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Exogenous contaminating DNA in *Taq* polymerases: A method to avoid false-positive results when detecting the bla_{TEM} gene

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ABSTRACT

In the course of developing an assay to identify genes responsible for antibiotic resistance in gram-negative bacteria, it has been found that standard (not DNA-free) *Taq* DNA polymerases were contaminated with bla_{TEM} gene fragments that varied in length and quantities. The complete bla_{TEM} gene sequence was either absent or was detected in infinitesimal amounts. We developed an approach to avoid false-positive findings caused by contaminating bla_{TEM} gene sequences in conventional polymerases. The method is based on selection of a target sequence to be detected within the bla_{TEM} gene in such a way that the chosen sequence is amplified with primers incapable of amplifying contaminating DNA sequences of the polymerase.

1. Introduction

The cogent success of diagnostic methods based on nucleic acid amplification technology has been largely due to its high sensitivity and swiftness in comparison to many traditional detection techniques. The most common amplification technique, polymerase chain reaction (PCR), is widely used for the purpose of multiplication of specific DNA sequences for basic research as well a s for a number of applications, including gene expression analysis, DNA mutation detection, cloning and sequencing. Laboratory diagnostics of infectious disease was one of the first fields to embrace PCR methodology as a powerful and precise diagnostic tool.

The routine use of amplification techniques have accumulated evidence that DNA isolation kits, PCR components, molecular biology grade reagents, and other laboratory supplies used in processing and analysis of DNA could be contaminated with bacterial DNA (Rand and Houck 1990; Carroll et al. 1999; Corless et al. 2000, 2001; Zhang and Fang 2006). With its high affinity for DNA, *Taq* polymerase is particularly prone to contaminating DNA that is presumably derived from the expression vector used for recombinant production of the polymerase and/or genome DNA of the host cells (Rand and Houck 1990; Nogami et al., 1998; Corless et al. 2000; Kulakov et al. 2002). Antibiotic resistance genes (ARG) are the most commonly used as a selective marker in multicopy vectors to express a recombinant protein in *Escherichia coli* (Sambrook and Russell, 2001). Most probably, during the *Taq* polymerase purification process, the DNA containing the ARG is not completely eliminated. Even 'DNA-free' reagents often come with a caveat limiting the quality assurance guarantee to a certain range of organisms, notably the recombinant host for polymerase expression, or a threshold level still above that expected in some clinical samples (Humphrey et al. 2015).

We encountered the problem of exogenous contaminating DNA when a method for identification of ARG sequences in gram-negative bacteria was being developed. Since we used a sensitive analysis based on the low-density oligonucleotide microarray platform (for review, see (Mikhailovich et al. 2008; Gryadunov et al. 2011), it was noticed that the negative (water) controls tested by hybridization to detect bla_{TEM} genes produced positive results. Meanwhile, no similar artifacts were observed if other *bla* genes were analyzed. As our routine laboratory practice includes permanent monitoring of internal cross-contamination, we aimed to discover an alien exogenous source of the false-positive results.

Taking into account that a number of studies have reported the presence of contaminating DNA in commercial *Taq* DNA polymerases (Rand and Houck 1990; Bottger 1990; Schmidt et al. 1991; Hughes et al. 1994; Corless et al. 2000; Newsome et al. 2004; Chiang et al. 2005; Song et al. 2006; Salter et al. 2014), we focused our effort on checking our PCR system ingredients and identification of the origin of

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contamination. Our findings allowed us to suggest a new methodical approach to obtain accurate identification results using standard (not DNA-free) *Taq* DNA polymerases.

2. Materials and methods

2.1. Polymerase samples used in the study

Different lots of three commercially available polymerases: HotStarTaq Plus DNA Polymerase (Qiagen, Chatsworth, CA, USA), HS *Taq* DNA polymerase (Evrogen, Moscow, Russia), and *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania) were used as samples to be investigated. Hereinafter in the study, the tested polymerases are referred to as Pol Q, Pol E, and Pol F, respectively. We used 2.5 units of the tested polymerases to perform reactions in a final 25 µl volume.

2.2. Positive and negative control samples

Genomic DNA isolated from a TEM ß-lactamase producing *E.coli* strain was used as a positive control for PCR reactions (GenBank: CP023387.1 (79087..79947), https://www.ncbi.nlm.nih.gov/nucleotide/CP023387.1). The strain was obtained from the collection of National Medical Research Center for Children's Health, Moscow, Russian Federation. The DNA was isolated using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's manual. DNA-free water (Qiagen) was used as a negative control for PCR reactions.

