Published in final edited form as:

Methods Mol Biol. 2016 ; 1373: 117–130. doi:10.1007/7651_2014_191.

Pulse Field Gel Electrophoresis

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Abstract

Pulse Field Gel Electrophoresis (PFGE) is a powerful genotyping technique used for the separation of large DNA molecules (entire genomic DNA) after digesting it with unique restriction enzymes and applying to a gel matrix under the electric field that periodically changes direction. PFGE is a variation of agarose gel electrophoresis that permits analysis of bacterial DNA fragments over an order of magnitude larger than that with conventional restriction enzyme analysis. It provides a good representation of the entire bacterial chromosome in a single gel with a highly reproducible restriction profile, providing clearly distinct and well-resolved DNA fragments.

Keywords

Pulse field gel electrophoresis; Restriction enzyme; Genomic DNA; Genotyping technique

1 Introduction

Genotyping of microorganisms is very important in evaluating the global evolution of the pathogens and studying their genetic relatedness to determine their point source during epidemiological investigations (1, 2). A variety of genotyping methods exists for *Staphylococcus aureus*. Each has strengths and weaknesses. These methods include pulse field gel electrophoresis (PFGE), surface protein A typing (*spa*-typing), multi locus sequence typing (MLST), SCC mec typing, plasmid profile analysis, restriction fragment length polymorphism (RFLP), RFLP-Southern blot, Rep-PCR typing, Multilocus VNTR (Variable Number Tandem Repeat) analysis (MLVA), and whole-genome DNA sequence typing (3–9).

S. aureus is one of the most important causes of life-threatening bacterial infections in the industrialized world causing infections both in the hospitals and the community. *S. aureus* is the second most common overall cause of healthcare-associated infections reported to the National Healthcare Safety Network, the most common cause of surgical site infections (10), the leading cause of infections involving heart valves and cardiac devices (11, 12), and a leading cause of bacteremia (13, 14) and endocarditis (15, 16). Additionally, *S. aureus* routinely becomes resistant to many of the currently available antibiotic therapies. Recently, the Centers for Disease Control and Prevention estimated that 80,461 invasive MRSA infections and 11,285 related deaths occurred in 2011 in the USA, even more than HIV/AIDS (17). Thus, a reproducible and highly discriminatory genotyping technique to rapidly

differentiate and type these isolates is needed to prevent the illness and costs associated with these infections. In addition, the availability of a sensitive genotyping method for staphylococcal isolates is essential in understanding the epidemiological evolution and outbreak of several antibiotic resistant strains including MRSA.

Among the various DNA-based methods available for genotyping *S. aureus* and other bacterial pathogens, PFGE is often considered as a gold standard due to its discriminatory power, reproducibility, and ease of execution, data interpretation, cost, and availability (18). PFGE is a powerful genotyping technique used for the separation of large DNA molecules (entire genomic DNA) after digesting with unique restriction enzyme. First developed by Schwartz et al. (19) in yeast, PFGE is reported to be very sensitive, highly reproducible with a very good discriminatory power in genotyping of *S. aureus* isolates (20). PFGE involves the isolation of the intact chromosomal DNA by lysing bacterial cells embedded in an agarose plug to avoid the mechanical shearing of DNA molecules during the extraction (21). This is followed by digestion of the chromosomal DNA within the agarose plug by a rare cutting restriction enzyme to produce 12 high-molecular weight DNA fragments. Finally, the digested DNA samples (10–800 kb) are subjected to separation by alternating the electric field between spatially distinct pairs of electrodes (Fig. 1). This will facilitate megabase (mb) size DNAs to reorient and migrate at different speeds through the gel pores towards the anode in a size dependent manner. The time required for reorientation is also inversely proportional to the size of DNA fragment. Overall, this process will achieve a good resolution of large DNA fragments in the agarose gel (19, 22). The obtained gel images will then be normalized and patterns of the DNA fragments will be analyzed by BioNumerics Software following the criteria to interpret PFGE patterns developed by Tenover et al. (23). These patterns serve a virtual barcode (Fig. 2), which “types” the strains and allows for the determination whether isolates are closely related.

