Journal of Microbiological Methods 84 (2011) 144–146

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Development of a modified DNA extraction method for pulsed-field gel electrophoresis analysis of *Staphylococcus aureus* and enterococci without using lysostaphin

Mohammad Mehdi Feizabadi [a,*], Arash Ghodousi [a], Bizhan Nomanpour [a], Maryam Omrani [a], Fereshteh Shahcheraghi [b]

[a] Department for Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Bacteriology, Pasteur Institute of Iran, 12th of Farvardin Street, Tehran, Iran

ARTICLE INFO

Article history:
Received 23 July 2010
Received in revised form 25 October 2010
Accepted 5 November 2010
Available online 13 November 2010

Keywords:
Pulse-field gel electrophoresis(PFGE)
MRSA
VRE
Nosocomial infections

ABSTRACT

A modified pulsed-field gel electrophoresis (PFGE) protocol was developed and applied to clinical isolates of *Staphylococcus aureus* and enterococci to reduce the cost of using lysostaphin. This protocol reduces the expenses of PFGE typing of *S. aureus* and enterococci as it removes the use of lysostaphin during the spheroplast formation from these bacteria.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) are pathogens often associated with nosocomial infections (Emori and Gaynes, 1993; Engemann et al., 2003; Schramm et al., 2006). Nosocomial infections have a tremendous impact on the morbidity, mortality and costs of hospitalization. Annually, two million patients are estimated to develop nosocomial infections in the United States (Richards et al., 1999).

Molecular typing methods are used to study the outbreak investigations at hospital and to trace the source of infections. Pulsed-field gel electrophoresis (PFGE) is a gold standard and a powerful typing technique for contradistinction of bacterial strains causing hospital-acquired infections (Birren and Lai, 1993; Leonard et al., 1995; Maslow et al., 1993; Swaminathan and Matar, 1993; Tenover et al., 1995). Problems arise when this technique is applied to differentiate MRSA isolates since spheroplast formation from this organism needs lysostaphin (McDougal et al., 2003; Wenming et al., 2008), which is very expensive. Similarly, this enzyme also is used for DNA extraction from enterococci (Hogan et al., 2008; Murray et al., 1990; Saeedi et al., 2002; Sulakvelidze et al., 2000).

In this study we successfully developed a novel protocol to eliminate lysostaphin from the DNA extraction.

We used protocol described by Murray et al. (1990) for DNA extraction of enterococci and protocol described McDougal et al. (2003) in order to gain genomic DNA of *S. aureus*.

The bacterial strains used in this study were isolated from nosocomial infection specimens (clinical isolates of *S. aureus* and clinical isolates of *Enterococcus faecalis*). *S. aureus* ATCC29213 and *E. faecalis* ATCC29212 were used as controls. We used *Salmonella* serotype *Branderup* strain H9812 as marker (Hunter et al., 2005). *Enterococcous faecium* strain D366 with van B phenotype (kindly provided by B. Murray, Texas University) was also included as control in this experiment.

Initially, one colony from each strain was sub-cultured from plates containing trypticase soy agar with sheep blood into 5 ml Brain Heart Infusion Broth (BHI) in 15×100 mm screw-cap tubes. The tubes were then incubated for 16–18 h at 35–37 °C with gentle shaking until the optical density of suspension reached to 0.8–1.2 at 600 nm for *S. aureus* strains and 1.3–1.7 for enterococci. From this suspension, 350 µl was transferred into 1.5 ml micro-centrifuge tube and the cells were spinned at 12,000 rpm (13,684.32 g) for 5 min. The supernatant was removed and the pellet was re-suspended in 300 µl TEN buffer (100 mM EDTA, 0.15 M NaCl, 100 mM Tris pH 7.5) and centrifuged at 12,000 rpm (13,684.32 g) for 5 min.

* Corresponding author. Tel./fax: +98 21 88955810.
E-mail address: mfeizabadi@tums.ac.ir (M.M. Feizabadi).

