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Simultaneous detection of methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by multiplex PCR

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Abstract. A PCR-based assay that can simultaneously detect and differentiate five different types of nosocomial bacterial pathogens was developed. Six pairs of selected primers targeting *femA* (132 bp) and *mecA* (310 bp) of methicillin-resistant *Staphylococcus aureus*, *gltA* (722 bp) of *Acinetobacter baumannii*, *phoA* (903 bp) of *Escherichia coli*, *mdh* (364 bp) of *Klebsiella pneumoniae* and *oprL* (504 bp) of *Pseudomonas aeruginosa* were used in this study. The conditions were optimized for the multiplex PCR to ensure specific amplification of the selected targets. Sensitivity and specificity tests were also carried out using a blind test approach on 50 bacterial cultures and resulted in 100% for both positive and negative predictive values.

INTRODUCTION

Most of the multidrug-resistant bacterial isolates causing nosocomial infections are of public concern worldwide and spread rapidly (Hsueh *et al.*, 2002). Nosocomial infection is an infection that develops in a patient at least forty-eight to seventy-two hours following admission at which the infection agents or its toxins are not present or incubating during the time of admission to the hospital (Emori & Gaynes, 1993). There are various types of nosocomial infections such as wound infections, respiratory infections, genitourinary tract infections and gastrointestinal infections. Immunocompromised patients, the elderly, infants and young children are more susceptible than others since they are usually in direct contact with the hospital staff, other ill patients, incompletely sterilized instruments and the food or water provided at hospital. Most nosocomial infections are caused by aerobic Gram negative bacilli or the Gram positive *Staphylococcus aureus*, which are usually multidrug resistant and contribute significantly to the patients' morbidity and mortality in the intensive care unit (ICU) (Potgieter, 1990). Five types of nosocomial pathogens namely, methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were targeted in the development of an easy one step multiplex PCR detection assay.

MRSA is a Gram positive bacterium and responsible for a large proportion of nosocomial infections that makes treatment

difficult due to the increasing resistance to multiple antibiotics (Hassanain *et al.*, 2009). It causes both nosocomial and community-acquired infections including endocartiditis, osteomycelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles (Geha *et al.*, 1994; Savitha *et al.*, 2006). Methicillin resistance in staphylococci is mediated by the over-production of PBP2a, a protein called penicillin-binding protein (78kDa) with low affinity for β-lactam antibiotics. The *mecA* gene is the structural determinant for methicillin resistance that encodes for PBP2a and therefore used as a molecular marker of putative MRSA (Geha *et al.*, 1994; Towner *et al.*, 1998; Menon & Nagendra, 2001; Perez-roth *et al.*, 2001). *Acinetobacter baumannii* is ubiquitous in the hospital environment, has simple growth requirements and high tolerance of environmental conditions (Marcos *et al.*, 1995; Chang *et al.*, 2009). It is a Gram negative, aerobic, non-glucose fermenting and oxidase negative bacteria and opportunistic pathogen that contributes to large proportions of nosocomial infections (Chang *et al.*, 2009). Both *E. coli* and *P. aeruginosa* have emerged as important nosocomial pathogens in intensive care units (ICUs) and cause both community and nosocomial urinary tract infection (UTI) (Blot *et al.*, 2003; Safa *et al.*, 2006; Bean *et al.*, 2008; Ding *et al.*, 2009;). It has been reported that hospital equipments and utensils are reservoirs for *P. aeruginosa* (Curran *et al.*, 2004). *Pseudomonas aeruginosa* infections are also commonly found in burn patients, mechanically ventilated patients and cystic fibrosis (CF) patients.

Klebsiella pneumoniae is also an opportunistic, Gram negative bacteria responsible for up to 10% of nosocomial infections (Prathiba *et al.*, 2004; Diancourt *et al.*, 2005; Ko *et al.*, 2008). It has been reported that *Klebsiella* species can grow rapidly in poor environments, particularly in a low nutrition and slightly damp environment. Consequently, outbreaks of *K. pneumoniae* infection often occur since they may contaminate the food and infusion fluids (Sardan *et al.*, 2004).