2.3. Oligonucleotide synthesis and microarray fabrication

Oligonucleotides for immobilization on a microarray and primers for target sequences amplification were synthesized on an automated 394 DNA/RNA synthesizer (Applied Biosystems, Carlsbad, CA, USA). Probes for hybridization contained a spacer with a free amino group 3'-Amino-Modifier C6 CPG 500 (Glen Research, Sterling, VA, USA) for subsequent immobilization in hydrogel. Oligonucleotides were designed using the softwares 'Oligo v. 6.31' (Molecular Biology Insights, Colorado Springs, CO, USA) and 'Bioedit' v. 7.09 (Ibis Biosciences, Carlsbad, CA, USA). The specificity of the resulting oligonucleotides was further examined by BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/). Microarrays were manufactured as described earlier (Rubina et al. 2004). The nucleotide sequences of the primers and probes are shown in Tables 1 and 2. The oligonucleotide probes X, Y, and Z immobilized on the microarray were designed in such a way to detect the majority of variable sequences inside the bla_{TEM} gene variants.

2.4. PCR for hybridization analysis

The PCR for hybridization was performed on a C1000 Touch Thermal Cycler (Biorad, Hercules, CA, USA). The reaction was carried out in a final 25 µl reaction volume containing 2 µl of template DNA, 1 µl (5 pmol/µl) of each forward and reverse primer, 15.3 µl of nuclease free water, 2.5 µl of dNTP mix (2.5 mmol of each dATP, dCTP, dGTP, and dUTP, Sileks, Moscow, Russia), 2.5 µl of 10 × PCR buffer, 0.5 µl of *Taq* polymerase (5 U/µl) and 0.2 µl of fluorescent dye dU49 (Biochip-IMB, Moscow, Russia). The final concentration of Mg^{2+} was 3 mM. The PCR program was initially held for 10 min at 95 °C for polymerase activation, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, followed by 30 cycles of 95 °C for 30 s, 70 °C for 30 s and 72 °C for 30 s, and then 5 min at 72 °C for the final extension step.

Table 1
Primers used for the bla_{TEM} gene fragment* amplification followed by electrophoretic detection.

Name	Sequence 5′→3′
For27	CCT TAT TCC CTT TTT TGC GGC ATT
For254	GGC AAG AGC AAC TCG GTC
Rev856	AAT GCT TAA TCA GTG AGG CAC C
Rev543	AGG CAT CGT GGT GTC AC

* Fragment A was amplified using For27 and Rev856 primers, Fragment B via For27 and Rev543, Fragment C via For254 and Rev543, and Fragment D via For254 and Rev856 primers.

Table 2
Primers and probes used for the bla_{TEM} gene fragment* amplification followed by hybridization analysis.

Name	Sequence 5′→3′
For27	CCT TAT TCC CTT TTT TGC GGC ATT
For254	GGC AAG AGC AAC TCG GTC
HybrRev856	GGC TGT ACG CTG TCA ATG CTT AAT CAG TG AGG CAC C
HybrRev543	GGC TGT ACG CTG TCA GGC ATC GTG GTG TCA C
probe207 (X)	TTT TAA AGT TCT GCT ATG TGG
probe302A (Y)	TTA AGT ACT CAC CAG TCA CAG
probe302G (Z)	TTG AGT ACT CAC CAG TCA CAG

* Fragment A was amplified using For27 and HybrRev856 primers, Fragment B via For27 and HybrRev543, Fragment C via For254 and HybrRev543, and Fragment D via For254 and HybrRev856 primers.

2.5. Hybridization on a microarray

Hybridization was performed by adding 25 µl of the reaction mixture after amplification to 8 µl of solution containing 2.5 M guanidine thiocyanate, 75 mM HEPES pH 7.5, and 7.5 mM EDTA (Sigma-Aldrich, St. Louis, MO, USA). The mixture was infused into a hybridization chamber, and the microarray was incubated for 6 h at 37 °C. After hybridization, the microarrays were washed with distilled water twice for 30 s at 37 °C and dried.

2.6. Image acquisition and processing

Hybridization images were acquired and processed using a fluorescence analyzer setup and specialized software "ImaGeWare" (Biochip-IMB).

