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Pulsed Field Gel Electrophoresis in Molecular Typing and Epidemiological Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA)

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important threat to hospitalized patients worldwide and is responsible for a wide range of human diseases, including septicemia, endocarditis, pneumonia, osteomyelitis, toxic shock syndrome, and bacteremia (Tenover & Gaynes, 2000). This species nevertheless represents a serious public health burden, particularly the clones which are resistant to methicillin and other classes of antibiotics; the emergence of penicillin-methicillin-, and recently high-level vancomycin-resistant strains emphasize the importance and urgency of such rational prescribing policy for the treatment of MRSA infections (Appelbaum, 2007; Goldstein 2007). Multiple studies have shown clonal spreads of epidemic MRSA strains within hospitals, between hospitals within a country (Breurec et al., 2011; Nübel et al., 2010), and also between countries and continents (Breurec et al., 2011; Deurenberg et al., 2009; Diekema et al. 2001). There are only a limited number of nosocomial MRSA clones spread worldwide (the Iberian [ST247-SCCmec I], the Brazilian [ST239-IIIa], the Hungarian [ST239-III], the New York/Japan [ST5-II], the Pediatric [ST5-VI], the Berlin [ST45-IV], EMRSA-15 [ST22-IV], and the EMRSA-16 [ST36-II] clones) (Enright et al., 2002; Oliveira et al., 2001).

Molecular typing of MRSA is used to support infection control measures. Although Pulsed-field gel electrophoresis (PFGE) is well known and considered as golden standard, for establishing clonal relationships at the local level, its detection capacity seems to make it also too discriminative for global comparisons (McDougal et al. 2003; Murchan et al., 2003). Recently multilocus sequence typing (MLST) has been proven to be the most adequate method both for long-term and global epidemiologic studies and for population genetic studies. Typing methods based on sequencing of more stable housekeeping genes (MLST) allow the creation of Internet-based curate databases and inter-laboratory data exchange

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(Enright et al., 2000) The combination of these methods allows the unambiguous assignment of collections of MRSA isolates or new MRSA clones (Enright et al., 2000).

The prevalence of MRSA in Mexico differs widely from one hospital to another and according to different studies performed; an increasing frequency of MRSA (7% in 1989, 14% in 2001 and 36% in 2004) are documented by reports of routine oxacillin disk diffusion tests only (Alpuche et al., 1989; Calderón et al., 2002; Chávez, 2004). This is of great concern, because it is a common experience that once MRSA is introduced in a hospital it is difficult to eradicate it (Creamer et al., 2011; Rebmann & Aureden, 2011). However, reports from Mexico documenting the clonality of MRSA isolates are very scarce, Aires de Sousa *et al.* in 2001 (Aires de Sousa et al., 2001) reported dominant and unique MRSA clone designated the Mexican clone (I::NH::M), identified by PFGE among isolates collected in 1997, 1998 from a pediatric hospital in Mexico, which had a rather limited resistance profile. In more recent studies which involve strains collected for the period 1997 to 2003 in two Mexican hospitals, PFGE distributed the MRSA isolates into two types M (clone EMRSA-16-U.K) and C (clone New York/Japan) these two clones were distinguished by antibiogram and other molecular properties (Echaniz et al., 2006; Velazquez et al., 2004).

The aim of this study was to identify MRSA clones circulating in a tertiary care hospital in Mexico City and their prevalence in the course of time 2002-2009. For this purpose, we used a phenotypic characterization and a combination of different molecular typing methods, including PFGE, hybridization with a Tn554 and *mecA* probes, staphylococcal cassette chromosome *mec* (SCC*mec*) and MLST.

2. Material and methods

2.1 Hospital setting

The Instituto Nacional de Cardiología “Dr. Ignacio Chavez” (CAR) is a tertiary-care cardiology hospital located, in Mexico City with 246 beds, distributed 10 wards: surgery, adults and pediatric cardiology, neumology, nephrology, coronary unit and others. In addition the hospital has 17 external services. The microbiology laboratory receives an average of 18,000 samples annually. The hospital has 5,800 admission and 5,700 discharges per year.

2.2 Bacterial isolates

We studied a total of ninety single-patient clinical MRSA isolates, between January 2002 and December 2009. The strains were collected from several clinical sources: bronchial secretions (n=34); wound secretions (n= 25), blood (n= 16); catheter (n=3); pleural liquid (n=3); peritoneal fluid (n=1) and others (n=13). MRSA strains were collected from different wards: pediatric surgery, adult surgery, coronary unit, nephrology, surgery and cardiology. Of the 90 MRSA isolates, 24 were from children and 66 were from adults.

2.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for MRSA isolates was performed using the automated method of MicroScan® (DADE-BEHRING, Sacramento, CA) for: penicillin, oxacillin,

amoxicillin, cefotaxime, cephalothin, cefazolin, imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, clarithromycin, gentamicin, rifampin, tetracycline and vancomycin, following the Clinical Laboratory Standards Institute guidelines (Clinical Laboratory Standards Institute [CLSI], 2009).

2.4 Molecular typing

The whole genomic DNA was prepared as described previously (Chung et al., 2000). After digestion with *Sma*I endonuclease, DNA was separated in a CHEF-DRII apparatus (Bio-Rad, Birmingham, U.K) (Chung et al., 2000). Strains HU25, HPV107, HDE288, BK2464, JP27 and 96/32010, representing the Brazilian, Iberian, Pediatric, New York/Japan-USA, New York/Japan-Japan and EMRSA-16-U.K clones, were included in the PFGE gels as controls. The control strains were kindly provided by Prof. Herminia de Lencastre from the Molecular Genetics laboratory Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa. Criteria of Tenover were used to compare different clones (Tenover et al., 1995). Strains BK2464 and HDE288 were used as *SCCmec* controls. Hybridization of *Sma*I digests with *mecA* and Tn554 probes (de Lencastre et al., 1994), *SCCmec* typing (Oliveira & de Lencastre, 2002) and MLST (Enright et al., 2000) were performed as previously described. Briefly, MLST is based in internal fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *yqiL*) for each isolates, the alleles at the seven loci defined the allelic profiles, which corresponded to a sequence type (ST). ST designations were those assigned the MLST data base (<http://www.mlst.net>). The *SCCmec* typing system is defined by combining the class of the *mec* gene complex with the cassette chromosome recombinase gene (*ccr*) allotypes. The polymorphism in the vicinity of the *mecA* gene detected by probe *Clal*-digested DNAs with a *mecA* probe and transposon Tn554 insertion patterns detecting by probing *Clal* digestion DNAs with a specific probe (de Lencastre et al., 1994, Enright et al., 2000; Oliveira & de Lencastre, 2002).