2 Materials

Prepare all the reagents using ultrapure deionized water and analytical grade reagents. Follow all waste disposal regulations when disposing of waste materials.

2.1 Preparation of Bacterial Cells

1. Trypticase soy agar (TSA) plates.
2. 37 °C shaking incubator.
3. Turbidity meter or spectrophotometer for preparation of cell suspensions.
4. Microcentrifuge to pellet cell suspensions.
5. Vortex mixer.

2.2 Preparation of Agarose Plugs

1. 55–60 °C stationary water bath.
2. 37 °C stationary water bath.
3. SeaKem Gold agarose (Bio-Rad #161–3109).

4. *TE buffer*: 10 mM Tris, 1 mM EDTA, pH 8.0. Mix 20 ml of 1 M Tris, pH 8.0 with 4 ml of 0.5 M EDTA, pH 8.0 and add water to make a final volume of 2,000 ml in a graduated cylinder. Transfer to two 1,000 ml glass screw-top bottles and autoclave. Store at room temperature for up to 6 months.
5. Clean beaker/container for “working” TE buffer.
6. 250 ml screw-capped Erlenmeyer flask.
7. Microwave oven.
8. PFGE plug mold (Bio-Rad, Hercules, CA).
9. Lysostaphin enzyme. Prepare a 1 mg/ml suspension in 20 mM sodium acetate (pH 4.5), aliquot, and freeze at -20°C for up to 6 months.
10. Stainless steel spatulas.
11. Other basic lab supplies.

2.3 Plug Lysis

1. 55–60 and 37 $^{\circ}\text{C}$ stationary water bath.
2. *EC Lysis buffer*: 6 mM Tris, 100 mM EDTA, pH 8.0, 1 M NaCl, 0.5 % Brij-58, 0.2 % Deoxycholate, 0.5 % Sarcosyl. Mix 5.4 ml of 1MTris, pH 8.0; 180 ml of 5MNaCl; 180 ml of 0.5 M EDTA, pH 8.0; 4.5 g of Brij-58 (Polyoxyethylene 20 Cetyl Ether); 1.8 g of sodium deoxycholate and 4.5 g of sodium lauroyl sarcosinate with 500 ml of water in a glass beaker using magnetic stir bar and low heat. Once completely dissolved, add water to make a final volume of 900 ml. Transfer to a screw-top bottle and autoclave. Store at room temperature for up to 6 months.
3. Tubes to hold plug and buffer.
4. Spatula to remove plugs from mold.
5. 5 ml pipettes.

2.4 Plug Washing

1. TE buffer (*see above*).
2. Spatula or equivalent to hold plug in tube.
3. 5 ml pipettes.
4. Orbital rocker, rotator, or equivalent at room temperature.

2.5 Restriction Enzyme Digestion

1. Pretested *Salmonella* serotype Braenderup strain H9812 plugs (outlined in Section 4).
2. Microcentrifuge tubes.
3. 5 ml pipettes.

4. Standard Pipetmans.
5. Restriction endonuclease SmaI and XbaI with packaged 10× restriction buffer and 100× bovine serum albumin (BSA).
6. Commercial enzyme buffer appropriate for enzyme.
7. Sterile, Type I water.
8. Sterile plastic tubes (5–15 ml) for preparing buffer-water and enzyme-buffer-water mixtures.
9. Spatula or equivalent to hold plug in tube.
10. 5 ml pipettes.
11. Cutting dish (sterile disposable petri dish or equivalent).
12. Sharp scalpel or razor blade for cutting agarose plugs.
13. Bucket of ice or –20 °C insulated storage box.
14. Orbital rocker, rotator, or equivalent at room temperature.

2.6 Preparing and Running the Gel

1. 10× TBE buffer.
2. 55–60 °C stationary water bath.
3. Sterile distilled water, pre-warmed to 55 °C.
4. SeaKem Gold agarose (Bio-Rad # 161–3109).
5. Gel-casting platform and accessories.
6. Appropriate comb and comb holder.
7. Gel leveling bubble or equivalent.
8. 1.8 % SeaKem Gold agarose gel (For sealing wells).
9. CHEF-DR II system (Bio-Rad) for running pulsed-field gels.
10. Spatula, Kimwipes, and related basic lab supplies.