2.7. PCR for electrophoretic detection

The PCR was performed on a C1000 Touch Thermal Cycler (Biorad). The reaction was carried out in a final 50 µl reaction volume containing 1 µl of template DNA, 2 µl of each forward and reverse primer (5 pmol/µl), 34 µl of nuclease free water, 5 µl of dNTP mix (2.5 mmol of each dATP, dCTP, dGTP, and dUTP, Sileks), 5 µl of 10 × PCR buffer and 1 µl of *Taq* polymerase (5 U/µl). The final concentration of Mg^{2+} was 3 mM. The PCR program was initially held for 10 min at 95 °C for the polymerase activation, followed by 45 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and then 5 min at 72 °C for the final extension step.

2.8. Electrophoresis

Fifty microliters of PCR product was mixed with 17 µl of 4 × Gel Loading Dye, Blue buffer (Evrogen). Thirty microliters of the mix per well was loaded into 2% agarose gel (with EtBr, 0.5 µg/ml). Electrophoresis was run at 100 V for ~25 min.

2.9. Sequencing

Sequencing was performed using an ABI PRISM® BigDye™ Terminator v. 3.1 Kit (Applied Biosystems) on an automatic sequencer Applied Biosystems 3730 DNA Analyzer (Applied Biosystems).

2.10. Evaluation of copy number by real-time qPCR

The qPCR was performed on a DTlite Real-Time PCR System (DNA-Technology, Moscow, Russia) according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin et al. 2009). The assay was carried out in a final 25 µl reaction volume in Ultra Flux i PCR Strip Tubes (SSI, Lodi, CA, USA) containing 2 µl of template DNA, 1 µl of each forward and reverse primer (5 pmol/µl), 14 µl of nuclease free water, 2.5 µl of dNTP mix (Sileks), 0.25 µl of SYBR Green I (Evrogen), 1.25 µl of DMSO (Evrogen), 2.5 µl of 10× PCR buffer and 0.5 µl of *Taq* polymerase (5 U/µl). The final concentration of Mg^{2+} was 3 mM. The qPCR program was initially held for 10 min at 95 °C for polymerase activation, followed by 45 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and then 3 min at 72 °C for the final extension step. All samples were run in 3 technical replicates. For each reaction, the efficiency of the assay was calculated by using the measured slope of the standard curve ($E = 10^{(-1/slope)} - 1$). A reaction was considered applicable if the slope (−3.1 to −3.6), efficiency (90–110%) and R2 (> 0.99) values were within the recommended range.

Standard curves were acquired by amplification of a ten-fold dilution series of lambda phage DNA (from *E. coli* strain W3110, Sigma-Aldrich), ranging from 10 to 10^4 copies and quantified using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Grand Island, NY, USA). The expected amplicon size was 373 bp; the primer sequences were: forward: 5′- GGA CTC CTC CAC AGA GAA ACA A -3′; reverse: 5′- GTG AAA GAC CTG GGC AAA G -3′.

The assay was run for each *Taq* polymerase and included no template control (NTC) and positive control with bla_{TEM-1} template and primers for bla_{TEM}: forward: 5′- GGC AAG AGC AAC TCG GTC -3′; reverse: 5′- AGG CAT CGT GGT GTC AC -3′. The copy number of the contaminating DNA in NTC for each *Taq* polymerase was evaluated using the software included in the DTlite Real-Time PCR System (DNA-Technology).

3. Results

3.1. Detection of exogenous contaminating DNA in polymerases

During an identification trial of gram-negative isolates associated with nosocomial infections, we repeatedly observed false-positive results when the bla_{TEM} gene sequence was identified. Other target genes belonging to the *bla* family did not produce false-positive results (data not shown). Different lots of three *Taq* DNA conventional polymerases were tested. The results were obtained by PCR followed with electrophoretic detection and by hybridization with three oligonucleotide probes, X, Y, and Z, that had been immobilized in gel pads of a microarray (Table 2).

3.1.1. Identification of bla_{TEM} gene fragments in contaminating DNA by electrophoresis

Four primers were used to amplify contaminating DNA sequences in the analyzed polymerases (see Table 1). The sensitive PCR assays (45 cycles) were performed to reveal all expected contaminating DNA fragments. Sizes and locations of these fragments within the bla_{TEM} gene sequence are shown on the left in Fig. 1. Purified PCR-grade water was added instead of template to amplify exogenous DNA in polymerases (Fig. 2A). A DNA sample containing the bla_{TEM} gene sequence was used as a positive control (Fig. 2B). None of the whole bla_{TEM} gene sequences (Fragment A) were found in all three polymerase samples. Fragments B, C, and D were found in Pol E and Pol Q and were not observed in Pol F. To have reproducible results, each polymerase specimen was replicated in five assays. We chose the most typical product out of these five assays to arrange the electrophoretic picture in Fig. 2.