2.5 Computer-fingerprinting analysis

The computer analysis of the banding patterns obtained by PFGE was done using the NTSYSpc software version 2.0.2.11 (Applied Biostatistics Inc.) after visual inspection. Each gel included reference strain *S. aureus* NCTC 8325 to normalize the PFGE profiles. For clusters analyses, the Dice coefficients were calculated to compute the matrix similarity and were transformed into an agglomerative cluster by the unweighted pair group method with arithmetic average (UPGMA).

3. Results

3.1 Antimicrobial susceptibility

The 90 isolates showed resistance to penicillin (100%), oxacillin (99.3%), amoxicillin (100%) , cefotaxime (100%), cephalothin (100%), cefazolin (100%), chloramphenicol (100%), imipenem (99.3%), ciprofloxacin (87.7%); eleven strains (12.2%) showed low susceptibility for clindamycin, erythromycin, clarithromycin and were susceptible to ciprofloxacin; two strains (2.2%) showed low susceptibility for oxacillin (MIC 4µg/mL) and imipenem. All strains were susceptible to rifampin, tetracycline, gentamicin, trimethoprim-sulfamethoxazole and vancomycin.

3.2 Molecular typing

3.2.1 PFGE analysis

The PFGE analysis separated the MRSA strains into three types, A (5 subtypes), B (3 subtypes) and C (6 subtypes) (Figure 1). PFGE pattern C and subtypes were predominant in this isolates n=72 (80%), Clone A, n=11 (12.2%) and B, n=7 (7.8%) were only found in the isolates of 2002, and these two clones (A and B) were totally replaced by clone C in 2004 and continue until 2009. The results produced by a computer analysis of the banding patterns show clearly the division of the three clone groups (A, B and C); interestingly, the A and B clone isolates have very similar PFGE patterns (coefficient similarity 95%). Nevertheless, the three clones A, B and C could easily be distinguished by antibiograms and other molecular properties as well (Table 1). The three clones were multiresistant, however, each one of them showed a characteristic resistance pattern; clone A was resistant to β -lactams and showed a low susceptibility to clarithromycin, clindamycin, erythromycin and was susceptible to ciprofloxacin; while clones B and C were resistant to β -lactams, clarithromycin, clindamycin, erythromycin and ciprofloxacin; only the strains with subtypes B1 and B2 showed low susceptibility for oxacillin and imipenem.

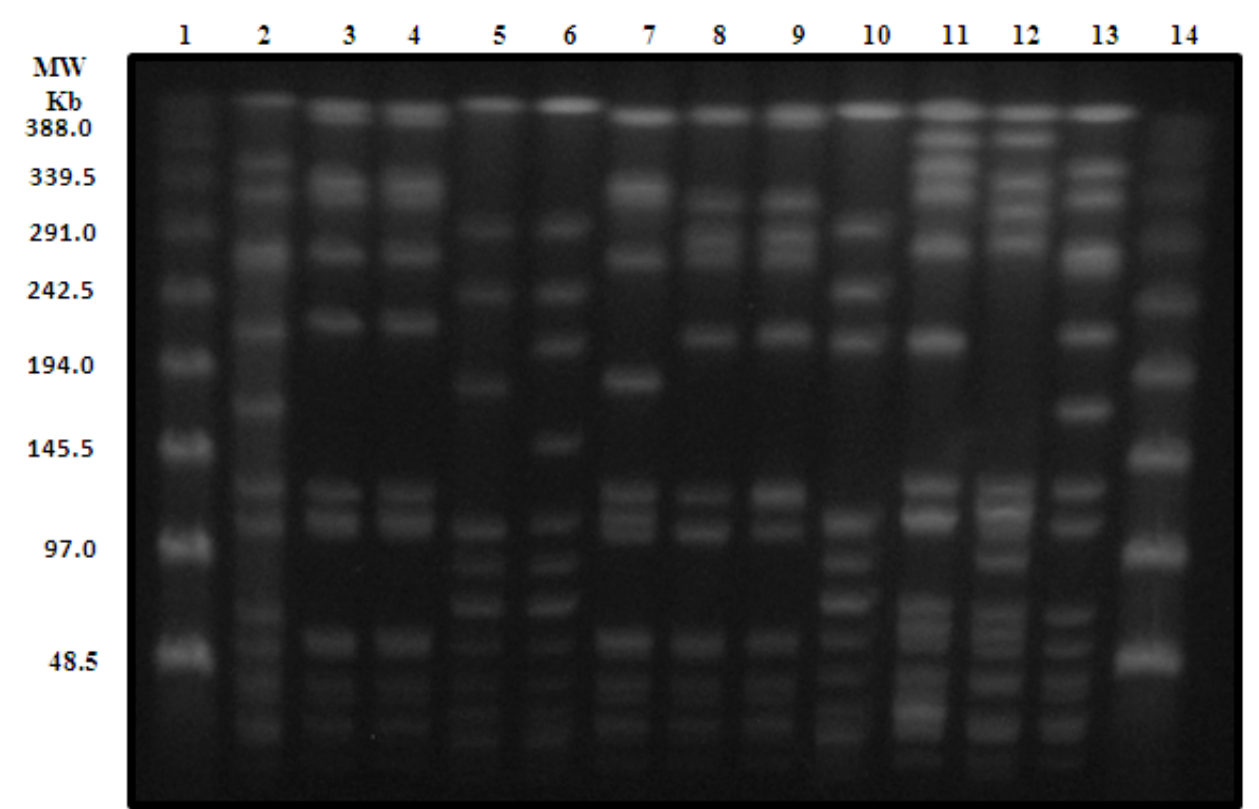


Fig. 1. Pulsed field gel electrophoresis profiles of MRSA clinical isolates from the Instituto Nacional de Cardiología “Dr. Ignacio Chávez”, Mexico and representatives of international clones. Lanes: 1-14 lambda ladder used a molecular size (MW) markers; 2 and 13 reference strain NCTC8325; 3-4 (44CAR and 47CAR) pattern C; 5 (2CAR) pattern A; 6 (20CAR) pattern B; 7-12 (HDE288, BK2464, JP27, EMRSA16, HPV107 and HU25) control strains representative of Pediatric, New York/Japan-USA, New York/Japan-Japan, EMRSA16-U.K, Iberian and Brazilian clones.

