

## Full Length Research Paper

# A new PCR strategy for multilocus sequence typing of methicillin-resistant *Staphylococcus aureus*

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**A single PCR protocol for methicillin-resistant *S. aureus* (MRSA) housekeeping genes, carbamate kinase, shikimate dehydrogenase, glycerol kinase, guanylate kinase, phosphate acetyltransferase, triosephosphate isomerases, and acetyl coenzyme-A acetyltransferase for multilocus sequence typing was developed. This PCR strategy provides a simple, rapid and cost effective amplification of the multiple loci of MRSA, with better quality data.**

**Key words:** Methicillin-resistant *Staphylococcus aureus*, PCR, multilocus sequence typing.

## INTRODUCTION

In last 60 years, *Staphylococcus aureus* has become truly a global challenge due to its high rate of morbidity and mortality. Besides infecting 20 to 30% of human population (van Belkum et al., 2009), it causes diseases ranging in severity from minor skin infection to life threatening conditions such as endocarditis, pneumonia, soft tissue infections, osteomyelitis, food poisoning and toxic shock syndrome (Monecke et al., 2011). Thus, it is better to refer to it as a *well-armed pathogen* rather than an *opportunistic pathogen*.

Numerous molecular typing methods have been designed to investigate the local and global epidemiology of *S. aureus*. Undoubtedly, Multilocus Sequence Typing (MLST) provides "gold standard" for long term population studies and for determining genetic relationships (Maiden et al., 1998). A single setup of PCR for seven housekeeping genes carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme-A acetyltransferase (*yqiL*) can greatly facilitate the design of MLST scheme enabling the efficient identification of candidate allelic profile from sequencing. Different PCR

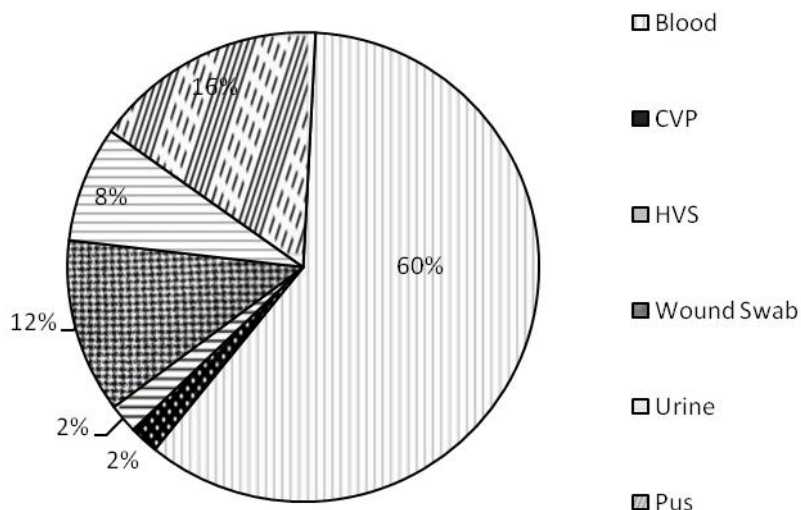
conditions for each locus are not only time consuming but also labor-intensive (Enright et al., 2000). Many times it has been noticed that amplification protocol is revised for an allele of different strains by lengthy reoptimization and redesigning of primers (Crisóstomo et al., 2005). These approaches generally increase the cost of MLST which is already an expensive technique.

This poses need for an efficient protocol that would be universally applicable for the amplification of seven housekeeping genes and that must be cost effective, less time consuming and encountering all the concerns related to MLST. To resolve this issue, the present study was designed to develop and validate a new PCR strategy that may exploit single PCR run for multiple loci of *S. aureus* to produce better quality data.

