

Pulsed-field gel electrophoresis analysis, toxin types and plasmid profile for Methicillin resistant Staphylococcus aureus

賴潔賢、張基郁；曾浩洋

E-mail: 9417972@mail.dyu.edu.tw

ABSTRACT

ABSTRACT Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens infected in community hospitals, and the incidence of MRSA strains is increasing and becomes a common problem among the hospitals in Taiwan recently. In this study, the polymer chain reaction (PCR) was used to detect the *mecA* resistant gene, and the *Sma I* restriction enzyme was used to digest the chromosomal DNA of MRSA. In addition, the plasmid profile and the pulsed field gel electrophoresis (PFGE) analysis were used to investigate the distribution of the toxin types for MRSA strains from Veterinary General Hospital (VGH) in Taichung and Center for Disease Control, Taiwan, ROC. (TCDC), and trace the MRSA infection in the hospitals. The PCR results showed that 45 (59.21 %) clinical strains from VGH and 23 (16.3 %) human *S. aureus* strains from food-poisoning cases provided by TCDC possessed *mecA* resistant gene. The ratio of MRSA strains from hospitals was higher than that from food-poisoning cases. As for the distribution for enterotoxin types, it was found that the major enterotoxin types for the strains from hospitals and food-poisoning cases were the enterotoxin A and B, respectively. From the results of the plasmid profiles for MRSA strains, totally 29 distinct plasmid types were found. Of them, no co-shared types were found in the isolates from hospitals and food-poisoning cases. The PFGE results showed that 18 PFGE types were found in the 68 MRSA strains from hospitals and food-poisoning cases in 2003, and the major types for the strains from hospitals were different from those for the strains from food-poisoning cases. In addition, the clinical strains isolated between 1998 and 2003 were found high similarity in PFGE type. The results of this study reveal that the MRSA with same PFGE type still prevail in hospitals.

Keywords : Methicillin-resistant *Staphylococcus aureus*, Pulsed-field gel electrophoresis, Enterotoxin types, Plasmid profiles.

Table of Contents

系統編號: 093DYU00111004 出版年: 94 研究生: 賴潔賢 研究生(英文姓名): Chieh-hsien Lai 論文名稱: 台灣地區1998-2003年間食品中毒案例及臨床樣本分離之Methicillin抗藥性金黃色葡萄球菌菌株脈衝式膠體電泳、毒素型分佈及質體圖譜之分析
英文論文名稱: Pulsed-field gel electrophoresis analysis, toxin types and plasmid profile for Methicillin resistant *Staphylococcus aureus* strains isolated in Taiwan from food-poisoning cases and clinical samples between 1998-2003 指導教授: 張基郁；曾浩洋 指導教授(英文姓名): Chi-Yue Chang ; Hau-Yang Tsen 學位類別: 碩士 校院名稱: 大葉大學 系所名稱: 生物產業科技學系 學號: R9214010 學年度: 93 語文別: 中文 論文頁數: 91 關鍵詞: methicillin抗藥性金黃色葡萄球菌、脈衝式膠體電泳、毒素型分佈、質體圖譜 英文關鍵詞: Methicillin-resistant *Staphylococcus aureus*, Pulsed-field gel electrophoresis, Enterotoxin types, Plasmid profiles. 被引用次數: 0 [摘要] 中文摘要 Methicillin抗藥性金黃色葡萄球菌(Methicillin-resistant *Staphylococcus aureus*, MRSA)為造成院內感染之主要病原菌之一，所導致之各種感染有逐漸增加之趨勢，為國內各醫院之共同問題。本研究利用聚合鏈鎖反應檢測*mec A*抗性基因以及利用*Sma I*限制性剪切MRSA菌株染色體DNA並配合脈衝式膠體電泳分析、質體圖譜分析方法及毒素型分佈探討不同來源及不同時間MRSA菌株之相關性，以瞭解菌株移轉情形及醫院內的感染情形。本研究所收集之226株金黃色葡萄球菌分別來自台中榮民總醫院以及行政院衛生署疾病管制局中部檢驗站之食品中毒案例人體分離檢體。研究結果顯示，以PCR方法檢測醫院來源之臨床金黃色葡萄球菌菌株，有45株菌帶有*mecA*抗性基因(59.21%)，而在食品中毒案例人體分離之菌株中則有25株菌(16.67%)帶有*mec A*抗性基因，結果顯示，醫院來源之金黃色葡萄球菌有較高的MRSA分離比例；在毒素型分佈方面，醫院臨床菌株所帶有之腸毒素類以A型腸毒素為主；而食品中毒案例人體分離菌株則以帶有B型腸毒素居多。在質體圖譜分析方面可將MRSA分為29種質體圖譜類型，醫院臨床與食品中毒案例來源之間沒有完全相同的質體圖譜，因此可配合其他分型方法如PFGE等用以調查MRSA菌株之間的相關性。在PFGE分型結果方面，2003年醫院臨床來源與食品中毒案例人體分離之MRSA菌株可得18種分型(type)，由分型結果得知醫院來源主要流行菌株與食品中毒來源主要流行菌株並不相同且主要流行菌株之間並無相互流傳情形產生。另一方面與本研究亦利用PFGE對1998及2003年醫院臨床分離來源之MRSA菌株進行分型，結果顯示2003年醫院來源主要流行菌株型態與1998年的流行菌株型態相似度極高。由此可推論此類型的MRSA菌株為此二個時期台灣地區醫院的主要流行菌株。由研究結果得知，相同分型的菌株所造成的MRSA感染情形依然在醫院內流傳。 [英文摘要] ABSTRACT Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens infected in community hospitals, and the incidence of MRSA strains is increasing and becomes a common problem among the hospitals in Taiwan recently. In this study, the polymer chain reaction (PCR) was used to