0167-7012/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2010.11.001

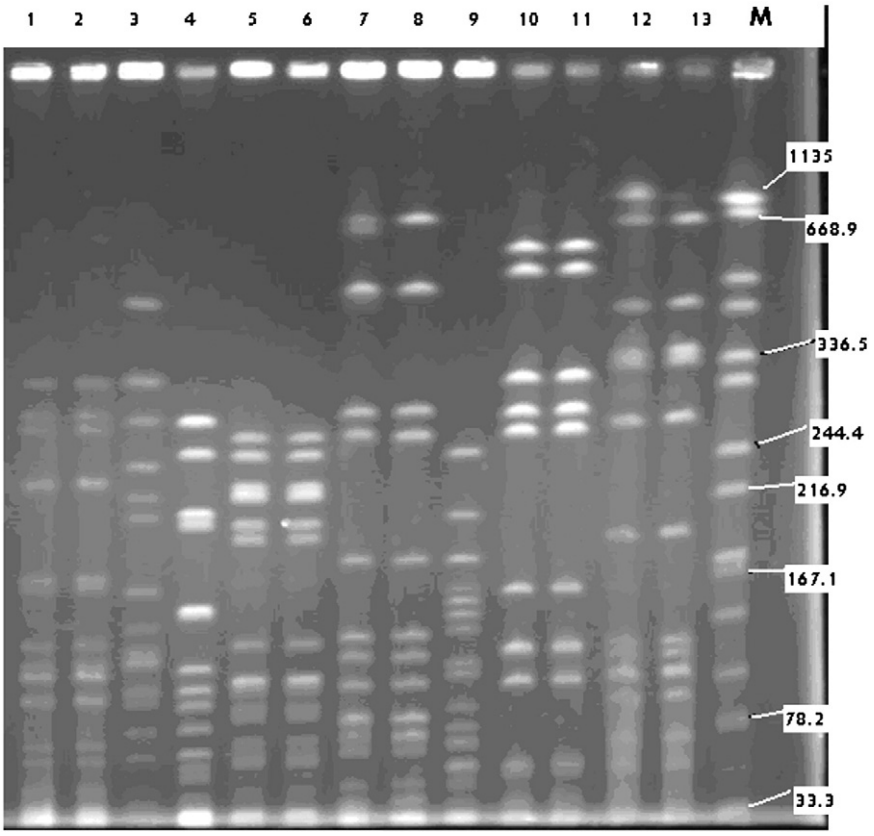

Fig. 1. DNA fingerprinting of *Staphylococcus aureus* and enterococci after digestion of genomic DNA with *Sma*I and resolution by PFGE. M: *Salmonella* serotype *Branderup* strain H9812 as marker. Lanes: 1, 10 *E. faecalis* ATCC29212 and *S. aureus* ATCC29213 (standard DNA extraction protocol); 2, 11: *E. faecalis* ATCC29212 and *S. aureus* ATCC29213 (new method without lysostaphin); 3, 5: clinical isolates of *E. faecalis* (standard DNA extraction protocol); 4, 6: clinical isolates of *E. faecalis* (new method without lysostaphin); 7, 12: clinical isolates of *S. aureus* (standard DNA extraction protocol); 8, 13: clinical isolates of *S. aureus* new method without lysostaphin), and 9: *Enterococcus faecium* D366 (new method without lysostaphin). Molecular sizes are shown in kilo base pairs on the right.

Subsequently, the sediments were suspended in 150 µl of EC buffer (100 mM EDTA, 1 M NaCl, 6 mM Tris–HCl (pH 7.6), 0.2% deoxycholate–0.5% Sarkosyl and 0.5% Brij-58). The suspension was heated in boiling water for 5–10 min before mixing it with an equal volume of 2% low melting point agarose (LMP). The LMP agarose was prepared in EC buffer and placed in a 50 °C water bath until use. The mixture was poured into plug molds. The plugs were solidified for 5 min at 4 °C. They were then transferred into 50 ml falcon tubes containing 10 ml EC buffer and lysozyme (25 mg/ml) and then placed in 36 °C water bath overnight with gentle shaking. The plugs were transferred to 9900 µl of lysis buffer II (0.5 mM EDTA, 1% Sarkosyl) plus 100 µl of 20 mg/ml proteinase K (the final concentration of proteinase K was 0.2 mg/ml) and incubated in water bath at 50 °C for 4 h with gentle shaking. They were washed three times with 10 ml of TE buffer for 30 min. The restriction digestion step was done by adding 30 U *Sma*I enzyme for 4 h incubation at 25 °C after 30 min preincubation at 30 °C.

The plugs were placed in wells of 1.0% agarose gels (Bio-Rad) made with 0.5X TBE, sealing with the same agarose. Restriction fragments of DNA are separated in 1% agarose in 0.5X TBE using clamped homogeneous electric fields (CHEF DRIII, Bio-Rad). The electrophoretic conditions were 6 V/cm; 120° switch angle at 14 °C, and a run time of 24 h divided into two different blocks. The first block had a run time of 20 h, an initial switching time of 5 s, and a final switching time of 35 s, whereas the second block used a switch time ramp of 5 s–10 s for 4 h. The gel was stained with 0.5 mg/ml ethidium bromide for 20 min and de-stained in water for 20 min.