2.7 Staining and Documentation

1. Ethidium bromide solution, 10 mg/ml (AMRESCO #X328 or equivalent).
2. Containers (Covered glass dishes) to stain and destain gels.
3. Distilled water, 2 l.
4. Gel Doc 2000 (Bio-Rad) or equivalent gel documentation apparatus

2.8 Data Analysis

1. BioNumerics software version 4.0 (Applied Maths, Belgium).

3 Methods

3.1 Overview

Day 0: Streak plates.

Day 1: Make plugs.

Day 2: Wash plugs.

Day 3: Restriction digest and electrophoresis.

Day 4: Stain, take photograph, analyze in Bionumerics.

3.2 Day 0: Streak Plates

1. Streak *Staphylococcus aureus* isolates onto TSA plates and incubate at 37 °C for 18–24 h.

3.3 Day 1: Make Plugs

1. Turn on 37 and 55 °C water baths, and prepare two boxes of ice.
2. Label a 15 ml conical tube, a 5 ml polystyrene round-bottom tube, and a 1.5 ml microcentrifuge tube for each sample.
3. Add 2 ml sterile water to each 5 ml polystyrene round-bottom tube.
4. Add 3 ml EC lysis buffer to each 15 ml conical tube.
5. Use a sterile swab to collect cells from plate and suspend them in water in the 5 ml polystyrene round-bottom tube. Vortex briefly. Place the tube in the turbidity meter. Aim for a reading of 0.80–0.89. If the reading is too high, add more sterile water (in 1.0 ml increments); if the reading is too low, add more cells. Store the tubes on ice.
6. Transfer 200 µl of suspended cells to a 1.5 ml microcentrifuge tube. Centrifuge at 13,000 rpm for 6 min.
7. While samples are being centrifuged, prepare the gram-positive agarose (Gram negative—Salmonella agarose described separately in Section 4).
8. Combine 0.9 g SeaKem Gold agarose and 50 ml TE buffer in a 200 ml screw-top flask. Screw lid on tightly and microwave for 1 min 50 s. Gradually loosen the lid and swirl agarose. If not completely melted, retighten the lid and continue microwaving for 25 s intervals, loosening lid and swirling after each time, until agarose has thoroughly melted. Place the flask in the 55 °C water bath to equilibrate for 30 min (*see* Note 1).

¹The purpose of screw capped flask is to reduce the amount of evaporation. If the volume is diminished during agar melting, readjust the volume to the original level by adding TE buffer.

9. When centrifugation is complete, use a SAMCO disposable sterile pipette to aspirate the entire supernatant from microcentrifuge tubes and discard. The pellet should be 2–3 mm in diameter (*see* Note 2).
10. Add 300 μ l TE buffer to each microcentrifuge tube and vortex to resuspend the cells. Place in 37 °C water bath for 10 min.
11. For each isolate, label two wells on the plug mold. Remove the microcentrifuge tubes from the 37 °C water bath.
12. The following must be done one tube at a time: add 3 μ l Lysostaphin (1 mg/ml) and 300 μ l 55 °C agarose (made in step 8) to the microcentrifuge tube. Quickly but gently mix with pipettor ten times. Using a SAMCO disposable sterile pipette, fill two of the plug mold wells, overfilling slightly to produce a rounded top.
13. Repeat step 12 for each sample, and then allow plugs to harden for 10–15 min at room temperature (or 5 min in the 8 °C refrigerator).
14. When plugs are hardened, use the snap-off tool provided with each mold (or a spatula cleaned with ethanol) to push the plugs (two per sample) into the 15 ml conical tubes containing ~3 ml of EC lysis buffer. Make sure the plugs are fully immersed in the buffer.
15. Incubate tubes in 37 °C water bath for 4 h (preferably overnight).