In general, many monoplex or duplex PCR tests have been established for species identification for some of these nosocomial bacteria (Menon & Nagendra, 2001; Jonas *et al.*, 2002; Theodore *et al.*, 2004; Safa *et al.*, 2006). However, a conventional single step multiplex PCR for these nosocomial pathogens has yet to be reported although there are published reports on multiplex approach using real-time PCR (Negar *et al.*, 2007). The reagents used for the later approach are more costly and the approach is only applicable to those laboratories that are equipped with real-time thermal cycler. Therefore, the objective of the study was to develop a conventional cost effective single step multiplex PCR for the simultaneous detection of MRSA, *A. baumannii*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. These multidrug resistant bacterial pathogens were selected for study because they are the most common causes of nosocomial infections in Malaysia. Multiplex PCR is a complicated technique that requires further optimization in the concentrations of *Taq* DNA polymerase, $MgCl_2$, dNTPs, and primers, and PCR cycling parameters such as annealing temperature (Henegariu *et al.*, 1997). Following PCR optimization, the sensitivity and specificity of the multiplex PCR were evaluated by blind-tests.

MATERIALS AND METHODS

Bacterial strains and multiplex PCR detection system development

Twenty five known nosocomial bacteria were used in this study. The bacterial strains used were MRSA (n=5), *A. baumannii* (n=5), *E. coli* (n=5), *P. aeruginosa* (n=5) and *K. pneumoniae* (n=5). All the strains were retrieved from the culture collection of Laboratory of Biomedical Science and Molecular Microbiology, Institute of Graduate Studies, University of Malaya. Initially, a monoplex PCR was carried out for each of the

selected primers to determine its specificity, followed by intensive optimization to develop a multiplex PCR detection assay.

Initially 13 sets of primers (MecA, FemA, GltA, Cpn, RecA, Gdh, Gpi, PhoA, UspA, GroES, OprL, GyrB and Mdh) that target the 5 different bacterial species were selected based on thorough literature search for particular bacterial house-keeping genes or resistant determinants (Table 1). The primer pairs were checked by BLAST search (http://www.ncbi.nih.gov) to minimize the likelihood of nonspecific amplification from non target loci. The selected primers were then tested through *in-silico* PCR (http://insilico.ehu.es/PCR/) as described by Teh *et al*. (2009) for prediction of amplicons. Overall, the sizes of the PCR amplicons and the sequences are listed in Table 1.

DNA extraction

DNA template from Gram negative bacteria (*A. baumannii*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*) was prepared by boiling method as previously described (Teh *et al*., 2008) while lysostaphin-lysis method was used to prepare DNA template from MRSA. The procedures for lysostaphin-lysis method were the same as boiling method except for the addition of lysostaphin (1 mg/ml). Following that, the DNA template was quantitated by using a biophotometer