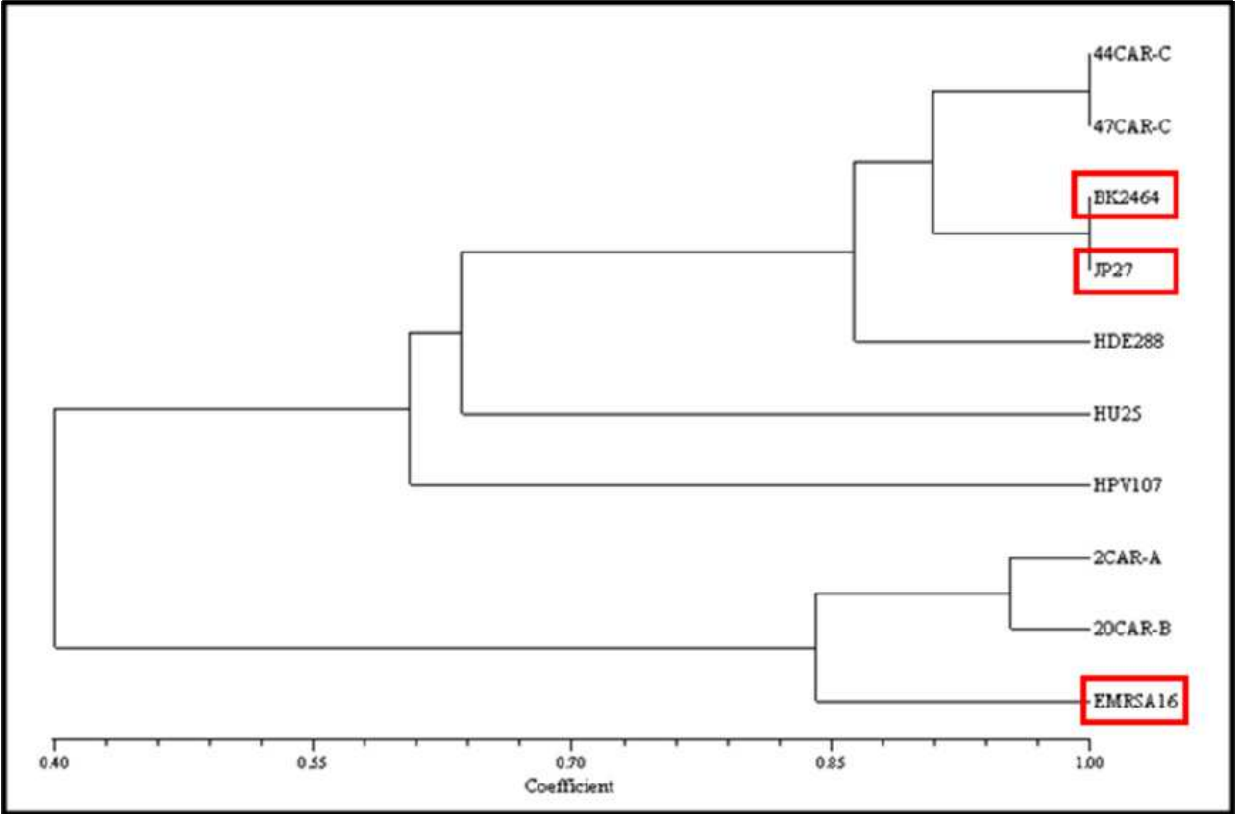


Fig. 2. Dendrogram comparing MRSA clones A, B and C from the Instituto Nacional de Cardiología “Dr. Ignacio Chávez”, Mexico with different international MRSA clones: BK2464-New York/Japan-USA clone; JP27-New York/Japan-Japan clone; HDE288-Pediatric clone; HU25-Brazilian-clone; HPV107-Iberian clone; EMRSA-16-U.K. clone. For cluster analysis, Dice coefficients were calculated to compute matrix similarity a transformed into an agglomerative cluster with the unweighted pair group method with arithmetic average.

Property	Clone A	Clone B	Clone C
Antibiotype ¹ (Resistance)	β-lactams, (CLA,CD,ERY) ²	β-lactams, (CIP, CLA,CD, ERY)	β-lactams, (CIP, CLA,CD, ERY)
Number of subtypes	5	3	6
SSCmec type ³	IV	II	II
Hybridization bands(Kb) <i>SmaI-mecA</i>	~180	~211 ⁴	~211
Hybridization bands(Kb) <i>Sma-Tn554</i>	~180	~211-640	~211-640
ST	30	30 ⁵ /36	5

¹Antibiotic abbreviations: CLA- clarithromycin; CD - clindamycin; ERY - erythromycin GEN - gentamicin; CIP - ciprofloxacin. ²Intermediate resistance pattern. ³Staphylococcal cassette chromosome *mec*. ⁴Except B1 and B2; ⁵Sequence typing (MLST), only the patterns B1 and B2.

Table 1. Antibiotype and Genotypic characterization of the MRSA clones presented in the Instituto Nacional de Cardiología “Dr. Ignacio Chávez”, Mexico (2002-2009).

3.2.2 Hybridization pattern

The hybridization patterns with *mecA* and Tn554 probes indicated that the MRSA strains accompanying clone A carried the *mecA* gene on a *SmaI* fragment of approximately 180 Kb, while the *mecA* gene of the clones B and C were found on a fragment of approximately 211 Kb (Figure 3-A). One Tn554 copy was identified usually on the fragment approximately 180 Kb between the isolates of clone A; while the MRSA strains accompanying clones B and C usually carried two identified Tn554 copies between the *SmaI* fragments of approximately 211 and 640 Kb; only the strains 3CAR, (B1) and 8CAR, (B2), carried the transposon Tn554 in a fragment of 640 Kb (Figure 3-B).