3.1.2. Detection of bla_{TEM} gene fragments by microarray analysis

More precise results regarding the presence of the bla_{TEM} gene fragments were obtained by hybridization on the microarray. No contaminating DNA was detected when the primers specific to the whole bla_{TEM} gene sequence were used. Fluorescence signals in the gel pads that corresponded to the whole bla_{TEM} gene (Fragment A) did not exceed background signals (row A in Fig. 1). No specific signals were observed when the primers specific to the Fragment D were used to amplify a blank water control with Pol F. Meanwhile, it was found that Pol F was contaminated with other fragments (B and C). All three fragments (B, C, and D) of the bla_{TEM} gene were revealed in both Pol Q and Pol E.

Positive control DNA samples were tested for every polymerase using all four primers pairs. At the bottom of Fig. 1, an example of PCR amplification of positive controls is presented. Line charts reproduced below the hybridization pictures allow estimating fluorescence in the corresponding gel pads.

Thus, identification of contaminating DNA performed with the microarray-based method allowed obtaining more accurate results: fragments B and C in Pol F were detected by hybridization analysis but were not revealed by electrophoretical analysis.

3.2. Sequencing

Since a Fragment C of 290 bp in length was the most commonly encountered contaminating sequence in the analyzed enzymes, this fragment was subjected to Sanger sequencing. The acquired sequences, along with the corresponding sequence of the positive control DNA sample, were aligned with the reference bla_{TEM-1} sequence (GenBank NC_022885.1 (95393.96250)) https://www.ncbi.nlm.nih.gov/nuccore/NC_022885.1. It was found that sequences acquired from Pol Q and Pol E differed in pos. 244 (G > A) relative to the reference one (see Fig. 3). The 244 position polymorphism results in an Ile/Val amino-acid substitution. The sequences were identified using BLAST analysis as bla_{TEM-1} and $bla_{TEM-116}$, respectively, to polymerases Pol Q and Pol E. We did not succeed in sequencing the Fragment C from the Pol F due to the small amount of DNA. Notably, the DNA specimen used in our laboratory as a positive control had nucleotide substitution in pos. 474. Contaminating DNA of polymerases analyzed did not contain the substitutions. This fact could be considered additional and conclusive proof that we did not encounter intra-laboratory cross-contamination.

3.3. Quantification of contaminating DNA fragments in polymerases

The copy number of a contaminating Fragment C in polymerases Pol Q, Pol E, and Pol F were quantified using the qRT-PCR method with ten-fold Lambda phage DNA dilutions as a quantitative reference. Specific primers for Lambda phage were designed to obtain a PCR product roughly matched in size with the target Fragment C. It was found that Pol Q and Pol F contained 10^2–10^3 copies of a contaminating Fragment C per unit of enzyme. Pol E contained 10^3–10^4 copies per unit.

3.4. Strategy to detect bla_{TEM} gene sequences using conventional polymerases contaminated with exogenous bacterial DNA

All conventional *Taq* DNA polymerases that were analyzed in the study contained exogenous DNA, particularly the bla_{TEM} gene sequences. A significant issue was the fact that the polymerases did not contain the complete bla_{TEM} gene but bore its fragments, which varied in length and amount. This peculiarity was utilized to develop a strategy to use standard polymerases contaminated with bacterial DNA for the identification of bla_{TEM} gene fragments in specimens.