3.4 Day 2: Wash Plugs

1. Pour off EC lysis buffer into glass beaker, holding cap close to rim to prevent plugs from escaping. Check beaker for escaped plugs.
2. Add 5 ml TE buffer to each tube, ensuring that all plugs are immersed. Place securely capped tubes horizontally in glass tray on rocker table for 60 min at room temperature. Set the speed of the rocker table to ~60 rpm.
3. Remove buffer as described in step 1. Repeat wash two more times for a total of three washes. The last wash may be done overnight. This can be stored refrigerated until all reagents are prepared for the enzyme digestion.

3.5 Day 3, Part 1: Restriction Digest

1. Turn on 37 and 55 °C water baths and prepare one box of ice. Remove 10 $\times$ multicore, SmaI, XbaI, and BSA from freezer and allow to thaw at room temperature. Centrifuge for 1 min, then place on ice.
2. Label a 1.5 ml microcentrifuge tube for each sample, plus one staphylococcal control (NCTC 8325—reference standard for data normalization) and four Salmonella size standards.
3. In a 15 ml conical tube, prepare the restriction buffer:

²Pellet size is crucial, if too small, add more suspension and recentrifuge; if too large, resuspend the pellet, remove some of the suspension, and recentrifuge.

10× multicore stock	420 µl
Sterile water	3,780 µl

This makes enough for 21 samples. Adjust the volumes accordingly based on the number of samples.

4. Add 200 µl of restriction buffer to each tube.
5. Clean a petri dish, spatula, and scalpel with ethanol. Place a plug in the petri dish and, using the scalpel and spatula, cut two 2–3 mm slices (Five slices for *Salmonella*). Place these slices in the corresponding microcentrifuge tube, and return what is left of the plug to its original tube. Repeat for each sample, cleaning the dish, spatula, and scalpel every time. Store the slices and remaining plugs at 8 °C.
6. Allow the staphylococcal samples to equilibrate at room temperature for 30–45 min, and the *Salmonella* samples (preparation described later) in a 37 °C water bath for 30–45 min.
7. Keeping reagents and prep tube on ice at all times, prepare restriction enzyme for staphylococcal samples by mixing (in a 15 ml conical tube):

10× multicore stock	360 µl
SmaI (10 U/µl)	54 µl
Acetylated BSA	36 µl
Sterile water	3,150 µl

Invert tube, vortex, and then return to ice. This makes enough for 18 samples. Adjust the volume accordingly based on the number of samples.

8. Keeping reagents and prep tube on ice at all times, prepare restriction enzyme for *Salmonella* samples by mixing (in a 1.5 ml microcentrifuge tube):

10× multicore stock	100 µl
XbaI (10 U/µl)	20 µl
Acetylated BSA	10 µl
Sterile water	869 µl

Keep on ice at all times. Invert tube, vortex, and then return to ice. This makes enough for five samples. Again, adjust the volume accordingly based on the number of samples.

9. Remove buffer from the plugs using disposable SAMCO pipettes.
10. Add 200 µl of the appropriate restriction enzyme/buffer mix to each tube (SmaI for staphylococcal samples, XbaI for *Salmonella* samples).