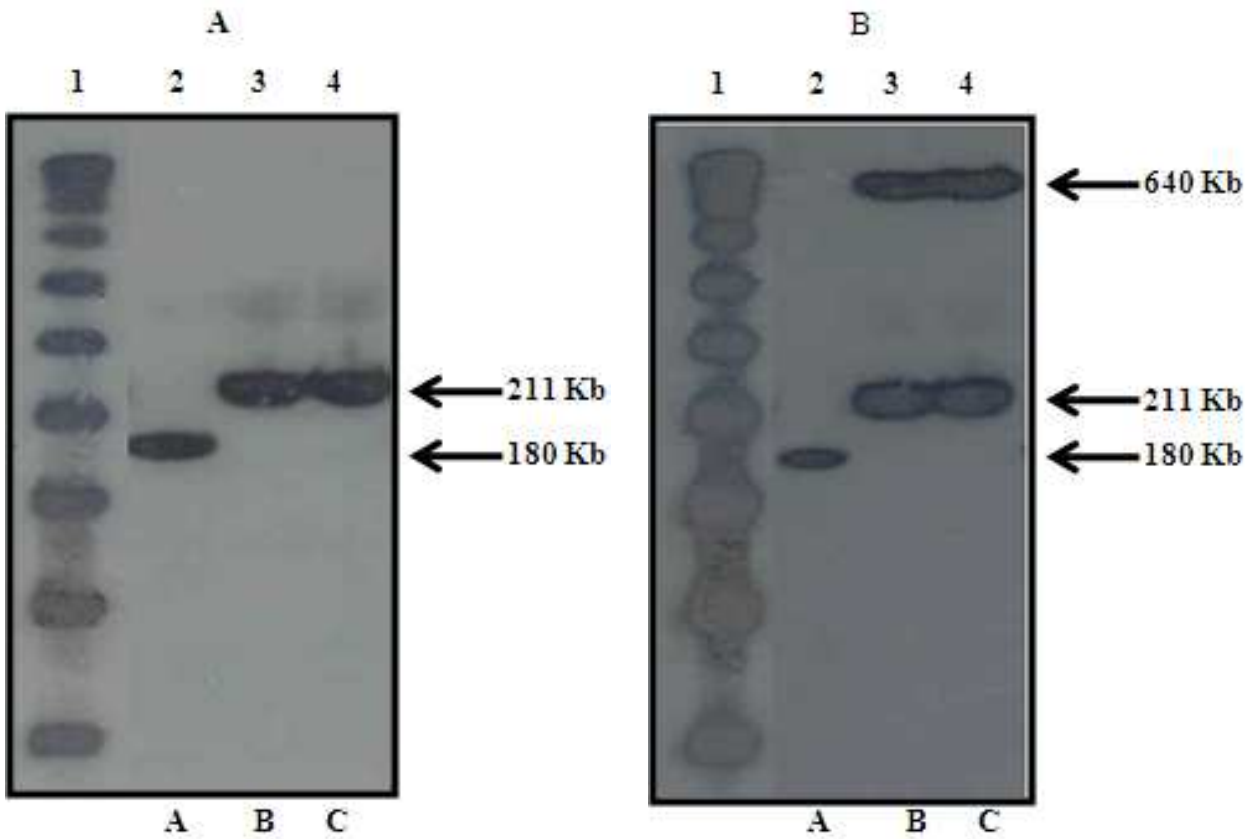


Fig. 3. (A) *SmaI-mecA* and (B) *SmaI* Tn554 patterns identified among the clones A, B and C of the Instituto Nacional de Cardiología “Dr. Ignacio Chávez, Mexico. Lane 1, molecular weight markers, lambda ladder; lane 2, 2CAR (pattern A); lane 3, 20CAR (pattern B) and lane 4, 44CAR (pattern C).

Two strains collected in this hospital 3CAR, (pattern B1) and 8CAR, (pattern B2) did not hybridize with the *mecA* DNA probe, interestingly both presented a low susceptibility to oxacillin and these isolates were subtypes of pattern B. The only difference was found in the *SmaI* hybridization fragment, which contains the *mecA* gene: in the two isolates, this fragment had a smaller molecular size (145 instead of 180 Kb) and did not react with the *mecA* probe, indicating a deletion of approximately 35 Kb, which must have included both the *mecA* gene and part of the *mec* element (Figure 4A and 4B). All the isolates accompanying clone A presented SCC*mec* type IV and sequence type 30 (ST30) whereas the

MRSA strains of clones B and C had SCCmec type II, sequences type 36 and 5 (ST36 and ST5) respectively, except B1 and B2 which did not amplify SCCmec, this isolates showed sequence type 30 (ST30) .