detect the *mecA* resistant gene, and the *Sma* I restriction enzyme was used to digest the chromosomal DNA of MRSA. In addition, the plasmid profile and the pulsed field gel electrophoresis (PFGE) analysis were used to investigate the distribution of the toxin types for MRSA strains from Veterinary General Hospital (VGH) in Taichung and Center for Disease Control, Taiwan, ROC. (CDC), and trace the MRSA infection in the hospitals. The PCR results showed that 45 (59.21 %) clinical strains from VGH and 23 (16.3 %) human *S. aureus* strains from food-poisoning cases provided by CDC possessed *mecA* resistant gene. The ratio of MRSA strains from hospitals was higher than that from food-poisoning cases. As for the distribution for enterotoxin types, it was found that the major enterotoxin types for the strains from hospitals and food-poisoning cases were the enterotoxin A and B, respectively. From the results of the plasmid profiles for MRSA strains, totally 29 distinct plasmid types were found. Of them, no co-shared types were found in the isolates from hospitals and food-poisoning cases. The PFGE results showed that 18 PFGE types were found in the 68 MRSA strains from hospitals and food-poisoning cases in 2003, and the major types for the strains from hospitals were different from those for the strains from food-poisoning cases. In addition, the clinical strains isolated between 1998 and 2003 were found high similarity in PFGE type. The results of this study reveal that the MRSA with same PFGE type still prevail in hospitals.