11. Incubate for 3–4 h at room temperature for staphylococcal samples, and in 37 °C water bath for Salmonella samples.

3.6 Day 3, Part 2: Electrophoresis

1. Make 2,200 ml of 0.5× TBE by combining: 110 ml 10× TBE in 2,090 ml purified water in a 4 l plastic beaker with a large magnetic stir bar. Set on magnetic stirrer to mix while completing the next steps.
2. To make the 1 % agarose gel, combine: 1.05 g SeaKem Gold agarose with 100 ml 0.5× TBE in a 200 ml screw-top flask, then dissolve and equilibrate as in Section 3.3, step 8.
3. Assemble the leveling table, gel casting stand, and combs. Clean thoroughly with alcohol and Kimwipes to remove lint. Use 360° level to ensure table is levelled.
4. Pour the remaining 0.5× TBE into the PFGE system. Turn on (in order) the command system, pump, and chiller. Set temperature to 14 °C.
5. When the plug slices have completed their incubation period, place them on the comb according to the layout on the log sheet. Scoop a slice out of its microcentrifuge tube with a spatula, and blot the slice with a Kimwipes. Slide the slice onto the end of a comb “tooth.” Be sure to clean spatula with ethanol between tubes. Allow the slices to set on the comb for 10 min.
6. Set the comb upright in the casting stand, with the slices facing forward. Look closely to be sure that slices are uniformly positioned, and wait for a few moments to be sure that they have not and will not slide off the comb.
7. Gently pour the 55 °C agarose into the casting tray, saving a small amount (1–2 ml) to fill in the wells after the comb is removed. Keep this agarose at 55 °C while the gel solidifies at room temperature for 1 h.
8. When the gel is solid, remove the comb and fill the holes with the saved agarose. Let it to set for 5–10 min.
9. Remove the gel with the black bottom tray from the casting stand and clean the black tray of all residues. Place in the electrophoresis cell and start the cycle:
 - Press “Volts” and set to 5.6 (for system on right) or 5.8 (system on left).
 - Set time for 21 h (right) or 21.5 h (left).
 - Press “Block” and “Volt” together, and set at 5.0.
 - Press “Volts” and “Run Time” together, and set at 40.0.
 - Press “Start.”

Follow the manufacturer’s instruction depending on the available system.

3.7 Day 4, Part 1: Stain Gel

1. Add 30 µl of 1 mg/ml ethidium bromide to 300 ml purified water in a glass tray (Or equivalent amount based on the tray size).

2. Slide gel off its black backing and into the tray. Incubate at room temperature for 45 min.
3. Decolorize in 1 l of purified water for 90 min.

3.8 Day 4, Part 2: Photograph Gel (Picture Can Be Captured in Any Equivalent Way)

1. Slide gel back on to the black backing to remove it from water and transfer it to the UV transilluminator.
2. Slide gel off black backing and on to the transilluminator table. Position the camera and hood over the table and turn on the UV light and camera.
3. Set the image type to RAW and the exposure time to 15 s.
4. Zoom in to frame the gel and take two pictures.

3.9 Processing PFGE Images Using BioNumerics

1. Open the .TIF image file of the gel in BioNumerics Software (Applied Maths) by clicking on “Add new experiment file.”
2. Follow the instructions to process the TIFF image using the software mainly through the following four steps:
 - a. Convert a TIFF to gel strips.
 - b. Define curves.
 - c. Normalize the gel.
 - d. Find gel bands.
3. For cluster analysis, select the isolates to be compared, click the “Calculate Cluster Analysis” and follow the instructions.

4 Preparation of *Salmonella* PFGE Plugs

Salmonella plugs should be used as standards in each gel. Thus plugs of standard strain *Salmonella* serotype Braenderup strain H9812 needs to be made and pretested. Once the plugs are made and have passed as controls on plug preparation, they can be stored and used in each gel as a test for enzyme efficacy. Prepare all the reagents using ultrapure deionized water and analytical grade reagents. Follow all waste disposal regulations when disposing of waste materials.