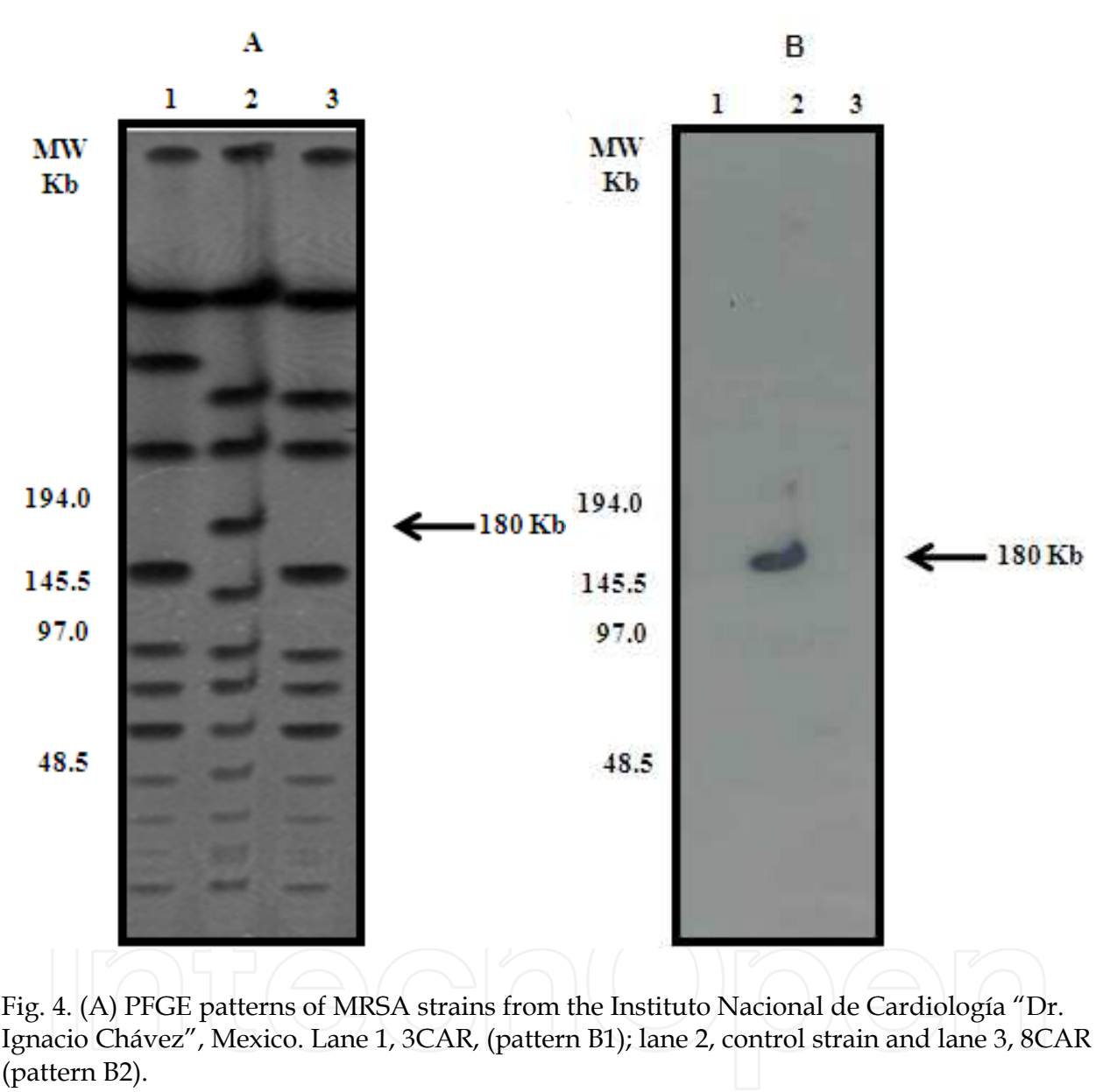


Fig. 4. (A) PFGE patterns of MRSA strains from the Instituto Nacional de Cardiología “Dr. Ignacio Chávez”, Mexico. Lane 1, 3CAR, (pattern B1); lane 2, control strain and lane 3, 8CAR (pattern B2).

3.2.3 Homology pattern

One isolate belonging to each type of clones (A, B and C) were compared to strains belonging to previously characterized MRSA clones, i. e., representatives of the pediatric clone and isolates belonging to the New York-Japan clone and also to other international pandemic clones, namely, the Iberian, Brazilian and EMRSA-16 clones (Figure 1). Clone C showed a high degree of similarity to the pediatric (85.5%) and the New York-Japan (89.5%) clones. Clones A and B showed a high degree of similarity to the EMRSA-16 (80%) clone (Figure 2).

4. Discussion

The emergence of strains resistant to methicillin and other antibacterial agents has become a major concern especially in the hospital environment, because of the higher mortality due to systemic methicillin-resistant *S. aureus* infections (Cosgrove et al., 2003; Handberger et al., 2011). Seven major pandemic MRSA (the so-called Brazilian, Hungarian, Iberian, New York-Japan, pediatric, EMRSA 16 and Berlin clone (EMRSA15) have been identified as the cause for the majority of hospital-acquired *S. aureus* infections in the world (Oliveira et al., 2002), indicating that they represent successful clones in terms of their ability to cause infections, persist and spread from one geographic zone to another, including across continents.

The combination of different molecular typing methods used in the present study allowed us to register epidemiologically relevant features of MRSA populations in the Instituto Nacional de Cardiología “Dr. Ignacio Chávez” in Mexico and document the coexistence of MRSA clones of international distribution.

All 90 strains were resistant to at least eleven antibiotics (amoxicillin, cefotaxime, cephalothin, cefazolin, chloramphenicol, imipenem, clindamycin, erythromycin and clarithromycin) in addition to penicillin and oxacillin and 94.4% were resistant to ciprofloxacin as well. The phenotypes of resistance to the antimicrobial agents are shown in the Table 1.

As a response to the emergence and worldwide spread of antibiotic-resistant *S. aureus* there was an urgent need for the creation of international surveillance systems with methodologies that could help hospital infection prevention and control such organisms. MRSA causing nosocomial infections have been reported in other hospitals in Mexico showing a wide geographic spread of MRSA specific clones (Aires de Sousa et al., 2001; Echaniz et al., 2006; Velazquez et al. 2004) similar spread has been observed by other clones in USA and Europe (Da Silva, 2003; Johnson, 2011; Oliveira & de Lencastre 2002).

Interestingly, only three PFGE types were found during the period of the study, designed A, B and C. Previous studies had documented that MRSA clones may spread in and between hospitals, cities and countries and even intercontinental spread may occur (Auken et al., 2002; Nübel et al., 2010). The multiresistant clone C (New York/Japan clone) was present in more than 50% of MRSA that were recovered from a variety of infections sites and hospital wards. Previously, this clone had already been reported in two hospitals in Mexico: Hospital Civil de Guadalajara “Fray Antonio Alcalde” and Hospital de Pediatría del Centro Medico Nacional Siglo XXI-IMSS and it has been circulating in these hospitals since 1999, and 2001 respectively (Echaniz et al., 2006; Velazquez et al. 2004). The results of these studies showed that clone C (New York/Japan clone) had, sequence type 5 and SCC_{mec} type II. In this study we found that pattern C was very similar (89.5%) to the multiresistant New York-Japan clone (Figure.2), which correspond to our last year's results, proving with this the capacity of this clone to persists for long periods of time within the hospitals; as well as its capacity to spread to other hospitals (epidemic clone). whose evidence is the existence of this clone in other hospital of third level in Mexico Instituto Nacional de Cancerología (INCan). It is important to mention that the existence of clone C (New York/Japan clone) had not been present in the INCan before 2006 (Cornejo et al., 2010). All these results are of relevant importance if we consider that the first high-level VRSA (vancomycin-resistant *S. aureus*) (MIC = 1024 µg/mL vancomycin), belonged to the New York lineage (Weigel et al., 2003) and the fact that the descending MRSA strains of this clone are circulating in our

population, together with the few means of antibiotic restriction it could represent a potential short term risk for the VRSA appearance in the hospitals of our country. Clone A and B were only found in the isolates in 2002, these clones showed a high degree of similarity to the EMRSA-16 clone, this clone is one of the dominant types of MRSA found in a UK hospital (Moore & Lindsay, 2002) and was widely disseminated in Canada (Simor et al., 2002), Greece (Aires de Sousa et al., 2003) and Mexico (Aires de Sousa et al., 2001). Interestingly, both clones (A and B) are very similar (95%) (Figure 2), nevertheless, clone A showed a reduced resistance profile as clone B, and this is because of the existence of the *SCCmec* IV in these isolates, this chromosomal cassette was found in relation to isolated MRSA strains in the community (CA-MRSA) (Coombs et al., 2011). Different reports of several infections caused by CA-MRSA in Latin America (Uruguay, Rio de Janeiro, Colombia, Argentina and Mexico) have been published (Alvaréz et al., 2006; Ma et al., 2005; Reyes et al., 2009; Ribeiro et al., 2005; Velazquez et al., 2011). All pattern of PFGE of the clones A, B and C showed subtypes. Probably the PFGE subtypes indicate the continued evolutionary divergence of these clones during its massive geographic expansion.

