). Amplicons are the result of using 200 pg crude DNA as template. (b) Adaptation of the multiplex PCR. Lanes designated as lower case ‘m’ and upper case ‘M’ represent 50 and 100 bp molecular weight standards (GIBCO), respectively. Lanes and strains are identical to that described for Fig. 2a, lanes 1–8.

choleraesuis and typhi (Fig. 2a). The overall specificity is consistent with data observed from colony blotting (Table 2).

Adaptation of the multiplex PCR

Our inability to detect other multiresistant (ACSSuT or ASSuT) Salmonella in the multiplex PCR documents the specificity of this protocol. However, the detection of these pathogens is important and should not be overlooked. Consequently, we modified the multiplex PCR such that other multiresistant Salmonella could be detected. Previous studies indicate that TEM genes can also confer ampicillin resistance to Salmonella. Therefore, we substituted TEM primers for the PSE-1 primers in the modified multiplex PCR. This adaptation, as depicted in Fig. 2b, enabled the detection of other multiresistant Salmonella, including S. dublin, S. typhimurium 208 and 771, using the TEM amplicon. Additionally, the adaptation facilitated the detection of an ampicillin resistant S. enteriditis and allowed for the discrimination between multiresistant DT104 and our limited inventory of multiresistant S. typhimurium U302 (lane 2 vs lane 3).

Fluorogenic PCR using a cmIA/tetR fluorescent-labelled probe

To enhance our ability to detect DT104 using PCR, we designed a fluorogenic PCR strategy that could complement amplicon visualization using agarose gel electrophoresis. Additionally, we wished to evaluate the detection of DT104 DNA in the
Table 2. Uniform presence of the cmlA/tetR segment in DT104 isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number tested</th>
<th>Positives cmlA/tetR</th>
<th>Positives SipB/C</th>
<th>Most common serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT104 All species</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>Typhimurium, Typhimurium Coppenhagen</td>
</tr>
<tr>
<td>Clinical cattle isolates</td>
<td>191</td>
<td>8</td>
<td>186</td>
<td>Typhimurium, Typhimurium Coppenhagen, Coppenhagen, Dublin</td>
</tr>
<tr>
<td>Non-clinical cattle</td>
<td>221</td>
<td>0</td>
<td>214</td>
<td>Cerro, Montevideo, Typhimurium, Enteritidis, Heidelberg</td>
</tr>
<tr>
<td>isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic lab. chicken</td>
<td>111</td>
<td>3</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>569</td>
<td>57</td>
<td>557</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and non-clinical antibiotic resistant Salmonella isolates were screened for the presence of the cmlA/tetR and SipB/C sequences. Strains were obtained from the National Antimicrobial Resistance Monitoring System. Colony blots were performed using digoxigenin-labelled cmlA/tetR or SipB/C digoxigenin-labelled probes as described in ‘Materials and Methods’.

a Among the eight positives: three were confirmed DT104; two were not phagetyped, they may be DT104 isolates but phagetyping was only performed on those with ACSSuT phenotypes and these two both have ACT phenotypes; one was a DT120 with the ACSSuT phenotype; one was designated with an untypable phagetype, ACSSuT phenotype; and one was Salmonella infantis with an ASSuT phenotype.

b There were no DT104 or pentaresistant isolates among the non-clinical cattle isolates.

c Two of the three are pentaresistant DT104 and one is a pentaresistant DT104b.

d The 12 isolates found to be negative for SipB/C were Salmonella montevideo (3), senftenberg (2), thompson (2), bovismobificans (2), cerro (1), mbandaka (1) and kentucky (1).

DISCUSSION

The ability of S. typhimurium DT104 to display antibiotic resistance is an important clinical property. The identification of this organism has previously been based on phagetyping which is restricted to a limited number of laboratories. While phagetyping obviously can accurately distinguish DT104 from other S. typhimurium phagetypes, this method alone does not elucidate the antibiogram of DT104. That is, phagetyping and the determination of resistance profiles must be used together to detect multiresistant DT104.

Here we report the development of a sensitive multiplex PCR-based assay that specifically and nearly exclusively detects multiresistant DT104. The use of the multiplex PCR permits the detection of three genetic regions found in multiresistant DT104. This assay is specific for multiresistant DT104 and S. typhimurium U302, a rare but potentially emerging organism that has evidently acquired the same resistance integrons as DT104. This method can be completed in less than 5 h after presumptive colonies have been grown in broth whereas phagetyping and antibiogram determinations may take up to 24 h.