Despite omitting lysostaphin in our DNA extraction procedure, there were no differences of DNA bands using this new method compared to the standard protocol (Fig. 1) The results were reproducible when further experiments were performed on more clinical strains (Fig. 2). Another advantage of our method is cost saving of proteinase K which is also expensive as we used 0.2 mg/ml of this reagent compared with other studies that used at least 1 mg/ml (Murray et al., 1990; Sulakvelidze et al., 2000).

The PFGE patterns obtained for strains of *S. aureus* and enterococci are shown in Fig. 1. As the picture shows there is no significant difference between using lysostaphin, or boiling, in order to obtain whole DNA. The type ability and repeatability of our new method were checked with further experiments using other isolates of *S. aureus* (n = 30) and enterococci (n = 30) (data is not shown).

In conclusion, the protocol described in this article allows a cost-saving and reproducible alternative to previously published PFGE typing protocols for typing of staphylococci, enterococci and, potentially, other gram-positive bacteria. We expect that the procedure will be of value during epidemiological investigations of MRSA and VRE outbreaks and for comparative characterization of MRSA and VRE strains isolated in different geographic loci.

This project was supported by Tehran University of Medical Sciences, project No. 8740.

References

Birren, B., Lai, E., 1993. Pulsed Field Gel Electrophoresis: A Practical Guide. Academic Press, Inc., San Diego, Calif.