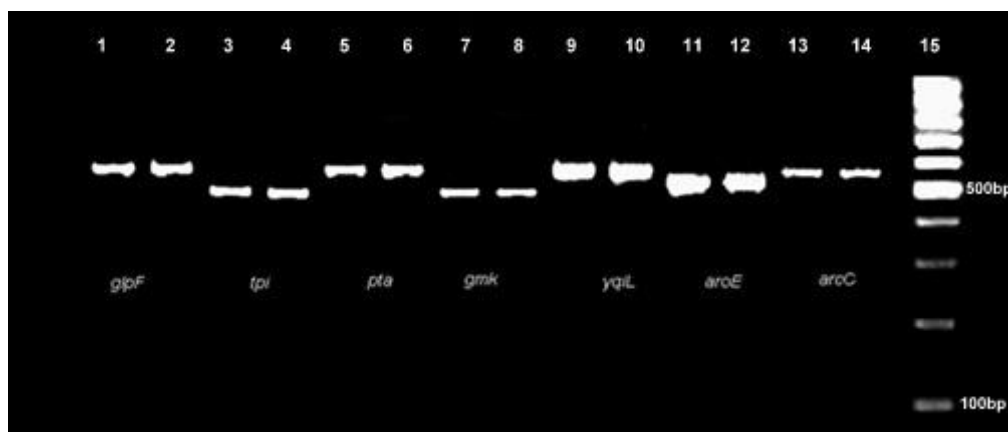
## MATERIALS AND METHODS

To establish the touch-down PCR (TD-PCR) strategy, a total of 50 non-duplicated, clinical *S. aureus* isolates were collected from local tertiary care hospitals in Karachi, Pakistan. The strains were isolated from a variety of clinical sites (Figure 1). All isolates were characterized biochemically. MRSA were determined using Kirby-

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**Figure 1.** Isolates from different clinical sites including blood, pus, wound swab, urine, HVS and CVP.



**Figure 2.** The seven housekeeping genes amplified by using touchdown PCR approach.

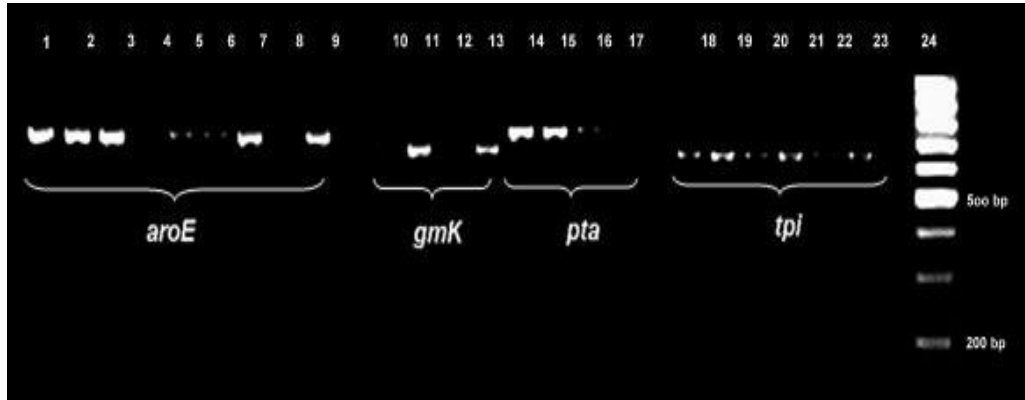
bauer disc diffusion in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006 and 2007). DNA was extracted from *S. aureus* isolates using MasterPure™ Gram Positive DNA purification protocol (Epicentre Biotechnologies Cat. MGP04100). The seven housekeeping genes *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL* were amplified by using conventional PCR method and specific primers as reported earlier (Enright et al., 2000).