[論文目次]	1 第二節 金黃色葡萄球菌檢測方式	3 第三節 金黃色葡萄球菌腸毒素分佈情形
目錄 封面內頁 簽名頁 授權書 -iii- 中文摘要 -iv- 英文摘要 -vi- 誌謝 -viii- 目錄 -ix- 圖目錄 -xi- 表目錄 -xii- 第壹章 文獻回顧	6 第五節 抗生素之介紹	7 第六節 金黃色葡萄球菌對methicillin之抗藥性
第一節 葡萄球菌的特色及所造成的疾病	9 第七節 抗生素感受性試驗	12 第八節 聚合?
鏈鎖反應	12 第九節 質體圖譜之分析	14 第十節 脈衝式膠體電泳
15 第十一節 影響脈衝式膠體電泳的因素	18 第十二節 脈衝式膠體電泳patterns判定標準	20 第貳章 材料方法
第一節 菌種來源	23 第二節 藥品	24 第三節 緩衝液及試劑
24 第四節 儀器	26 第五節 研究方法	27 第參章 結果討論
第一節 醫院臨床來源與食品中毒案例人體所分離出之 <i>S. aureus</i> 中Methicillin抗藥性金黃色葡萄球菌(MRSA)之探討	32 第二節 Methicillin抗藥性金黃色葡萄球菌之產腸毒素類型分佈	33 第三節 醫院臨床來源與食品中毒案例人體分離之Methicillin抗藥性金黃色葡萄球菌(MRSA)之質體圖譜之分析及探討
36 第四節 利用脈衝式膠體電泳對MRSA進行分子分類	37 第五節 綜合PFGE及質體圖譜等分型結果	39 第四章 結論
41 參考文獻	42 圖目錄 圖一 抗生素的抗性機制	58 圖二 Sma?剪切2003年自台中榮總及中檢站之methicillin抗藥性金黃色葡萄球菌gnomic DNA之PFGE圖譜
59 圖三 <i>Staphylococcus aureus</i> 分離株以SmaI作用後所得圖譜之樹狀圖	60 表目錄 表一 民國70年至93年台灣地區食品中毒案件病因物質分類表	61 表二 以脈衝式膠體電泳基因型態鑑定之準則
62 表三 台中榮總臨床檢體分離之Methicillin抗藥性金黃色葡萄球菌菌株表	63 表四 2001-2003年中檢站食品中毒案件人體分離之Methicillin抗藥性金黃色葡萄球菌菌株表	71 表五 Methicillin紙錠劑量及抗性判定標準
72 表六 本研究所使用之引子組條件	72 表七 2003年由台中榮總臨床樣本及中檢站食品中毒案例人體分離之Methicillin抗藥性金黃色葡萄球菌菌株基因型及表現型	73 表八 2003年榮總臨床檢體分離之Methicillin抗藥性金黃色葡萄球菌腸毒素型基因分佈
74 表九 2003年中檢站食品中毒案件臨床檢體分離之Methicillin抗藥性金黃色葡萄球菌腸毒素型基因分佈	76 表十 由台中榮總及中檢站收集之MRSA質體譜之比較	77 表十一 Methicillin抗藥性金黃色葡萄球菌以Sma I剪切之脈衝式膠體電泳(PFGE)圖譜類型及分佈
79 表十二 2003年Methicillin抗藥性金黃色葡萄球菌之脈衝式膠體電泳及質體圖譜分型	80	

REFERENCES

- 參考文獻 1. 張立同。2004。金黃色葡萄球菌新型腸毒素K、L、M基因引子之設計及其應用於食品及臨床菌株腸毒素型之分佈調查。碩士論文。中興大學食品科學研究所。台中。2. 李詩益。2004。台灣地區萬古黴素中度抗藥性金黃色葡萄球菌之出現與其特性之探討。碩士論文。國防大學國防醫學院病理暨寄生蟲學研究所。台北。3. 林麗秋。2003。抗生素在畜牧產業之應用。碩士論文。大葉大學食品工程研究所。彰化。4. 張玉芳。1998。Methicillin抗藥性金黃色葡萄球菌之毒素型分佈、抗生素型、質體圖譜及脈衝式電場膠體電泳之分析。碩士論文。中興大學食品科學研究所。台中。5. 張美瑩。1996。臺灣地區收集自食品及臨床樣品來源之金黃色葡萄球菌其脈衝式電場膠體電泳圖譜之多樣性。碩士論文。中興大學食品科學研究所。台中。6. 張進祿。2003。臨床使用抗生素手冊(第二版)。天津科學技術出版社。台北。7. 陳依雯，張靜美，嚴小燕等。1999。某醫學中心各加護中心院內感染金黃色葡萄球菌之調查。感控雜誌。9:245-53. 8. 陳孟娟。1999。院內抗藥性菌種感染之防護措施。感控雜誌。9:98-107. 9. 程秉輝。2004。應用脈衝式電泳探討*Staphylococcus aureus*多重抗藥性之基因型變化。碩士論文。嘉義大學生物科技研究所。嘉義。10. 虞積凱。1995。食品與臨床樣品分離之葡萄球菌腸毒素型分佈及腸毒性金黃色葡萄球菌之分子分類研究。博士論文。中興大學食品科學研究所。台中。11. 蘇遠志。1999。應用微生物學。華香園出版社。台北。12. Abigail, A. S. and Whitt, D. D. 2001. *Bacterial pathogenesis: a molecular approach*, second edition. p. 216-231. ASM Press, Washington, D. C. 13. Al-Thawadi, S. I., Kessie, G., Dela Cruz, D. and Al-Ahdal, M. N. 2003. A comparative study on the application of 3 molecular methods in epidemiological typing of bacterial isolates using MRSA as a prototype. Saudi. Med. J. 24:1317-1324. 14. Bannerman, T. L., Hancock, G. A., Tenover, F. C. and Miller, J. M. 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. 33:551-555. 15. Bayles K. W and J. J. Iandolo. 1989. Genetic and molecular