Relative genetic instability of the *mecA* element was observed in two strains and this was associated with an apparent deletion of the *mec* element, these isolates were very similar to profile B (B1 and B2) and presented a low susceptibility to oxacillin. In the literature there are reports of *S. aureus* strains with low-level methicillin resistance (MIC 2-4 µg/mL) which are not associated to the presence of *mecA* gene, Tomasz et al. reported one class of borderline methicillin-resistant strains having PBP1 and PBP2 with altered methicillin-binding affinities and overproduction of PBP4 (Tomasz et al., 1989). Another class of low-susceptibility has been reported and was attributed to overproduction of penicillinase (McDougal & Thornsberry, 1986). Hackbarth et al. studied the nucleotide sequence of the PBP2 gene and identified a point mutation near the penicillin-binding motive of transpeptidase (Hackbarth et al., 1995). An MRSA clinical strain with significant methicillin resistance (MIC 64µg/mL) despite absence of *mec A* was reported (Yoshida et al., 2003).

5. Conclusion

The combination of molecular typing methods (PFGE, *mecA*, Tn554 probes, *SCCmec*, and MLST) with epidemiologic and clinical information allows the detection of MRSA clusters and outbreaks and therefore provides a rationale for appropriate infection control intervention. Our study emphasizes the need of national and international collaborations to monitor the spread of current epidemic strains as well as the emergence of new ones in our country. The mechanisms of spread in different areas are poorly understood and further studies are necessary to understand the dynamics involved in the predominance of unique MRSA clones.

6. Acknowledgment

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7. References

Aires de Sousa, M. Miragaia, M. Santos, I. Avila, S. Adamson, I. Casagrande, S. Brandileone, C. Palacio, R. Dell'Acqua, L. Hortal, M. Camou, T. Rossi, A. Velazquez, ME.

- Echaniz, G. Solorzano, F. Heitmann, I. & de Lencastre, H. (2001). Three-year assessment of methicillin-resistant *Staphylococcus aureus* clones in Latin America from 1996 to 1998. *Journal Clinical Microbiology*, Vol.39, No.6, (June 2001), pp. 2197-3205, ISSN 0095-1137
- Aires de Sousa, M. Bartzavali, C. Spiliopoulou, I. Santos, I. Crisostomo, I & de Lencastre, H. (2003). Two international methicillin-resistant *Staphylococcus aureus* clones endemic in a university hospital in Patras, Greece. *Journal of Clinical Microbiology*, Vol.41, No.5, (May 2003), pp. 2027-2031, ISSN 0095-1137
- Alpuche, C. Avila, C. Espinoza, LE. Gómez, D. & Santos, I. (1989). Perfiles de sensibilidad antimicrobiana de *Staphylococcus aureus* en un hospital pediátrico: prevalencia de resistencia a meticilina. *Boletín Medico del Hospital Infantil de México*, Vol.11, pp. 697-699. ISSN 1665-1146
- Alvaréz, C. Barrientes, O. Leal, A. Contreras, G. Barrero, L. Rincón, S. Díaz, L. Vanegas, N. & Arias, C. (2006). Community-associated methicillin-resistant *Staphylococcus aureus*, Colombia. *Emerging Infectious Diseases*, Vol.12, No.12, (December 2006), pp. 2000-2001, ISSN 1080-6040
- Appelbaum, P. (2007). Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *International Journal of Antimicrobial agents*, Vol.30, No.4, (November 2007), pp. 398-408, ISSN 0924-8579
- Auken, H. Ganner, M. Murchan, S. Cookson, D. & Johnson, P. (2002). A new UK strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-17) resistant to multiple antibiotics. *Journal Antimicrobial Chemotherapy*, Vol.50, No.2, (August 2002), pp.171-175, ISSN 0305-7453
- Breurec, S. Zriouil, . Fall, C. Boisier, P. Brisse, S. Djibo, S. Etienne, J. Fonkoua, M Perrier-Gros-Claude, J. Pouillot, R. Ramarokoto, C. Randrianirina, F. Tall, A. Thiberge, J, Working Group on *Staphylococcus aureus* infections, Laurent, F. & Garin, B. (2011) Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. *Clinical Microbiology and Infectious Diseases*, Vol.17, No.2, (February 2011), pp. 160-165, ISSN 1198-743X
- Calderón, E. Espinoza, LE. & Avila, R. (2002). Epidemiology of drug resistance: The case of *Staphylococcus aureus* and coagulase negative staphylococci infections. *Salud Pública de México*, Vol.44, No.2, (October 2001), pp. 108-12. ISSN 0036-3634
- Chávez, B. (2004). Infecciones intrahospitalarias ¿Que ha pasado en 23 años? *Enfermedades Infecciosas y Microbiología*, Vol.24, No.3, (July-September 2004), pp. 89-92.
- Chung, M. de Lencastre, H. Matthews, P. Tomasz, A. Adamsson, I. Aires de Sousa, M. Camou, T. Cocuzza, C. Corso, A. Couto, I. Dominguez, A. Gniadkowski, M. Goering, R. Gomes, A. Kikuchi, K. Marchese, A. Mato, R. Melter, O. Oliveira, D. Palacio, R. Sá-Leão, R. Santos, I. Song, J. Tassios, P. & Villari, P. (2000). Molecular typing of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis: comparison of results obtained in a multilaboratory effort using identical protocols and MRSA strains. *Microbial Drug Resistance*, Vol.6, No.3, (October 2000), pp. 189-98, ISSN 1076-6294
- [CLSI] Clinical and Laboratory Standards Institute. (2009). Performance Standards for Antimicrobial Susceptibility Testing. Nineteenth Informational Supplement; M100-S19. *Clinical and Laboratory Standards Institute*, Vol.29, No.3, (January 2009), pp. 1-149, ISBN 1-56238-690-5, Wyne, Pennsylvania.