The new TD-PCRs protocols were carried out with 50 µl reaction volumes containing 0.5 µl of chromosomal DNA (approximately 0.5 µg), 0.5 µg of each primer (7), 5 µl of 10X buffer (50 mM KCl, 15 mM Tris HCl, pH 8.8), 0.2 mM of deoxynucleotide triphosphate (Gene DireX, Cat. DN001025), 2.5 µg of MgCl<sub>2</sub> and 1 U of *Taq* polymerase (GeneAid, Cat. TQ251). The PCR cycling conditions were: initial denaturation at 94°C for 5 min followed by 10 cycles of 1 min each at 94, 55, 72°C and other 25 cycles of 1 min each at 94, 52 and 72°C with final extension of 10 min at 72°C. Aliquots of 5 µl PCR products were loaded onto agarose gel (2% agarose, 1X Tris-buffered EDTA; 100 V for 60 min). The gels were stained with 0.5 µg/ml ethidium bromide.

## RESULTS AND DISCUSSION

Amplification in a single PCR setup produced distinct bands, corresponding to their respective molecular sizes that were recognizable in stained agarose gels. Explicit and high yield products were obtained by using newly developed TD-PCR approach. In addition, two different cycles of annealing temperature were adjusted so that the aforementioned primers (Enright et al., 2000) could be used for all *S. aureus* MLST loci. Each allele of all *S. aureus* strains were amplified satisfactorily by using new TD-PCR strategy (Figure 2).

The characterization of pathogenic isolates plays an essential role in the epidemiology of infectious diseases, generating the necessary information for identification, tracking, and intervening against disease outbreaks. MLST has been applied to all the major human bacterial



**Figure 3.** Conventional PCR for MLST of *S. aureus*. Lane 1, 2, 3, 7, 9 for *aroE*; lane 11, 13 for *gmK*; lane 14, 15 for *pta*, lane 18, 19, 21 for *tpi* (gave high yield product). Lanes, 4, 8, 10, 12, 17, 22 were not amplified and lane 5, 6, 16, 20, 23 gave very low yield PCR products. Lane 24; 100 bp ladder.

pathogens, and many new schemes for pathogenic and environmental taxa are in development (Turner et al., 2007). In a large number of studies, the molecular characterization of *S. aureus* in different regions of the world has been conducted primarily by RT-PCR, pulsed-field gel electrophoresis (PFGE) and plasmid analysis, which showed the existence of internationally disseminated clones, reflecting epidemiological and geographical relatedness. The ability to correctly identify the *S. aureus* strains is important for epidemiological understanding and decision making in public health (Peterson et al., 2012; Carmo et al., 2011; D'Souza et al., 2010).

For MLST studies, the amplification of seven housekeeping genes was performed using conventional PCR strategies as described earlier (Enright et al., 2000). In earlier studies, the conventional PCR and/or RT-PCR strategies have failed to produce high yield for all the seven housekeeping genes with complete allelic profile in a single PCR run, using similar PCR conditions and set of primers. The earlier researchers need to design some alternative pairs of primers, due to the possible alterations in the binding sites of reporter primers. These studies found inconsistent PCR conditions along with intra-assay variations for all seven housekeeping genes (Crisóstomo et al., 2005; Berglund et al., 2005; Huletsky et al., 2005).

During this study, a new TD-PCR strategy was developed and validated successfully that amplified all the seven housekeeping genes of MRSA strains. Prior to the development of TD-PCR approach, conventional PCR was used to amplify seven housekeeping genes. Few strains were amplified with high yield of product whereas, some strains were found to have very low PCR product. On the other hand, a group of strains were not amplified (Figure 3).

To address applicability and validity, we further applied our PCR strategy to amplify about 350 reactions of *arcC*, *aroE*, *glpF*, *gmK*, *pta*, *tpi* and *yqiL* by following TD-PCR.

Products accuracy was also endorsed by sequence analysis of all samples.

## Conclusion

This study concludes the establishment of an efficient, cost effective and rapid amplification protocol that would be universally applicable for the amplification of seven housekeeping genes of *S. aureus* encountering all the concerns related to MLST. This TD-PCR strategy needs single PCR run for the amplification of the multiple loci of MRSA, and produces better quality data in lesser time.

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