Table 1. Primers tested in *in-silico* PCR

Target gene	Primers	Sequence (5' to 3')	Amplicon size (bp)	Reference
femA	**FemA-F**	**AAAAAAGCACATAACAAGCG**	**132**	**Mehrotra *et al.*, 2000**
	FemA-R	**GATAAAGAAGAAACCAGCAG**		
mecA	**MecA-F**	**GTAGAAATGACTGAACGTCCGATAA**	**310**	**Geha *et al.*, 1994**
	MecA-R	**CCAATTCCACATTGTTTCGGTCTAA**		
gltA	**GltA-F**	**AATTTACAGTGGCACATTAGGTCCC**	**722**	**Bartual *et al.*, 2005**
	GltA-R	**GCAGAGATACCAGCAGAGATACACG**		
cpn 60	Cpn-F	ACTGTACTTGCTCAAGC	479	Bartual *et al.*, 2005
	Cpn-R	TTCAGCGATGATAAGAAGTGG		
recA	RecA-F	CCTGAATCTTCYGGTAAAAC	425	Bartual *et al.*, 2005
	RecA-R	GTTTCTGGGCTGCCAAACATTAC		
gdhB	GdhB-F	GCTACTTTTATGCAACAGAGCC	775	Bartual *et al.*, 2005
	GdhB-R	GTTGAGTTGGCGTATGTTGTGC		
gpi	Gpi-F	AATACCGTGGTGCTACGGG	508	Bartual *et al.*, 2005
	Gpi-R	AACTTGATTTTCAGGAGC		
phoA	**PhoA-F**	**GTGACAAAAGCCCGGACACCATAAATGCCT**	**903**	**Kong *et al.*, 1999**
	PhoA-R	**TACACTGTCATTACGTTGCGGATTTGGCGT**		
uspA	UspA-F	CCGATACGCTGCCAATCAGT	884	Osek, 2001
	UspA-R	ACGCAGACCGTAAGGGCCAGAT		
oprL	**OprL-F**	**ATGGAAATGCTGAAATTCGGC**	**504**	**Daniel *et al.*, 1997**
	OprL-R	**CTTCTTCAGCTCGACGCGACG**		
groES	GroES-F	ATGAAGCTTCGTCCTCTGCAT	536	Clarke *et al.*, 2003
	GroES-R	GTCTTTCAGCTCGAT		
mdh	**Mdh-F**	**GCGTGGCGGTAGATCTAAGTCATA**	**364**	**Sun *et al.*, 2007**
	Mdh-R	**TTCAGCTCCGCCACAAAGGTA**		
gyrB	GyrB-F	GCGCGACGGCAAAGAAGA	233	Sun *et al.*, 2007
	GyrB-R	GGAAGCCGGCGAGGTGAG		

Primers selected for multiplex PCR optimisation and analysis are in bold

(Eppendorf, Germany) and 2 µl of supernatant (≈100 ng of DNA template) was used for PCR reactions.

Multiplex PCR

A multiplex PCR was performed in a final volume of 25 µl containing 100 ng of DNA template, 1X of buffer, 0.3 µM of each GltA, PhoA, OprL and Mdh primers, 0.5 µM of MecA primer, 0.4 µM of FemA primer, 200 µM of dNTP mix, 1.5 mM of $MgCl_2$ and 1U of *Taq* DNA polymerase (Promega, USA). The multiplex PCR cycling parameters consisted of denaturation at 95ºC for 5 min, followed by 30 cycles of 96ºC for 1 min, 52ºC for 30 sec, 72ºC for 1 min and a final extension at 72ºC for 10 min. The PCR products were resolved on a 2.0 % (w/v) agarose gel stained with ethidium bromide, visualized under UV and analysed using a Gel Doc system (BioRad, CA, USA).

DNA sequence analysis of amplicons

PCR amplicons generated by the different primer sets were sequenced to validate their identities. In general, PCR products were purified using DNA purification kit (iNtRON Biotechnology, Korea) according to the manufacturer's protocol and sequenced using ABI PRISM Big Dye terminator cycle sequencing system (Applied Biosystems). The DNA sequences obtained were then compared with the GenBank database using BLAST (http://www.ncbi.nih.gov).

Further evaluation of optimized multiplex PCR and Blind Test

The optimized multiplex PCR assay was evaluated with 118 known bacterial cultures (Table 2). These strains included: MRSA (n=20), *A. baumannii* (n=13), *E. coli* (n=20), *P. aeruginosa* (n=20) and *K. pneumoniae* (n=16), *Salmonella* spp. strains (n=20), *Vibrio* spp. (n=4) and *Listeria* spp. (n=5). To further validate the sensitivity and specificity of the optimized multiplex PCR, a blind test on 50 known bacterial cultures as shown in Table 3 was carried out.