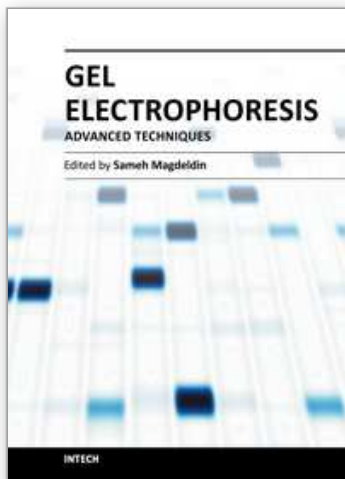
- Coombs, G. Monecke, S. Pearson, J. Tan, H. Chew, Y. Wilson, L. Ehricht, R. O'Brien, F. & Christiansen, K. (2011). Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region. *BMC Microbiology*, Vol.11, (September 2011), pp. 1-212 ISSN 1471-2180
- Cornejo, P. Volkow, P. Sifuentes, J. Echániz, G. Díaz, A. Velázquez, C. Bobadilla M. Gordillo, P. & Velazquez, ME. (2010). Tracing the source of an outbreak of methicillin-resistant *Staphylococcus aureus* in a tertiary-care oncology hospital by epidemiology and molecular methods. *Microbial Drug Resistance*, Vol.16, No.3, (September 2010), pp. 203-208, ISSN 1076-6294
- Cosgrove, S. Sakoulas, G. Perencevich, E. Schwaber, M. Karchmer, A. & Carmeli, Y. (2003). Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta analysis. *Clinical Infectious Diseases*, Vol.36, No.1, (January 2003), pp. 53-59, ISSN 1058-4838
- Creamer, E. Galvin, S. Dolan, A. Sherlock, O. Dimitrov, B. Fitzgerald-Hughes. D. Thomas, T. Walsh, J. Moore, J. Smyth, E. Shore, A. Sullivan. D. Kinnevey, P. O'Lorcain, P. Cunney, R. Coleman, D. & Humphreys, H. (2011). Evaluation of screening risk and nonrisk patients for methicillin-resistant *Staphylococcus aureus* on admission in an acute care hospital. *American Journal of Infection Control*, Vol.30, [Epub ahead of print] ISSN 0196-6553
- Da Silva, M. Silva, M. Wisplinghoff, H. Hall, G. Tallent. S. Wallance, S. Edmond, M. Figueiredo, A. & Wenzel. R. (2003). Clonal spread of methicillin-resistant *Staphylococcus aureus* in a large geographic area of the United States. *The Journal of Hospital Infection*, Vol.53, No.2, (February 2003), pp. 103-110, ISSN 0195-6701
- de Lencastre, H. Couto, I. Santos, I. Melo, J. Torres, A. & Tomasz, A. (1994). Methicillin-resistant *Staphylococcus aureus* disease in a Portuguese hospital: characterization of clonal types by a combination of DNA typing methods. *European Journal of Clinical Microbiology and Infectious Diseases*, Vol.13, No.1, (January 1994), pp. 64-73, ISSN 0934-9723
- Deurenberg, R. Nulens, E. Valvatne, H. Sebastian, S. Driessen, C. Craeghs, J. De Brauwert, E. Heising, B. Kraat, Y. Riebe, J. Stals, F. Trienekens, T. Scheres, J. Friedrich, A. van Tiel, F. Beisser, P. & Stobberingh, E. (2009). Cross-border dissemination of methicillin-resistant *Staphylococcus aureus*, Euregio Meuse-Rhin region. *Emerging infectious diseases*, Vol.15, No.5, (May 2009), pp. 727-734, ISSN 1080-604
- Diekema, D. Pfaller, M. Schmitz, F. Smayevsky, J. Bell, J. Jones, R. & Beach, M. (2001). Survey of infections due to *Staphylococcus* species: frequency, occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clinical of Infectious Diseases*, Vol.15, No.32, (May 2001), pp. S114-132, ISSN 1058-4838
- Echaniz, G. Velazquez, ME. Aires de Sousa, M. Morfin, R. Rodríguez-Noriega, E. Carnalla, N. Esparza, S. & de Lencastre H. (2006). Molecular characterization of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone in a Mexican hospital (1999-2003). *Clinical Microbiology and Infectious Diseases*, Vol.12, No.1, (January 2006), pp. 12-28, ISSN 1198-743X
- Enright, M. Day, N. Davies, C. Peacock, S. & Spratt, B. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of

- Staphylococcus aureus*. *Journal of Clinical Microbiology*, Vol.38, No.3, (March 2000), pp. 1008-1015, ISSN 0095-1137
- Enright, M. Robinson, D. Randle, G. Feil, E. Grundmann, H. & Spratt, B. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 99, No.11, (May 2002), pp. 7687-7692, ISSN 0027-8424
- Goldstein, F. (2007). The potential clinical impact of low-level antibiotic resistance in *Staphylococcus aureus*. *Journal Antimicrobial Chemotherapy*, Vol.59, No.1, (January 2007), pp. 1-4, ISSN 0305-7453.
- Hackbarth, C. Kocagoz, T. Kocagoz, S. & Chambers. H. (1995). Point mutation in *Staphylococcus aureus* PBP2 gene affects penicillin-binding kinetics and is associated with resistance. *Antimicrobial Agents and Chemotherapy*, Vol.39, No.1, (January 1995), pp. 103-106, ISSN 0066-4804
- Hanberger, H. Walther, S. Leone, M. Barie, P. Rello, J. Lipman J. Marshall, J. Anzueto A. Sakr, Y. Pickkers, P. Engoren, M. Vincent, J. & EPIC II Group of investigators. (2011). Increased mortality associated with methicillin-resistant *Staphylococcus aureus* (MRSA) infection in the intensive care unit: results from the EPIC II study. *International Journal of Antimicrobial Agents*, Vol.38, No.4, (October 2011), pp. 331-335, ISSN 0924-8579
- Johnson, A. (2011). Methicillin-resistant *Staphylococcus aureus*: the European landscape. *The Journal of antimicrobial chemotherapy*, Vol.66, No.4, (May 2011), pp. 43-48, ISSN 0305-7453
- Ma, XX. Galiana, A. Pedreira, W. Mowszowicz, M. Christophersen, I. Machiavello, S. Lopez, L. Benaderet, S. Buela, F. Vincentino, W. Albini, M. Bertaux, O. Constenla, I. Bagnulo, H. Llosa, L. Ito, T. & Hiramatsu, K. (2005). Community-acquired methicillin-resistant *Staphylococcus aureus*, Uruguay. *Emerging Infectious Diseases*, Vol.11, No. 6, (June 2005), pp. 973-976, ISSN 1080-604
- McDougal, L. & Thornberry, C. (1986). The role of β -lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *Journal of Clinical Microbiology*, Vol.23, No.5, (May 1986), pp. 832-839, ISSN 0095-1137
- McDougal, L. Steward, C. Killgore, G. Chaitram, J. McAllister, S. & Tenover, F. (2003). Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *Journal of Clinical Microbiology*, Vol.41, No.11, (November 2003), pp. 5113-5120, ISSN 0095-1137
- Moore, P. & Lindsay, J. (2002). Molecular characterization of the dominant UK methicillin-resistant *Staphylococcus aureus* strain, EMRSA-15 and EMRSA-16. *Journal of Medical Microbiology*, Vol.51, No.6, (June 2002), pp. 516-521, ISSN 0022-2615
- Murchan, S. Kaufmann, M. Deplano, A. de Ryck, R. Struelens, M. Zinn, C. Füssing, V. Salmenlinna, S. Vuopio-Varkila, J. El Solh, N. Cuny, C. Witte, W. Tassios, P. Legakis, N. van Leeuwen, W. van belkum, A. Vindel, A. Laconcha, I. Garaizar, J. Haeggman, S. Olsson-Liljequist, B. Ransjö, U. Coombes, G. & Cookson, B. (2003). Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *Journal of Clinical Microbiology*, Vol.41, No.4, (April 2003), pp. 1574-85, ISSN 0095-1137