4.1 Day 0: Streak Plates

1. Streak *Salmonella* serotype Braenderup strain H9812 onto TSA plates and incubate at 37 °C for 18–24 h.

4.2 Day 1: Make Plugs

1. Turn on 54 °C shaker incubator and water bath (55 °C); Prepare ice in a Styrofoam box.

2. To prepare 1 % SeaKim Gold (1 % SDS agarose gel for *Salmonella plugs*), mix 0.5 g SeaKim Gold agarose and 47.5 ml TE buffer in 200 ml screw-top flask, then dissolve in Section 3.3, step 8. Place the flask in the 55 °C water bath to equilibrate for 5 min before adding SDS.
3. Add 2.5 ml of 20 % SDS (preheated to 55 °C), mix well, and keep in the 55 °C water bath until ready to use.
4. Prepare *Cell Suspension Buffer*: 100 mM Tris, 100 mM EDTA, pH 8.0. Mix 10 ml of 1 M Tris, pH 8.0 with 20 ml of 0.5 M EDTA, pH 8.0 and add water to make a final volume of 100 ml in a graduated cylinder. Transfer to a screw-top bottle and autoclave. Store at room temperature for up to 6 months.
5. Transfer 2 ml of Cell suspension buffer to labeled 5 ml polystyrene round-bottom tubes, and keep tubes with the buffer on ice.
6. Use a sterile swab to collect cells from the TSA plate and suspend them in the Cell suspension buffer. Vortex briefly. Place the tube in the turbidity meter. Aim for a reading of 0.48–0.52. If the reading is too high, add more buffer (in 500 µl increments); if the reading is too low, add more cells. Store the tubes on ice.

4.3 Casting Plugs

1. Label wells of PFGE plug molds (40 plug molds or as little as 20 plug molds).
2. Transfer 400 µl of cell suspension with strain in Cell suspension buffer to labeled 1.5 ml microcentrifuge tubes (>15 tubes).
3. Add 20 µl of Proteinase K (20 mg/ml) to each microcentrifuge tube and mix gently with pipette tip, one or two tubes at a time.
4. Add 400 µl of melted 1 % SDS agarose from above to microcentrifuge tube containing cell suspension (Brought to the room temperature) and mix gently with fine tip transfer pipette. If cell suspensions are cold, place tubes containing cell suspensions in 37 °C water bath for a few minutes to warm.
5. Fill plug molds by immediately dispensing the agarose mix into appropriate well(s) of reusable plug mold. Do not allow bubbles to form. Two plugs of each sample can be made from these amounts of cell suspension and agarose. Allow plugs to solidify at room temperature for 10–15 min. They can also be placed in the refrigerator (4 °C) for 5 min.

4.4 Lysis of Cells in Agarose Plugs

1. Label 50 ml polypropylene screw-cap tubes with culture numbers (ten tubes).
2. Prepare *Cell Lysis Buffer*: 50 mM Tris, 50 mM EDTA, pH 8.0, 1 % Sarcosyl. Mix 25 ml of 1 M Tris, pH 8.0; 50 ml of 0.5 M EDTA, pH 8.0 and 5 g of Sodium Lauroyl Sarcosinate and add water to make a final volume of 500 ml in a graduated cylinder. Transfer to a 1 l screw-top bottle and warm to 50–60 °C for 30–60 min or leave at room temperature for 2 h to completely dissolve Sodium Lauroyl

Sarcosinate. Autoclave for 20 min and store at room temperature for up to 6 months.

3. Prepare Cell lysis buffer/Proteinase K mix by adding 250 µl of Proteinase K solution (20 mg/ml) to 50 ml of Cell lysis buffer.
4. Add 5 ml of Cell lysis buffer/Proteinase K mix to each tube labeled above (ten Orange screw-cap tubes from above).
5. Push out plugs (3–4 plugs) into each tube making sure that the plugs are immersed under buffer.
6. Place tubes in rack and incubate in 54 °C shaker incubator for 1.5–2 h with vigorous shaking at 150–175 rpm.

4.5 Washing of Agarose Plugs

1. Preheat sterile purified water (10 tubes × 10–15 ml × 2 times = 2–300 ml) to 50 °C for washing plugs.
2. Remove tubes from shaker incubator and pour off Cell lysis buffer/Proteinase K solution.
3. Add 10–15 ml preheated sterile purified water to each tube and shake tubes vigorously (150–175 rpm) in 50 °C shaker incubator for 10–15 min. Pour off water from plugs. Repeat the wash process one more time.
4. While the plug is being washed with water, preheat sterile TE buffer (10 tubes × 10–15 ml × 4 times = 4–600 ml) to 50 °C for washing plugs.
5. Add 10–15 ml of preheated (50 °C) TE buffer to each tube and shake tubes in 50 °C shaker incubator for 10–15 min. Pour off the TE buffer from plugs and repeat the wash process for a total of four times.
6. Store plugs in microcentrifuge tubes with TE buffer at 4 °C (2–4 plugs per tube) until needed.