analyses of the gene encoding staphylococcal enterotoxin D. J. Bacteriol. 171: 4799-4806. 16. Betley, M.J., Borst, D.W. and Regassa, L.B. 1992. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: a comparative study of their molecular biology. Chem Immunol. 55:1-35. 17. Betley, M. J. and T. O. Harris. 1994. Staphylococcal enterotoxins: genetic characterization and relationship between structure and emetic activity. J. Food Microbiol. 11: 109-121. 18. Bej, A. K., Dicesare, J. L., Haff, L. and Altas, R. M. 1991. Detection of *Escherichia coli* and *Shigella* spp. in water by using polymerase chain reaction and gene probes for uid. Appl. Environ. Microbiol. 57: 1013- 1017. 19. Bessene, M. T., Luo, Q. A., Rotbart, H. A., Blaser, M. J. and Ellison, R.T. 3rd. 1990. Detection of *Listeria monocytogenes* by using the polymerase chain reaction. Appl. Environ. Microbiol. 56:2930-2932. 20. Birgit, S., Christiane, K., Guido, W. and Wolfgang, W. 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J. Clin. Microbiol. 41: 4089-4094. 21. Birren, B., Hood, L. and Lai, E. 1989. Pulsed field gel electrophoresis: studies of DNA migration made with the programmable, autonomously-controlled electrode electrophoresis system. Electrophoresis 10:302-309. 22. Birren, B.W., Lai, E., Clark, S.M., Hood, L. and Simon, M. I. 1988. Optimized conditions for pulsed field gel electrophoresis separations of DNA. Nucleic Acids Res. 16:7563-7582. 23. Boerlin P., Kuhnert, P., Hussy, D. and Schaellibaum, M. 2003. Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. J. Clin. Microbiol. 41:767-771. 24. Brakstad O. G., Aasbakk, K. and Maeland, J. A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol. 30:1654-1660. 25. Caddick, J.M., Hilton, A.C., Rollason, J., Lambert, P.A., Worthington, T. and Elliott, T.S. 2005 Molecular analysis of methicillin-resistant *Staphylococcus aureus* reveals an absence of plasmid DNA in multidrug-resistant isolates. FEMS Immunol Med Microbiol. 44:297-302. 26. Caprioli, A., Busani, L. and Helmuth, R. 2000. Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int J Antimicrob Agents. 14:295-301 27. Chang, S. C., Hsu, L. Y., Pan, H. J., Luh, K. T. and Hsieh, W. C. 1992. Epidemiologic investigation of nosocomial outbreak of methicillin-resistant *Staphylococcus aureus* by plasmid pattern analysis. J. Formos. Med. Assoc. 91:945-950. 28. Chiou, C., Wei, H. and Yang, L. 2000. Comparison of pulsed-field gel electrophoresis and coagulase gene restriction profile analysis techniques in the molecular typing of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 2186-2190. 29. Czop, J. K. and Bergdoll, M. S. 1974. Staphylococcal enterotoxin synthesis during the exponential, transitional, and stationary growth phases. Infect. Immun. 9: 229-235. 30. Enright, M. C. 2003. The evolution of a resistant pathogen-the case of MRSA. Cur. Opin. Pharmacol. 3:474-479. 31. Fitzgerald, J. R., Monday, S.R., Foster, T.J., Bohach, G. A., Hartigan, P.J., Meaney, W.J. and Smyth, C.J. 2001. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J. Bacteriol. 183: 63 – 70. 32. Freed, R.C., Evenson, M.L., Reiser, R.F. and Bergdoll, M.S. 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. Appl. Environ. Microbiol. 44: 1349-1355. 33. Fueyo, J.M., Martin, M.C., Gonzalez-Hevia, M.A. and Mendoza, M.C. 2001. Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Relations between genetic types and enterotoxins. Int J Food Microbiol. 67:139-45. 34. Fung-Tomo, J. H., Radelski, E. G., Denbleyker, K., Bonner, D. P. and Kessler, R. E. 1991. Emergence of homogeneously methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 29: 2880-2883. 35. Gaskell, M. E. and Khan, S.A. 1988. Regulation of the enterotoxin B gene in *Staphylococcus aureus*. J. Biol. Chem. 263: 6276-6280. 36. Gemmill, R.M. (1991). Pulsed field gel electro- phoresis. In Advances of Electrophoresis (A. Chrambach, M.J. Dunn, and B.J. Radola, eds.), 4:1-48. VCH, Weinheim, Germany. 37. Genigeorgis, C. A. 1989. Present state of knowledge on staphylococcal intoxication. Int. J. Food Microbiol. 9: 327-360. 38. Gudding, R. 1983. Differentiation of Staphylococci on the basis of nuclelease properties. J. Clin. Microbiol. 18: 1098-1101. 39. Hayes, J. D. and Wolf, C. R. 1990. Molecular mechanisms of drug resistance. Biochem. J. 272: 281-295. 40. Hiramatsu, K., Aritaka, N., Hanaki, H., Kawasaki, S., Hosoda, Y., Hori, S., Fukuchi, Y. and Kobayashi, I. 1997. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet. 350:1670-1673. 41. Jaffe, R. I., Lane, J. D., Albury, S. V. and Niemeyer, D. M. 2000. Rapid extraction from and direct identification in clinical samples of methicillin-resistant Staphylococci using the PCR. J. Clin. Microbiol. 38:3407-3412. 42. Joppa, B., Li, S., Cole, S. and Gallagher, S. 2003. Pulsed field electrophoresis for separation of large DNA. Hoefer Scientific Instruments San Francisco, CA. 43. Katayama, Y., Ito, T., Hiramatsu, K. 2000. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 44:1549-55. 44. Kado, C.I. and Liu, S.T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-73. 45. Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H. 2005. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J. Vet. Med Sci. 67:107-10. 46. Kleppe, K., Ohtsuka, E., Kleppe, R., Molineux, I. and Khorana, H. G. 1971. Studies on polynucleotides. XCVI. Repair replications of short synthetic DNA's as catalyzed by DNA polymerases. J. Mol. Biol. 56:341-61. 47. Kluytmans, J., van Leeuwen, W., Goessens, W., Hollis, R., Messer, S., Herwaldt, L., Bruining, H., Heck, M., Rost, J. and van Leeuwen, N. 1995. Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. J Clin Microbiol. 33:1121-8. 48. Lam, T. J., Pengov, A., Schukken, A., Smit, Y. H. and Brand, A. 1995. The differentiation of *Staphylococcus aureus* from other Micrococcaceae isolated from bovine mammary glands. J. Appl. Bacteriol. 79:69-72. 49. Lee, H. J., Suh, J. T., Kim, Y. S., Lenz, W., Bierbaum, G. and Schaal, K. P. 2001. Typing and antimicrobial susceptibilities of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated in a hospital in Korea. J. Korean. Med. Sci. 16:381 – 385. 50. Lee, J.H. 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol. 69:6489-94. 51. Liu, S.L. and Sanderson, K.E. 1992. A physical map of the *Salmonella typhimurium* LT2 genome made by using XbaI analysis. J. Bacteriol. 174:1662-72. 52. Livermore, D. M. 2000. Antibiotic resistance in Staphylococci. Int. J. Antimicrobial Agents 16:S3 – S10. 53. Luzar, M. A., Coles, G. A. and Faller, B. 1990. *Staphylococcus aureus* nasal carriage