Before designing the PCR to detect the bla_{TEM} gene sequence, the performance of some additional preparatory procedures is recommended. At the first stage, the set of specific primers to the bla_{TEM} gene (see Table 1) is used to identify which bla_{TEM} gene fragments are

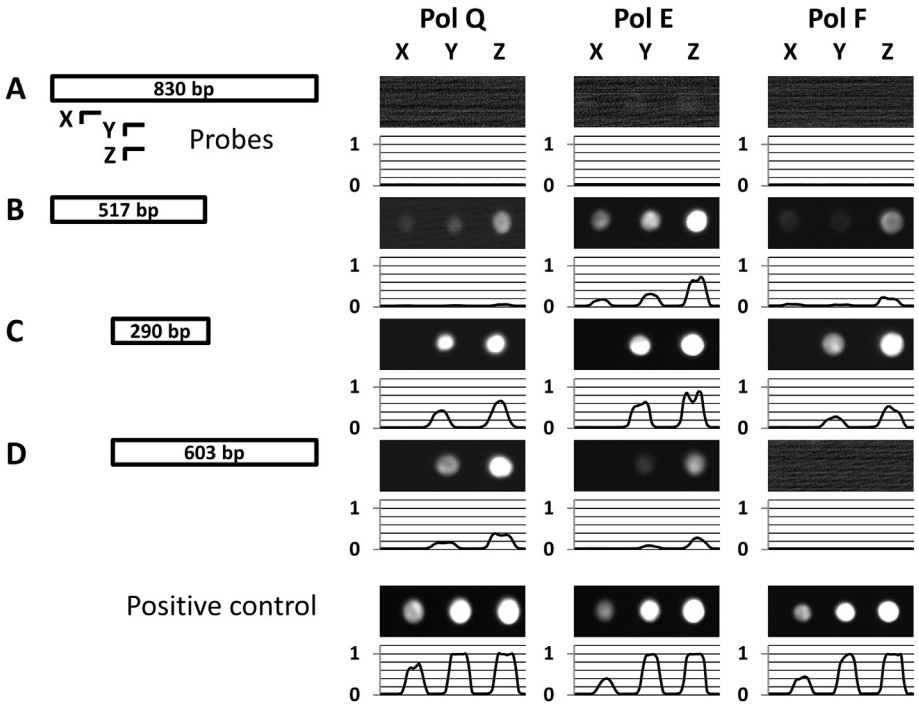

Fig. 1. Microarray-based analysis of contaminating fragments A, B, C, and D in polymerases Pol Q, Pol E, and Pol F. Layout of the fragments and hybridization probes (X, Y, Z) concerning the *bla*$_{TEM}$ gene sequence is shown on the left. Hybridization images of fragments A-D acquired from the tested samples of Pol Q, E, and F are on the right. No DNA templates were added in PCR reactions. Line charts below hybridization pictures indicate fluorescence signal distribution in the corresponding gel pads. Signal intensities were normalized to the maximum detected value. An example of typical hybridization pictures obtained by analysis of a positive control specimen (containing the *bla*$_{TEM}$ gene template) is shown in the lower part.

present in the enzyme as contaminating DNAs. Standard PCR performed with a negative control (a water sample) will produce a number of products in the range from 0 to 4. It is strongly recommended to test at least 3–5 negative controls to ensure that results are accurate. If all four fragments are detected, *e.g.*, by agarose gel electrophoresis, the tested enzyme cannot be utilized, and, conversely, if no specific products are amplified, the polymerase can be used without any limitations.

Indeed, a researcher could reveal 1–3 specific amplification products that correspond to contaminating fragments of the *bla*$_{TEM}$ gene in an analyzed enzyme. (The allegation concerning the numbers is

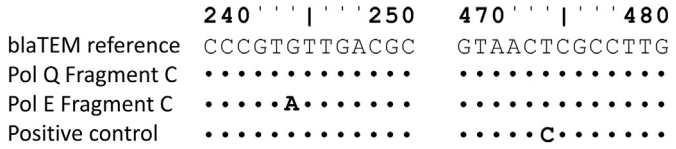

Fig. 3. Alignment of the contaminating Fragment C sequences acquired from Pol Q and Pol E and positive control DNA to the reference *bla*$_{TEM}$ sequence. Sequence of the analyzed fragments had unique nucleotide substitutions and differed from a DNA sequence used as a positive control.