References

1. Blanc DS. The use of molecular typing for epidemiological surveillance and investigation of endemic nosocomial infections. *Infect Genet Evol.* 2004; 4:193–197. [PubMed: 15450198]
2. Blanc DS, Petignat C, Wenger A, et al. Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a small geographic area over an eight-year period. *J Clin Microbiol.* 2007; 45:3729–3736. [PubMed: 17881551]
3. Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol.* 2000; 38:1008–1015. [PubMed: 10698988]
4. Koreen L, Ramaswamy SV, Graviss EA, et al. spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol.* 2004; 42:792–799. [PubMed: 14766855]
5. Mwangi MM, Wu SW, Zhou Y, et al. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *Proc Natl Acad Sci U S A.* 2007; 104:9451–9456. [PubMed: 17517606]

6. Trindade PA, McCulloch JA, Oliveira GA, et al. Molecular techniques for MRSA typing: current issues and perspectives. *Braz J Infect Dis.* 2003; 7:32–43. [PubMed: 12807690]
7. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2002; 46:2155–2161. [PubMed: 12069968]
8. Zhang K, McClure JA, Elsayed S, et al. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol.* 2005; 43:5026–5033. [PubMed: 16207957]
9. Struelens MJ, Hawkey PM, French GL, et al. Laboratory tools and strategies for methicillin-resistant *Staphylococcus aureus* screening, surveillance and typing: state of the art and unmet needs. *Clin Microbiol Infect.* 2009; 15:112–119. [PubMed: 19291142]
10. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol.* 2008; 29:996–1011. [PubMed: 18947320]
11. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. *Circulation.* 2005; 111:e394–e434. [PubMed: 15956145]
12. Wang A, Athan E, Pappas PA, et al. Contemporary clinical profile and outcome of prosthetic valve endocarditis. *JAMA.* 2007; 297:1354–1361. [PubMed: 17392239]
13. Fluit AC, Jones ME, Schmitz FJ, et al. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin Infect Dis.* 2000; 30:454–460. [PubMed: 10722427]
14. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis.* 2004; 39:309–317. [PubMed: 15306996]
15. Fowler VG Jr, Miro JM, Hoen B, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA.* 2005; 293:3012–3021. [PubMed: 15972563]
16. Miro JM, Anguera I, Cabell CH, et al. *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis.* 2005; 41:507–514. [PubMed: 16028160]
17. CDC. Antibiotic resistance threats in the United States. Atlanta, GA: Centers for Disease Control and Prevention; 2013.
18. Bannerman TL, Hancock GA, Tenover FC, et al. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol.* 1995; 33:551–555. [PubMed: 7751356]
19. Schwartz DC, Cantor CR. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell.* 1984; 37:67–75. [PubMed: 6373014]
20. Prevost G, Jaulhac B, Piemont Y. DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. *J Clin Microbiol.* 1992; 30:967–973. [PubMed: 1572985]
21. Matushek MG, Bonten MJ, Hayden MK. Rapid preparation of bacterial DNA for pulsed-field gel electrophoresis. *J Clin Microbiol.* 1996; 34:2598–2600. [PubMed: 8880529]
22. Reed KD, Stemper ME, Shukla SK. Pulsed-field gel electrophoresis of MRSA. *Methods Mol Biol.* 2007; 391:59–69. [PubMed: 18025669]
23. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol.* 1995; 33:2233–2239. [PubMed: 7494007]

Fig. 1.

An example comparison of PFGE versus conventional electrophoresis. In both cases, the gel (grey rectangle) is placed in a buffer inside a gel rig with anodes (+) and cathodes (–) (top diagrams). In the case of PFGE, the direction of current cycles between 1, 2, and 3. As depicted in the bottom graphs, unlike conventional electrophoresis where current only runs in a single direction, PFGE cycles between several directions, allowing for separation of large molecular weight DNA

Fig. 2.
A representative processed gel showing the different banding patterns of eight USA types. Image is a negative image of a processed gel with higher molecular weight (MW) DNA towards *top* of the image