RESULTS

Outcome of *in-silico* PCR

From the result of evaluation of primer sequences through BLAST and *in-silico* PCR programme, the selected target genes for the bacterial pathogens are shown in Table 1. For detection of MRSA, MecA and FemA primers were chosen. For detection of *A. baumannii*, the GltA primer was selected since other primers were not specific: Cpn60 primer could bind to the genome of other organisms such as *Vibrio* spp., RecA primer bound to the genome of *Pseudomonas fluorescens* strain 2-79, GdhB primer bound to *Vibrio cholerae* M66-2 chromosome I and Gpi primer bound to *Escherichia coli* IHE3034 genome.

Table 2. Strains used for evaluation of multiplex PCR

Organism	Total no. of strains	No. of strains tested positive					
		mecA	***femA***	***gltA***	***phoA***	***oprL***	***mdh***
MRSA	20	20	20	–	–	–	–
A. baumannii	13	–	–	13	–	–	–
E. coli	20	–	–	–	20	–	–
P. aeruginosa	20	–	–	–	–	20	–
K. pneumoniae	16	–	–	–	–	–	16
Salmonella spp.	20	–	–	–	–	–	–
Vibrio spp.	4	–	–	–	–	–	–
Listeria spp.	5	–	–	–	–	–	–
Total	**118**	**20**	**20**	**13**	**20**	**20**	**16**

For *E. coli*, the target of choice was *phoA* gene instead of *uspA* gene as there was no amplification for *uspA* gene in *in-silico* PCR (data not shown).

OprL primer was selected to amplify the target gene of *P. aeruginosa* as the GroES primer showed 100% similarity to *P. fluorescence* pfo-1. Mdh primer was selected to amplify the target gene of *K. pneumonia*e as the GyrB primer was not specific to *K. pneumonia* since it also amplified *gyrB* gene from *Salmonella* spp. in *in-silico* PCR (data not shown).

Each selected primer pairs was individually tested to determine the specificity. All the tested primers were specific for the targeted organisms as the expected genes were amplified. To validate the amplicons, DNA sequence analysis of the representative amplicons produced by each primer pair was carried out and the results showed high identity ranging from 98% to 100% to the available GenBank sequences (data not shown). Hence, the specificity of the primers was determined.

A multiplex PCR using all the selected primer sets was then optimized for each parameter. Initially, the optimum magnesium concentration was determined at 1.5 mM. Following that, the optimum annealing temperature of multiplex PCR was determined at 52.0°C. The concentrations of the primers were also adjusted to produce an equal amount of amplicons. Overall, the optimum concentrations for the 6 pairs of primers are as mentioned earlier in Materials and Methods.

Figure 1 indicates a representative agarose gel of multiplex PCR after optimization. The target gene for each organism was identified by its specific amplicons: MRSA (132 bp and 310 bp which represent *femA* gene and *mecA* genes, respectively.), *K. pneumoniae* (364 bp which represents *mdh* gene), *P. aeruginosa* (504 bp which represents *oprL* gene), *A. baumannii* (722 bp which represents *gltA* gene) and *E. coli* (903 bp which represents *phoA* gene).

The optimized multiplex PCR was evaluated with 118 known bacterial strains (Table 2). In this panel of bacterial strains, 89 strains (MRSA n=20, *A. baumannii* n=13, *E. coli* n=20, *P. aeruginosa* n=20, *K. pneumoniae* n=16) gave true positive results while the remaining bacteria isolates (*Salmonella* spp., n=20, *Vibrio* spp. n=4, *Listeria* spp. n=5) gave true negative results.