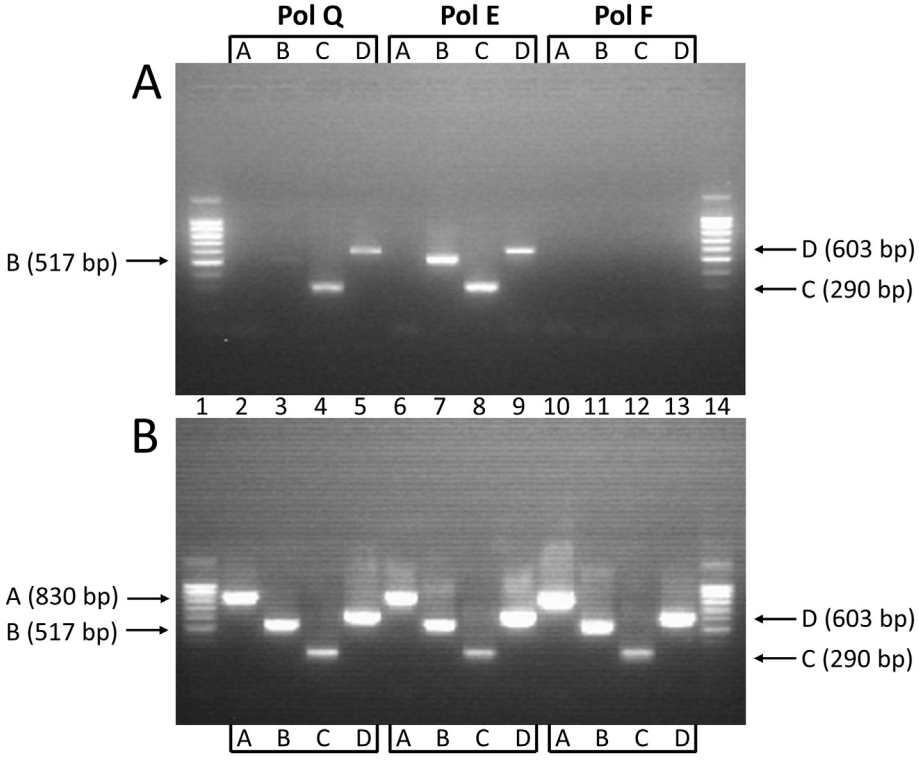

Fig. 2. PCR products amplified from tested polymerase samples Pol Q, Pol E, and Pol F without any template added (A) and positive controls obtained when the *bla*$_{TEM}$ gene template was added to the corresponding polymerase samples (B). Amplification products corresponding to fragments A, B, C, and D of the *bla*$_{TEM}$ gene were obtained with Pol Q (lanes 2–5, respectively), with Pol E (lanes 6–9), and Pol F (lanes 10–13). Lanes 1 and 14 contained a DNA 100 bp ladder. The arrows indicate amplified fragments and sizes.

considered in the Discussion.) Being aware of the contaminating bla_{TEM} gene sequences present in the polymerase, an investigator ought to choose a target for specific amplification (and corresponding primers) outside the fragments identified in the enzyme as contaminating sequences. The same recommendations could also be applied elsewhere, *e.g.*, to selecting specific oligonucleotide probes used in hybridization analysis.

An example of the utilization of the developed strategy was demonstrated in the current study. For instance, any sequence within the bla_{TEM} gene sequence could be chosen as a target to be identified if a researcher performs the standard 30–35 cycles of PCR with Pol F, and the results are detected electrophoretically. No false-positive results should be detected. However, if a researcher applies a more sensitive analysis, the whole Fragment A or Fragment D should be selected as target sequences to design specific primers.

4. Discussion

With their extreme sensitivity, PCR-based techniques are vulnerable to amplifying trace amounts of contaminating nucleic acids, potentially leading to false-positive results. Exogenous bacterial DNA, particularly from recombinant biotechnologically produced reagents such as *Taq* DNA polymerase, are a known limitation for analyzing samples with low bacterial load if highly conserved amplification targets, such as the bacterial 16S rRNA gene, are used for identification (Salter et al. 2014). Similar limitations occur when researchers identify some specific targets, *inter alia*, that are responsible for antibiotic resistance (Chiang et al. 2005; Song et al. 2006).

The impact of false-positive results due to exogenous contaminating DNA can be far-reaching if a sample of nucleic acid is being investigated for clinical diagnostics. This serious problem is often underestimated, and the greatest danger might not be contamination itself but rather ignoring or disregarding it. A striking example of such underestimation has been convincingly demonstrated by Koncan et al. (Koncan et al. 2007). They analyzed results obtained by Y.F., Ding et al. in an article originally published in Chinese (Y.F., Ding et al., 2004). Koncan and colleagues revealed a methodical error in that study which resulted in erroneous identification of the ß-lactamase TEM gene in *Streptococcus pneumonia*. As a result of the misidentification, Chinese researchers published wrong mutations in the bla_{TEM-1} and $bla_{TEM-129}$ genes in the domestic journal, and, moreover, the corresponding sequences were submitted to the GenBank database. R. Koncan and colleagues reasonably assumed that the Chinese researchers had performed PCR-based assays in the absence of adequate negative controls and that the contaminating bla_{TEM} gene sequences in amplification reagents had been neglected.