and infection patients on continuous ambulatory peritoneal dialysis. *N. Engl. J. Med.* 322:505-509. 54. Lyon, B. R. and Skurray, R. 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol. Rev.* 51:88-134. 55. Macfarlane, L., Walker, J., Borrow, R., Oppenheim, B. A. and Fox, A. J. 1999. Improved recognition of MRSA case clusters by the application of molecular subtyping using pulsed-field gel electrophoresis. *J. Hosp. Infect.* 41:29-37. 56. Mackay, A. D., Quick, A., Gillespie, S. H. and Kibbler, C. C. 1993. Coagulase-negative methicillin-resistant *Staphylococcus aureus* infection. *Lancet.* 342:492. 57. Maranan, M. C., Moreira, B. and Boyle-Vavra, S. 1997. Antimicrobial resistance in *Staphylococci*. Epidemiology, molecular mechanisms, and clinical relevance. *Infect. Dis. Clin. North Am.* 11:813-849. 58. Martin M. D., Paul M. O. and Patrick M. S. 2000. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev.*, p.16-34. 59. Martin, M.C., Fueyo, J.M., Gonzalez-Hevia, M.A. and Mendoza, M.C., 2004. Genetic procedures for identification of enterotoxigenic strains of *Staphylococcus aureus* from three food poisoning outbreaks. *Int J Food Microbiol.* 94:279-86. 60. McClelland, M., Jones, R., Patel, Y. and Nelson, M. 1987. Restriction endonucleases for pulsed field mapping of bacterial genomes. *Nucleic Acids Res.* 15: 5985-6005. 61. Miller, B.A., Reiser, R.F. and Bergdoll, M.S., 1978. Detection of staphylococcal enterotoxins A, B, C, D, and E in foods by radioimmunoassay, using staphylococcal cells containing protein A as immunoabsorbent. *Appl. Environ. Microbiol.* 36: 421-426. 62. National Committee for Clinical Laboratory Standards. 1998. Performance standards for antimicrobial susceptibility testing; eight informational supplements. NCCLS. PA. USA. 63. Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Decastelli, L., Mioni, R., Scuota, S., Bolzoni, G., Di Giannatale, E., Salinetti, AP., La Salandra, G., Bartoli, M., Zuccon, F., Pirino, T., Sias, S., Parisi, A., Quaglia, NC. and Celano, GV. 2005. Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *Int. J. Food biol.* 98:73-9. 64. Orwin P. M., Fitzgerald, J.R., Leung, D.Y., Gutierrez, L.A., Bohach, G.A. and Schlievert, P. M. 2003. Characterization of *Staphylococcus aureus* enterotoxin L. *Infec. Immun.* 71: 2916-2919. 65. Orwin, P. M., Leung, D. Y. M., Donahue, H. L., Novick, R. P. and Schlievert, P. M. 2001. Biochemical and biological properties of staphylococcal enterotoxin K. *Infect. Immun.* 69: 360-366. 66. Orwin, P. M., Leung, D. Y. M., Tripp, T.J., Bohach, G. A., Earhart, C. A. Ohlendorf, D. H. and Schlievert, P. M. 2002. Characterization of a novel staphylococcal enterotoxin-like superantigen, a member of the group V subfamily of pyrogenic toxins. *Biochemistry.* 41: 14033-14040. 67. Otero, A., Garcia, M. L., Farcia, M. C., Moreno, B. and Bergdoll, M. S. 1990. Production of staphylococcal enterotoxins C1 and C2 and thermonuclease throughout the growth cycle. *Appl. Environ. Microbiol.* 56: 555-559. 68. Park, C. E. and Szabo, R. 1986. Evaluation of the reversed passive latex agglutination (RPLA) test kits for detection of staphylococcal enterotoxins A, B, C, and D in foods. *Can. J. Microbiol.* 32: 723-727. 69. Petinaki, E., Arvaniti, A., Dimitracopoulos, G. and Spiliopoulou, I. 2001. Detection of *mecA*, *mecR1*, and *mecI* genes among clinical isolates of methicillin-resistant *Staphylococci* by combined polymerase chain reactions. *J. Antimicrob. Chemother.* 47:297-304. 