- Nübel, U. Dordel, J. Kurt, K. Strommenger, B. Westh, H. Shukla, S. Zemlicková, H. Leblois, R. Wirth, T. Jombart, T. Balloux, F & Witte, W. (2010). A timescale for evolution, population expansion, and spatial spread of an emerging clone of methicillin-resistant *Staphylococcus aureus*. *PLoS pathogens*, Vol.6, No.4, (April 2010), pp. 1-12, ISSN 1553-7366
- Oliveira, D. Tomasz, A. & de Lencastre, H. (2001). The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. *Microbial Drug Resistance*, Vol.7, No.4, (December 2001), pp. 349-361, ISSN 1076-6294
- Oliveira, D. & de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, Vol.46, No.7, (July 2002), pp. 2155-2161, ISSN 0066-4804
- Oliveira, D. Tomasz, A. & de Lencastre, H. (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *The Lancet infectious diseases*, Vol.2, No.3, (March 2002), pp. 180-189, ISSN 1473-3099
- Rebmann, T. & Aureden, K. (2011). Preventing methicillin-resistant *Staphylococcus aureus* transmission in hospitals: an Executive Summary of the Association for Professionals in Infection Control and Epidemiology, Inc, Elimination Guide. *American Journal of Infection Control*, Vol.39, No.7, (September 2011), pp. 595-598, ISSN 0196-6553
- Reyes, J. Rincón, S. Díaz, L. Panesso, D. Contreras, G. Zurita, J. Carrillo, C. Rizzi, A. Guzmán, M. Adachi, J. Chowdhury, S. Murray, B. & Arias, C. (2009). Dissemination of methicillin resistant *Staphylococcus aureus* USA 300 sequence type 8 lineage in Latin America. *Clinical of Infectious Diseases*, Vol.49, No. 12, (December 2009), pp. 1861-1867, ISSN 1058-4838
- Ribeiro, A. Dias, C. Silva-Carvalho, M. Berquó, L. Ferreira, F. Santos, R. Ferreira-Carvalho, B. & Figueiredo, A. (2005). First report of infection with community-acquired methicillin-resistant *Staphylococcus aureus* in South America. *Journal of Clinical Microbiology*, Vol.43, No.4, (April 2005), pp. 1985-1988, ISSN 0095-1137
- Simor, A, Ofner-Agostini, M. Bryce, E. McGeer, A. Paton, S. & Mulvey, M. (2002). Laboratory characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: results of 5 years of national surveillance, 1995-1999. *The Journal of Infectious Disiases*, Vol.186, No.5, (September 2002), pp. 652-660, ISSN 0022-1899
- Tenover, F. Arbeit, R. Goering, R. Mickelsen, P. Murray, B. Persing, D. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology*, Vol.33, No.9, (September 1995), pp. 2233-2239, ISSN 0095-1137
- Tenover, F. & Gaynes, R. The epidemiology of *Staphylococcus* infections, In: V. A. Fischetti, R. Novick, J. Ferretti, D. Portnoy, and J. Rood, (Ed.). (2000). *Gram-positive pathogens*. American Society for Microbiology, Washington, DC. pp. 414-421
- Tomasz, A. Drugeon, H. de Lencastre, H. Jabes, D. McDougall, D. & Bille, J. (1989). New mechanism for methicillin resistance in *Staphylococcus aureus*: clinical isolates that lack the PBP2a gene and contain normal penicillin-binding proteins with modified

- penicillin-binding capacity. *Antimicrobial Agents and Chemotherapy*, Vol.33, No.11, (November 1989), pp. 1869-1874, ISSN 0066-4804
- Velazquez, ME. Aires de Sousa, M. Echaniz, G. Solórzano, F. Miranda, G. Silva, J, & de Lencastre, H. (2004). Surveillance of methicillin- resistant *S. aureus* in a pediatric hospital in Mexico City during a 7-year period (1997 to 2003): clonal evolution and impact of infection control. *Journal of Clinical Microbiology*, Vol.42, No.8, (August 2004), pp. 3877-3880, ISSN 0095-1137
- Velazquez, ME. Ayala, J. Carnalla, N. Soto, A. Guajardo, C. & Echaniz, G. (2011). First report of community-associated methicillin-resistant *Staphylococcus aureus* (USA300) in Mexico. *Journal of Clinical Microbiology*, Vol.49, No.8, (August 2011), pp. 3099-3100, ISSN 0095-1137
- Weigel, L. Clewell, D. Gill, S. Clark, N. McDougal, L. Flannaga, S. Kolonay, J. Shetty, J. Killgore, G. & Tenover, F. (2003). Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science*, Vol.302, No.5650, (November 2003), pp. 1569-1571, ISSN 0036-8075
- Yoshida, R. Kuwahara-Arai, K. Baba, T. Cui, L. Richardson, J. & Hiramatsu, K. (2003). Physiological and molecular analysis of a *mecA*-negative *Staphylococcus aureus* clinical strain that expresses heterogeneous methicillin-resistance. *The Journal Antimicrobial Chemotherapy*, Vol.51, No.2, (February 2003), pp. 247-255, ISSN 0305-7453

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As a basic concept, gel electrophoresis is a biotechnology technique in which macromolecules such as DNA, RNA or protein are fractionated according to their physical properties such as molecular weight or charge. These molecules are forced through a porous gel matrix under electric field enabling uncounted applications and uses. Delivered between your hands, a second book of this Gel electrophoresis series (Gel Electrophoresis- Advanced Techniques) covers a part, but not all, applications of this versatile technique in both medical and life science fields. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

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