We have faced the similar problem of contaminating bacterial DNA when the bla_{TEM} gene sequence was permanently found in the negative controls. Since we used hybridization analysis, that is, *a priori*, more sensitive in comparison to agarose gel detection (Ehricht et al. 2006), false-positive fluorescence signals were immediately revealed in gel pads corresponding to the bla_{TEM} gene sequence.

Following the generally accepted practice, we endeavored to assemble our diagnostic tests with relatively inexpensive, preferably domestic-made, reagents, including *Taq* DNA polymerases. This practice provides significant benefits in cost, availability and delivery of components, while the diagnostic features remain sufficiently high.

Regarding to identification of the bla_{TEM} gene sequence, the domestic polymerase (Pol E) did not provide proper specificity. The ways to solve the problem were obvious: one ought to use another enzyme lacking residual bacterial contaminating DNA or containing it in infinitesimal, undetectable amounts.

Several lots of three enzymes produced by reputable manufacturers were tested with negative (water) controls. All these high-grade enzymes did not allow us to obtain pure negative controls: the bla_{TEM} gene contaminating DNA sequences were detected in the analyzed enzymes. We assumed the further large-scale testing of standard polymerases offered by other providers was unproductive, and the expected results would be comparable with those already obtained.

No universal method to completely purify DNA polymerases from contaminating DNA has been established (Corless et al. 2000; Philipp et al. 2010). Removal of DNA contamination from enzymes has been approached by physical, chemical, and enzymatic treatments using UV- and ɣ-irradiation (Deragon et al. 1990; Ou et al. 1991), psoralens with longwave UV light (Jinno et al. 1990; Cimino et al. 1991; Meier et al. 1993), hydroxylamine hydrochloride (Aslanzadeh 1993) or ethidium monoazide treatment (Rueckert and Morgan 2007), restriction endonuclease digestion (Carroll et al. 1999; Mohammadi et al. 2003), ultrafiltration (Mohammadi et al. 2003), and digestion with DNase I (Rochelle et al. 1992; Corless et al. 2000; Eshleman and Smith 2001). These methods have been tested individually, and inconsistent decontamination results have been reported for all of them (Corless et al. 2000; Klaschik et al., 2002; Philipp et al. 2010).

A number of reputed manufacturers produce so called “DNA-free” PCR reagents lacking contaminating bacterial, human, and plasmid DNA. More accurately, these reagents differ from conventional ones in the stringency of their quality control with regard to residual DNA level. Some commercial providers use rather sophisticated procedures, such as closed single-use systems, to purify enzymes from contaminating DNA. It is obvious that these biotechnological intricacies significantly increase the cost of DNA-free reagents. These reagents should no doubt be very useful to perform precise fundamental research, but the cost will impede their extensive introduction into routine clinical diagnostics.

The differences revealed in contaminated DNA sequences in the conventional polymerases inspired us to use this diversity to develop a novel approach that allows a researcher to utilize conventional polymerases for detection of the bla_{TEM} gene fragments without false-positive results.

Hereby, we proposed performing pre-analysis of contaminating DNAs in conventional polymerases using the developed set of primers to identify which fragment(s) of the bla_{TEM} gene sequences they contain. Possessing this information, a researcher could choose a fragment of the target sequence to detect the bla_{TEM} gene that is absent in the enzyme as the contaminating DNA. According to our data, conventional polymerases either do not contain the whole sequence of the bla_{TEM} gene (830 bp) or have it in undetectable quantities. Perhaps the relatively long whole fragment is hydrolyzed into shorter ones during the enzyme purification process. Meanwhile, the shorter fragments that vary in length and quantities remain in the final solution. Hence, we proposed testing at least 3–5 blank samples (with no template) to be certain of whether an enzyme is contaminated with one or more fragment(s).

In conclusion, we proposed the approach to solve the problem of contaminating bacterial DNA in conventional DNA polymerases. The possibility of using inexpensive, standard polymerases to detect the bla_{TEM} gene sequence would encourage the widespread deployment of PCR-based techniques into routine practice in clinical microbiological laboratories.

Acknowledgments

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We have no conflict of interest to declare.

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