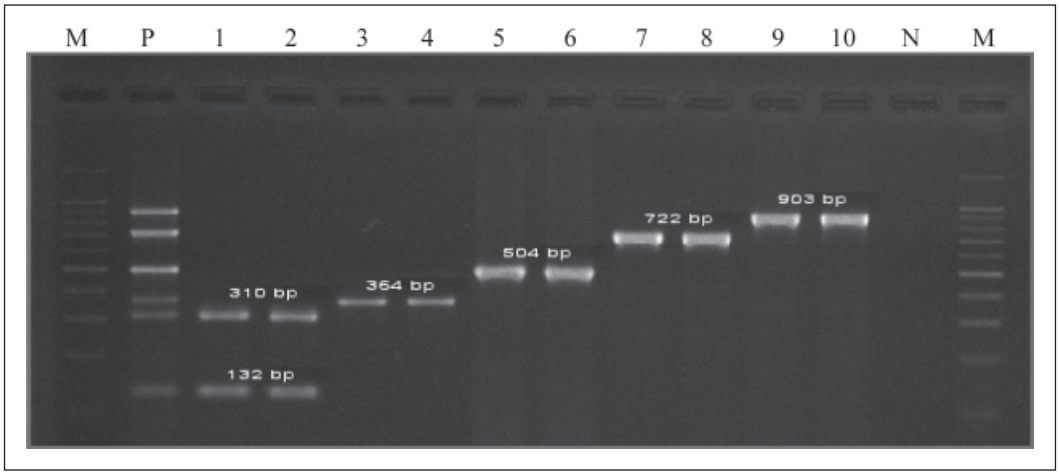

Figure 1. Multiplex PCR result for nosocomial bacterial pathogens. The desired amplification band for all of the strains was obtained, at which *femA* (132 bp) and *mecA* (310 bp) for MRSA, *gltA* (722 bp) for *A. baumannii*, *phoA* (903 bp) for *E. coli*, *oprL* (504 bp) for *P. aeruginosa* and *mdh* (364 bp) for *K. pneumoniae*. Lanes: M, Molecular size marker (100-bp DNA ladder); P, Positive control; N, Negative control; lanes 1-2, MRSA strains; lanes 3-4, *K. pneumoniae* strains; lanes 5-6, *P. aeroginosa* strains; lanes 7-8, *A. baumannii*; lanes 9-10, *E. coli* strains

Fifty known bacterial cultures (Table 3) were blind-coded and tested with the optimized multiplex PCR. In the panel of strains, 21 strains yielded true positive result while remaining yielded true negative result. The PCR data was then compared with the identity of the strains and the result showed that this multiplex PCR enabled to differentiate the five types of nosocomial pathogens from other microorganisms.

The multiplex PCR proved 100% sensitivity and specificity in this case in detecting all the tested true strains. Both positive and negative predictive values of the multiplex PCR were 100%.

DISCUSSION

PCR plays an important role as a powerful tool in clinical microbiology studies and has been widely applied to detect bacteria and genes of interest (Towner *et al*., 1998; Mehrotra *et al*., 2000; Perez-roth *et al*., 2001; Jonas *et al*., 2002; Amghalia *et al*., 2009). In this study, a multiplex PCR was developed to simultaneously detect and differentiate 6 targeted genes of 5 different nosocomial pathogens namely *mecA* and *femA* of MRSA, *gltA* of *A. baumanii*, *phoA* of *E. coli*, *oprL* of *P. aeruginosa* and *mdh* of *K. pneumoniae*.

To obtain good PCR results, a minimum amount of relatively good quality DNA template was needed. Overall, DNA extraction from Gram negative bacteria (*A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*) and Gram positive bacteria (MRSA) was different due to the difference in the cell wall composition. For Gram negative bacteria, the thin cell wall could be lysed by direct boiling the cells to release the genomic DNA but this method was not applicable to Gram positive bacteria such as MRSA due to their thick layer of peptidoglycan. An addition of specific enzymes such as achromopeptidase or lysostaphin was needed (Japoni *et al*., 2004). Therefore, DNA extraction of Gram negative bacteria and Gram positive bacteria in this study were carried out by two different methods, ie: direct boiling and lysostaphin-lysis method, respectively. In cases of an unknown bacterial isolate, lysostaphin-lysis approach followed by boiling method can be used to prepare the DNA template for PCR. On the other hand, future work will also be carried out to evaluate the DNA extraction procedures directly from clinical specimens.