70. Recsei, P., Kreiswirth, B., O ' Reilly, M., Schlievert, P., Gruss, A. and Novick, R. P. 1986. Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr*. *Mol. Gen. Genet.* 202: 58-61. 71. Ryffel, C., Tesch, W., Birch-Machin, I., Reynolds, P.E., Barberis-Maino, L., Kayser, F.H. and Berger-Bachi, B. 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Gene.* 94:137-8. 72. Regassa, L. B., Couch, J. L. and Betley, M. J. 1991. Steady-state staphylococcal enterotoxin type C mRNA is affected by a product of the accessory gene regulator (*agr*) and by glucose. *Infect. Immun.* 59: 955-962. 73. Robbins, R., Gould, S. and Bergdoll, M. S. 1974. Detecting the enterotoxicity of *Staphylococcus aureus* strains. *Appl. Microbiol.* 28: 946-950. 74. Roberson, J. R., Fox, L. K., Hancock, D. D., and Besser, T. E. 1992. Evaluation of methods for the differentiation of coagulase-positive *Staphylococci*. *J. Clin. Microbiol.* 30:3217-3219. 75. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491. 76. Saiki, R. K., Scharf, S., Falonna, F., Mullis, K. B., Horn, G. T. and Arnbeim, N. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350-1354. 77. Schmitz, F.J., MacKenzie, C.R., Geisel, R., Wagner, S., Idel, H., Verhoef, J., Hadding, U. and Heinz, H.P. 1997. Enterotoxin and toxic shock syndrome toxin-1 production of methicillin resistant and methicillin sensitive *Staphylococcus aureus* strains. *Eur J Epidemiol.* 13:699-708. 78. Schlichting, C., Branger, C. and Fournier, J. M. 1993. Typing of *Staphylococcus aureus* by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. *J. Clin. Microbiol.* 31:227-232. 79. Schwalbe, R. S., Ritz, W. J., Verma, P. R., Barranco, E. A. and Gilligan, P. H. 1990. Selection for vancomycin resistance in clinical isolates of *Staphylococcus haemolyticus*. *J. Infect. Dis.* 161:45-51. 80. Schwalbe, R. S., Stapleton, J. T. and Gilligan, P. H. 1987. Emergence of vancomycin resistance in coagulase-negative *Staphylococci*. *N. Engl. J. Med.* 316: 927-931. 81. Schwartz, D. C. and Cantor, C. R. 1986. Separation of yeast chromosome sized DNA by pulsed field gradient gel electrophoresis. *Cell* 37:67. 82. Sener, K., Saraci, M.A, Acikel, C.H. and Doganci, L. 2004. Evaluation of three methods in genotyping of nosocomial *Staphylococcus aureus* isolates. *Mikrobiyol Bul.* 38:363-75. 83. Smeltzer, M. S., Hart, M. E. and Iandolo, J. J. 1993. Phenotypic characterization of *xpr*, a global regulator of extracellular virulence factors in *Staphylococcus aureus*. *Infect. Immun.* 61: 919-925. 84. Smole, S. C., Aronson, E., Durbin, A., Brecher, S. M. and Arbeit, R. D. 1998. Sensitivity and specificity of an improved rapid latex agglutination tests for identification of methicillin-sensitive and -resistant *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* 36:1109-1112. 85. Chu, G., Vollrath, D. and Davis, R.W. 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. *Science* 234:1582-1585. 86. Strandén, A., Frei, R. and Widmer, A.F. 2003. Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? *J. Clin. Microbiol.* 41:3181-6. 87. Suzuki, Y., Saito, M. and Ishikawa, N. 1999. Restriction fragment length polymorphisms analysis by pulsed-field gel electrophoresis for discrimination of *Staphylococcus aureus* isolates from foodborne outbreaks. *Int. J. Food Microbiol.* 46:271-274. 88. Tayfour, M. A., Eris, F. N. and Alanazi, A. R. 2005. Comparison of antibiotic susceptibility tests, plasmid profiles and restriction enzyme analysis of plasmid DNA of methicillin susceptible and resistant-*Staphylococcus aureus* strains isolated from