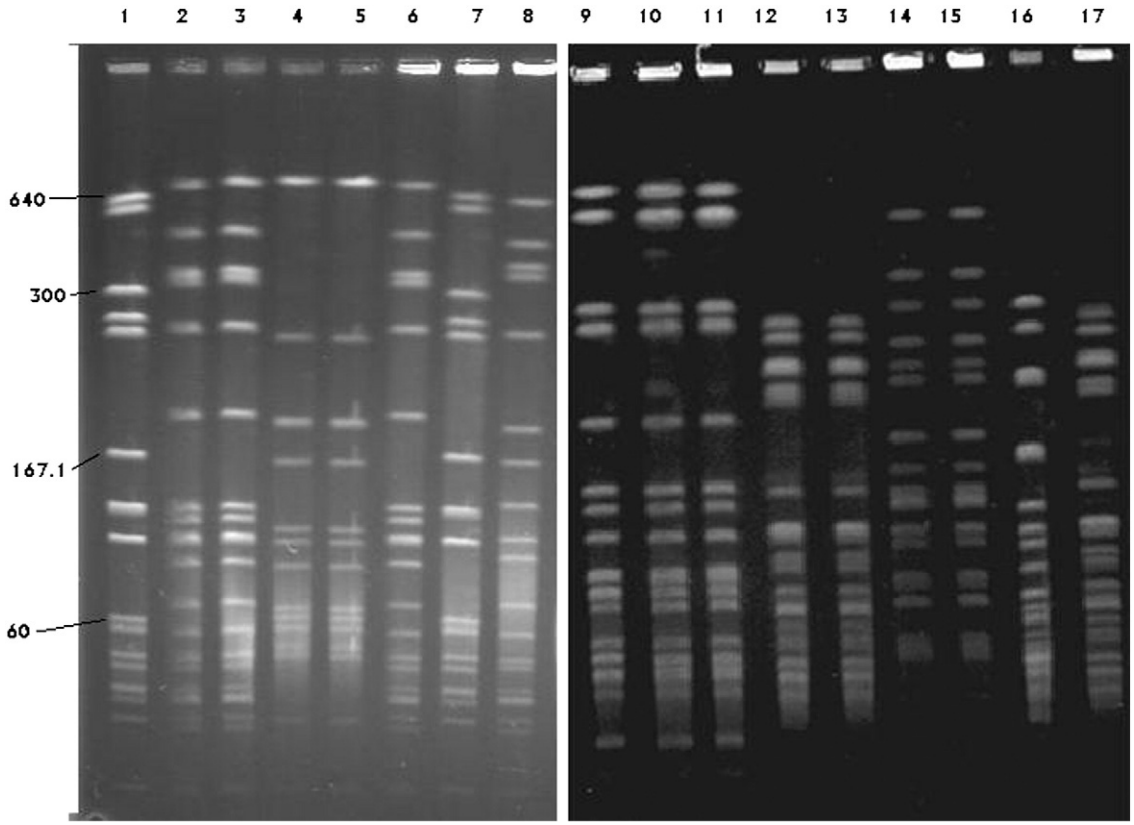

Fig. 2. DNA fingerprinting of *Staphylococcus aureus* and enterococci after digestion of genomic DNA with *Sma*I and resolution by PFGE. DNAs were extracted without using lysostaphin. Lanes; 1: *S. aureus* ATCC29213, 2–8: clinical isolates of *S. aureus*, 9–17: clinical isolates of *E. faecalis*, 12 and 13: Repetitive isolates from another lung transplanted patient at different times which equivalent to lane 4 of Fig. 1 (same patient), *14* and *15*: Repetitive Isolates from a lung transplanted patient at different times which equivalent to lane 3 of Fig. 1 (same patient). The numbers show the molecular sizes of fragments in kilo base pairs.

Emori, T.G., Gaynes, R.P., 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin. Microbiol. Rev. 6, 428–442.

Engemann, J.J., Carmeli, Y., Cosgrove, S.E., Fowler, V.G., Bronstein, M.Z., Trivette, S.L., Briggs, J.P., Sexton, D.J., Kaye, K.S., 2003. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin. Infect. Dis. 36, 592–598.

Hogan, J.S., Petersson-Wolfe, C.S., Adams, S., Wolf, S.L., 2008. Genomic typing of enterococci isolated from bovine mammary glands and environmental sources. J. Dairy Sci. 91, 615–619.

Hunter, S.B., Vauterin, P., Lambert-Fair, M.A., Van Duyne, M.S., Kubota, K., Graves, L., Wrigley, D., Barrett, T., Ribot, E., 2005. Establishment of a universal size standard strain for use with the pulsenet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J. Clin. Microbiol. 43, 1045–1050.

Leonard, R.B., Mayer, J., Sasser, M., Woods, M.L., Mooney, B.R., Brinton, B.G., Newcomb-Gayman, P.L., Carroll, K.C., 1995. Comparison ofMIDI Sherlock system and pulsed-field gel electrophoresis in characterizing strains of methicillin-resistant *Staphylococcus aureus* from a recent hospital outbreak. J. Clin. Microbiol. 33, 2723–2727.

Maslow, J.N., Slutsky, A.M., Arbeit, R.D., 1993. Application of pulsedfield gel electrophoresis to molecular epidemiology. In: Persing, D.H., Smith, T.F., Tenover, F.C., White, T.J. (Eds.), Diagnostic Molecular Microbiology: Principles and Applications. American Society for Microbiology, Washington, D.C, pp. 563–572.

McDougal, L., Steward, C.D., Killgore, G.E., Chaitram, J.M., McAllister, S., Tenover, F.C., 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. 11, 5113–5120.

Murray, B.E., Singh, K.V., Heath, J.D., Sharma, B.R., Weinstock, G.M., 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. 28, 2059–2063.

Richards, M.J., Edwards, J.R., Culver, D.H., Gaynes, R.P., et al., 1999. Nosocomial infections in medical intensive care units in the United States. Crit. Care Med. 27, 887–892.

Saeedi, B., Hällgren, A., Jonasson, J., Nilsson, L.E., Hanberger, H., Isaksson, B., 2002. Modified pulsed-field gel electrophoresis protocol for typing of enterococci. APMIS 110 (12), 869–874.

Schramm, G.E., Johnson, J.A., Doherty, J.A., et al., 2006. Methicillin-resistant *Staphylococcus aureus* sterile-site infection: the importance of appropriate initial antimicrobial treatment. Crit. Care Med. 34, 2069–2074.

Sulakvelidze, A., Turabelidze, D., Kotetishvili, M., Kreger, A., Morris, J.G., 2000. Improved pulsed-field gel electrophoresis for typing vancomycin-resistant enterococci. J. Clin. Microbiol. 4242–4245.

Swaminathan, B., Matar, G.M., 1993. Molecular typing methods. In: Persing, D.H., Smith, T.F., Tenover, F.C., White, T.J. (Eds.), Diagnostic Molecular Microbiology: Principles and Applications. American Society for Microbiology, Washington, D.C, pp. 26–50.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33, 2233–2239.

Wenming, Z., Clark, N.C., McDougal, L.K., Hageman, J., McDonald, L.C., Patel, J.B., 2008. Vancomycin-resistant *Staphylococcus aureus* isolates associated with Inc18-like *vana* plasmids in Michigan. J. Antimicrob. Agents Chemother. 52, 452–457.