The choice of PCR targets and primers were very important in the development of this multiplex PCR. The chosen genes should be present all the time in the particular bacteria and the sizes of amplicons should not overlap as that would complicate the detection analysis. Based on this premise, *mecA* and *femA* genes were selected for identification of MRSA, *gltA* gene for *A. baumannii*, *phoA gene for E. coli*, *oprL* gene for *P. aeruginosa* and *mdh* gene for *K. pneumonia* since the sizes of each of these targets were discernable (Figure 1).

The *mecA* gene is unique for MRSA (Hassanain *et al*., 2009) and is the primary structural requirement for methicillin resistance in staphylococci (Geha *et al*., 1994; Towner *et al*., 1998). In this study, the detection of *mecA* gene of MRSA was based on MecA primer previously described by Geha *et al*. (1994). The size of the amplicon was 310 bp. This primer has been used by other researchers (Perez-roth *et al*., 2001; Jonas *et al*., 2002; Wang *et al*., 2009).

femA gene is universally present only in *S. aureus* and is implicated in cell wall metabolism and pentaglycine-side chain formation (Judith *et al*., 2007; Hassanain *et al*., 2009). Hence, *femA* gene is a useful marker for confirmation of *S. aureus*. However, methicillin-sensitive *S. aureus* (MSSA) also harbor *femA* gene. Therefore, MRSA isolates were determined when genes, *mecA* and *femA* were simultaneously amplified (Mehrotra *et al*., 2000) and these primers have been validated by other researcher (Savitha *et al*., 2006).

Table 3. Fifty bacterial strains used for blind testing to evaluate the efficiency of mPCR

SAMPLE	PCR	IDENTITY
Sample 01	504 bp (PA)	*P. aeruginosa*
Sample 02	132 bp (MSSA)	Methicillin-sensitive SA (MSSA)
Sample 03	903 bp (EC)	*E. coli*
Sample 04	132 bp + 310 bp (MRSA)	MRSA
Sample 05	No amplification	*Vibrio cholerae*
Sample 06	132 bp (MSSA)	MSSA
Sample 07	No amplification	*S. typhi*
Sample 08	504 bp (PA)	*P. aeruginosa*
Sample 09	132 bp + 310 bp (MRSA)	MRSA
Sample 10	132 bp (MSSA)	MSSA
Sample 11	364 bp (KB)	*K. pneumoniae*
Sample 12	No amplification	*S.enteritidis*
Sample 13	No amplification	*Vibrio* spp.
Sample 14	No amplification	*S. enteritidis*
Sample 15	722 bp (AC)	*A. baumannii*
Sample 16	903 bp (EC)	*E. coli*
Sample 17	903 bp (EC)	*E. coli*
Sample 18	132 bp (MSSA)	MSSA
Sample 19	No amplification	*S. enteritidis*
Sample 20	No amplification	*Listeria* spp.
Sample 21	364 bp (KB)	*K. pneumoniae*
Sample 22	132 bp (MSSA)	MSSA
Sample 23	No amplification	*S.typhi*
Sample 24	No amplification	*S. enteritidis*
Sample 25	903 bp (EC)	*E. coli*
Sample 26	504 bp (PA)	*P.aeruginosa*
Sample 27	No amplification	*S. enteritidis*
Sample 28	364 bp (KB)	*K. pneumoniae*
Sample 29	No amplification	*S. enteritidis*
Sample 30	364 bp (KB)	*K. pneumoniae*
Sample 31	No amplification	*S. enteritidis*
Sample 32	722 bp (AC)	*A. baumannii*
Sample 33	No amplification	*S.typhi*
Sample 34	No amplification	*Listeria* spp.
Sample 35	132 bp (MSSA)	MSSA
Sample 36	No amplification	*Vibrio* spp.
Sample 37	722 bp (AC)	*A. baumannii*
Sample 38	364 bp (KB)	*K. pneumoniae*
Sample 39	No amplification	*S. typhi*
Sample 40	504 bp (PA)	*P. aeruginosa*
Sample 41	No amplification	*S. typhi*
Sample 42	132 bp (MSSA)	MSSA
Sample 43	132 bp (MSSA)	MSSA
Sample 44	No amplification	*Listeria* spp.
Sample 45	132 bp (MSSA)	MSSA
Sample 46	No amplification	*S. enteritidis*
Sample 47	722 bp (AC)	*A. baumannii*
Sample 48	504 bp (PA)	*P. aeruginosa*
Sample 49	722 bp (AC)	*A.baumannii*
Sample 50	No amplification	*Vibrio cholerae*