intensive care units. Saudi. Med. J. 26:57-63. 89. Tremaine, M. T., Brockman, D.B. and Betley, M. J. 1993. Staphylococcal enterotoxin A gene (sea) expression is not affected by the accessory gene regulator (agr). Infect. Immun. 61: 356-359. 90. Tenover, F. C., Arbeit, R. D. and Goering, R. V. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233-2239. 91. Towner, K. J. and Cockayne, A. 1993. Molecular methods for microbial identification and typing. P29-41. Chapman and Hall, London, United Kingdom. 92. Trindade, P.A., McCulloch, J.A., Oliveira, G.A. and Mamizuka, E.M. 2003. Molecular techniques for MRSA typing: current issues and perspectives. Braz. J. Infect. Dis. 7:32-43. 93. Udo, E. E., Al-Bustan, M. A., Jacob, L. E. and Chugh, T. D. 1999. Enterotoxin production by coagulase-negative Staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning. J. Med. Microbiol. 48:819-823. 94. Unal, S., Hoskins, J. Flokowitsch, J. E., Wu, C. Y., Preston, D. A. and Skatrud, P. L. 1992. Detection of Methicillin-Resistant Staphylococci by Using the Polymerase Chain Reaction. J. Clin. Microbiol. 30:1685-1691. 95. Vandenesch, F., Lebeau, C., Bes, M., Lina, G., Lina, B., Greenland, T., Benito, Y., Brun, Y., Fleurette J. and Etienne, J. 1994. Clotting activity in *Staphylococcus schleiferi* subspecies from human patients. J. Clin. Microbiol. 32:388-392. 96. Velculescu, V. E., Zhang, L., Vogelstein, B. and Kinzler, K. W. 1995. Serial analysis of gene expression. Science 270:484-487. 97. Villarda, L., Lamprell, H., Borgesa, E., Maurina, F., Noelb, Y., Beuvierb, E., Chambac, J.F. and A. Kodjoa . 2005. Enterotoxin D producing strains of *Staphylococcus aureus* are typeable by pulsed-field gel electrophoresis (PFGE).Food Microbiology p261 – 265. 98. Walsh, C. T. 2003. Opinion anti-infective: Where will new antibiotics come from? Nature Rev. 1:65-70. 99. Wang, J. T., Chen, Y. C., Yang, T. L. and Chang, S.C. 2002. Molecular epidemiology and antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus* in Taiwan. Diagn. Microbiol. Infect. Dis. 42:199-203. 100. Wei, H.L. and Chiou, C.S. 2002. Molecular subtyping of *Staphylococcus aureus* from an outbreak associated with a food handler. Epidemiol Infect. 128:15-20. 101. White, H. W. 1992. Rapid separation of DNA molecules by agarose gel electrophoresis: use of a new agarose matrix and a survey of running buffer effects. Biotechniques. 12:574-579. 102. Wieneke, A. A. 1991. Comparison of four kits for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. Int. J. Food Microbiol. 14: 305-312. 103. Wong, C., Dowling, C. E., Saiki, R. K., Higuchi, R. G., Erlich, H. A. and Kazazian, R. H. 1987. Direct genomic sequencing of amplified single copy DNA: rapid characterization of unknown O-thalassemia mutations. Nature 330:384-386. 104. Working Party Report. 1998. Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. J. Hosp. Infect. 39:253-290. 105. Yarwood, J. M., McCormick, J. K., Paustian, M. L., Orwin, P. M., Kapur, V. and Schlievert, P. M. 2002. Characterization and expression analysis of *Staphylococcus aureus* pathogenicity island 3. J. Biol. Chem. 277:13138-13147. 106. Ye, W. R., Wang, T., Bedzyk, L. and Croker, K. M. 2001. Applications of DNA microarrays in microbial systems. J. Microbiol. Methods 47:257-272.