The sensitivity of this assay can be enhanced by two different alterations. First, the threshold for detection can be lowered from 40 to 20 pg by purifying the DNA using RNaseA and proteinase-K in a commercially-available kit. However, there is no advantage to this approach as an extra 2–3 h is required to increase the sensitivity a mere two-fold.
Fig. 3. Fluorogenic polymerase chain reaction (PCR) using the cmlA/tetR fluorescent-labelled probe. Templates were composed of 20 μg DNA from either Salmonella typhimurium DT104 (D) or S. typhimurium U302 (U302) with a ten-fold excess of DNA from potential contaminating bacteria (MRSA, multiresistant Staphylococcus aureus; EPEC, enteropathogenic Escherichia coli; EHEC, enterohaemorrhagic E. coli; ETEC, enterotoxogenic E. coli; sources of strains described under ‘Materials and Methods’). Fluorescence was determined as described in ‘Materials and Methods’. Background fluorescence was determined using water as a template (none). Template derived from S. typhimurium SL1344 (SL1344) was used as a negative control. Assays were performed independently three times to derive mean ± SEM values. Similar results were observed using only DT104 or S. typhimurium U302 DNA as the template while an insignificant increase in fluorescence (similar to water or S. typhimurium controls) was observed when ‘contaminating’ DNA was the only template (data not shown).

multiplex procedure. The fluorogenic assay endows the ability to detect 20 pg crude DNA in approximately 3 h. This latter procedure can add an extra level of sensitivity, by decreasing the detection threshold, and specificity, by verifying the identity of the cmlA/tetR amplicon, to the overall detection protocol.

The specificity of the multiplex PCR can be also modified, if warranted, to detect multiresistant Salmonella other than DT104. The revision is based on differences in ampicillin resistance genes in Salmonella. The utilization of primers that amplify TEM genes enabled the detection of other resistant Salmonella including S. enteridis, S. dublin, S. typhimurium U302, 208 and 771. Since GenBank searches (BLAST sequence similarity) revealed that TEM or TEM variant genes are found in at least 12 different bacterial genera, the potential exists that contaminating bacteria may contribute to false positives using the modified multiplex PCR assay. Fortunately, however, S. typhimurium U302 and DT104 appear to nearly exclusively possess the same arrangement of cmlA and tetR genes yet two strains of S. typhimurium U302 additionally possess the TEM gene for ampicillin resistance. Therefore, the adapted multiplex PCR appears to be best suited for detecting both S. typhimurium U302 and DT104 and for distinguishing the two if TEM-based resistance continues to be unique as S. typhimurium U302 sample sizes accumulate. An S. dublin strain (ACSSuT) described in 1986 possesses chromosomally-integrated antibiotic resistance genes. The methods described in the present work establish Salmonella other than DT104. The revision is based on differences in ampicillin resistance genes in Salmonella. The utilization of primers that amplify TEM genes enabled the detection of other resistant Salmonella including S. enteridis, S. dublin, S. typhimurium U302, 208 and 771. Since GenBank searches (BLAST sequence similarity) revealed that TEM or TEM variant genes are found in at least 12 different bacterial genera, the potential exists that contaminating bacteria may contribute to false positives using the modified multiplex PCR assay. Fortunately, however, S. typhimurium U302 and DT104 appear to nearly exclusively possess the same arrangement of cmlA and tetR genes yet two strains of S. typhimurium U302 additionally possess the TEM gene for ampicillin resistance. Therefore, the adapted multiplex PCR appears to be best suited for detecting both S. typhimurium U302 and DT104 and for distinguishing the two if TEM-based resistance continues to be unique as S. typhimurium U302 sample sizes accumulate. An S. dublin strain (ACSSuT) described in 1986 possesses chromosomally-integrated antibiotic resistance genes. The methods described in the present work establish that this strain does not possess the same multiple resistance gene cluster as is found in DT104 and U302. This S. dublin strain possesses aadA and sul1 genes but does not possess the PSE-1 gene or the cmlA-like gene (unpubl. obs.). Thus, the mechanism of gene acquisition by early multiresistant S. dublin appears to be distinct from that observed in DT104.

The multiplex and fluorogenic PCR system described here was intended for the identification of multiresistant DT104 in culture. Future studies will be aimed at adapting these assays to detect multi-resistant DT104 in clinical specimens, diagnostic
isolates and in foods. In addition, the multiplex PCR assays described here may be useful to the study of the epidemiology of multiresistant DT104 and to the study of events that contributed to the emergence of multiresistant DT104.

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REFERENCES