For *A. baumannii*, *gltA* gene (encodes for citrate synthase) was used as the target gene. The GltA primer was adopted from Bartual *et al*. (2005) and size of the generated amplicon was 722 bp. Bartual *et al*. (2005) reported a development of MLST for characterization of clinical isolates of *A. baumannii* and seven housekeeping genes used as the targets *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD*. Each of the genes (*gltA*, *gdhB*, *recA*, *cpn60*, and *gpi*) was evaluated using *in-silico* PCR amplification and BLAST programmes. Only *gltA* gene was found to be the most suitable target gene for *A. baumannii* in this study. *gyrB* was not selected as target since the amplicon generated by GyrB primer (909 bp) overlapped with the 903 bp generated by PhoA primer. Also, *rpoD* was not selected in our study as the length of the forward and reverse primer sequences were too long (54 bases) and this would cause a lot of unspecific bands (Burpo, 2001).

In this study, *phoA* gene was used as the target for detecting *E. coli* as this has been proven to be a universal marker (Kong *et al*., 1999). Our previous studies have demonstrated the stability and usefulness of *pho*A for identification of *E. coli* (Rathi *et al*., 2009, Yu & Thong, 2009). In this study, we were able to correctly amplify the *phoA* gene at 903 bp as reported in the literature.

To detect *P. aeruginosa*, the *oprL* gene (encodes for membrane lipoprotein) was selected since it has been reported to be conserved in *P. aeruginosa* (Daniel *et al*., 1997).

To develop a multiplex PCR, optimization in the quantitities of $MgCl_2$, dNTPs, primers and annealing temperature was carried out to avoid unspecific amplification and to ensure that each of the selected targets was correctly amplified at about equal intensity. From all the results obtained, the best amplification conditions for this multiplex PCR were 1.5 mM $MgCl_2$, 200 µM dNTPs and the annealing temperature was 52°C. The validity of the amplicons was confirmed by the DNA sequencing analysis.

Although most of the conventional culture methods are still useful and valid, rapid methods for the identification of bacterial pathogens such as PCR become an inevitable goal for medical microbiology laboratories. Conventional culture and biochemical tests would take at least 5 days for confirmation of positive cultures as opposed to 5 hours using this method. Rapid diagnostics are really important to patients for appropriate treatment and reduce the hospitalization and medical cost. There are many advantages of using this multiplex PCR system. The technique is rapid since it could be used to detect and differentiate five types of nosocomial bacterial pathogens simultaneously compared to a monoplex PCR. However, the multiplex PCR developed in this study was only evaluated with pure cell cultures. Further work is therefore needed to directly apply this approach to clinical specimens. This multiplex PCR is also easily established in laboratories including medical and food laboratories as long as the laboratory is equipped with a PCR machine. In addition, the overall preparation process is not tedious and low cost since lysostaphin-lysis and direct boil method are being used for DNA extraction from cell culture.

In conclusion in this study, we have demonstrated development of a conventional multiplex PCR for simultaneous detection of 5 different types of nosocomial pathogens and further confirmed its sensitivity and specificity.

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