行政院所屬各機關因公出國人員出國報告書
（出國類別：開會研習）

參加「美國微生物學會第 105 屆年會」

服務機關：行政院衛生署藥物食品檢驗局
姓名職稱：方紹威薦任技正
派赴國家：美國喬治亞州亞特蘭大
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目　次　　頁數

摘　要

美國微生物學會（American Society for Microbiology, ASM**）為微生物學領域中會員規模位居世界首位的科學性組織，今年**(2005)**美國微生物學會年會於美國喬治亞州亞特蘭大市的喬治亞世界會議中心（**Georgia World Congress Center**）舉行，本會期由六月五日起至九日止共計五天，本次大會包括有會前研習會（**workshops**），分組演講和會議（**lectures and meetings**）與學術論文發表，以及參展廠商與相關團體的書籍、儀器設備、試劑之展覽會（**exhibits**）（表一）。今年的大會主題是"共生之研究（**Studies in Symbiosis**）"，專題演講是由華盛頓大學醫學院**Jeffrey Gordon**博士主講"人類腸道中的微生物相－與數以兆萬計的朋友共餐"，他特別提到細菌和真核細胞之間的共生關係是與地球上生命有關之主要論題，大部分的細菌共生體是居住在我們腸道中，在那裡它們提供我們所欠缺的代謝特性，未來若能鑑別這些宿主基因和其所表現和作用的微生物效應物質，對於易受其影響的宿主之各種感染性和非感染性疾病，將可在預防或治療上提供一些新的分子學目標和治療策略。**ASM**於年會前兩天（六月四日和五日）舉辦**27**場的研習會，其中有兩場是兩天研習會，有**6**場是半天的研習會，一天的研習會則共有**19**場，這些場次中有三場包含實驗室的實習，其餘只有演講不另含實習操作，演講是在喬治亞世界會議中心舉辦，而實習場地是移師至**Spelman college**實驗室，各研習會場次和主題如表二。考量時間及目前業務需要僅報名**6**月**4**日的**WS-04**：針對臨床微生物實驗室的快速循環、即時**PCR**（**Rapid cycle, real-time PCR for the clinical microbiology laboratory**）（**1-DAY**）以及**6**月**5**日的**WS-19**：以**DNA**序列為基礎的微生物鑑定和分型：資料數據的產生和分析及結果解析（**DNA sequence based identification and typing of microbes: generation and analysis of data and interpretation of results**）

兩場。學術論文發表分口頭及壁報兩種方式，討論主題分為四個群組：診斷微生物學及流行病學（包括C、F、L、U、Y分組）、致病性與宿主反應機制（包括A、B、D、E、G、V、Z分組）、普通微生物學（包括I、N、O、P、Q、R、W分組）以及分子微生物學、生理學與病毒學（包括H、J、K、M、S、T、X、AA分組）（表三）。依科學興趣及研究目標再細分為27組：A-抗微生物化學治療、B-微生物的致病性、C-臨床微生物學、D-普通醫學微生物、E-免疫學、F-醫用黴菌學、G-黴漿菌學、H-遺傳與分子生物學、I-普通微生物、J-超微構造與功能、K-微生物生理與代謝、L-院內感染、M-噬菌體、N-微生物生態學、O-發酵與生物技術、P-食品微生物、Q-環境與普通應用微生物、R-演化及基因體微生物學、S-去氧核糖核酸病毒、T-核糖核酸病毒、U-分枝桿菌學、V-臨床診斷免疫學、W-微生物學教育、X-真核生物的分子、細胞及普通生物學、Y-公共衛生、Z-動物健康微生物學、AA-自營、共生和寄生性原生生物，其中環境與普通應用微生物（Q）、微生物的致病性（B）、臨床微生物學（C）、微生物生態學（N）等組發表的壁報論文篇數最多，約佔五成。壁報論文之外，大會另外以各領域之熱門題材，邀請學者專家做專題演講或座談會。本屆年會所發表的壁報論文2843篇，而專題演講約有300場。本局今年發表的四篇壁報論文，其題目分別為「（P049）台灣地區食品中毒案分離之氣單胞菌毒素基因檢測」、「（Y035）台灣地區市售冷飲及中毒案金黃色葡萄球菌分離株凝固？及蛋白質A基因聚合？鏈反應-限制片段長度多型性分型」、「（P078）以新型多重組引子對聚合？反應分析法偵測台灣產毒性仙人掌桿菌」及「（C386）台灣地區禽肉產品和人體中曲狀桿菌分離菌株之增殖片段長度多型性分型、血清型、致病性和毒素基因」（表四），頗受一些研究學者專家關注，紛紛留下名片或電子郵件地址，要求提供完整之壁報論文報告，回國後業已分別以電子郵件一一回覆（附件一）。此行除了就發表之壁報論文和與會的學者專家互相交流相關經驗和

資訊之外，並藉由參與研習會、分組演講和會議、學術論文發表等相關學術活動，更進一步瞭解跟目前業務相關之重要食因性微生物的檢驗和研究之趨勢與精髓，同時也多方面蒐集到參展廠商最新的檢驗或研究用儀器耗材資訊，尤其是生物恐怖攻擊發生時能夠快速篩檢涉嫌食品之檢測套組，不但拓展了自身的視野，而且有助於提升本局之檢驗研究水準，維護食品之衛生安全，保障國民之健康。

壹、 前言及目的：

美國微生物學會（American Society for Microbiology，ASM）的前身是美國細菌學家協會（the Society of American Bacteriologists），ASM 會員人數從 1899 年創立的 59 位科學家，至今已經成長至 42000 人，會員遍佈全球，其規模的龐大和歷史的悠久，皆位居世界單一生命科學會員組織之首，學會的宗旨是透過科學知識的追求和基礎與應用研究結果的傳播，以促進微生物學發展。ASM 分為 7 個區域（Region）和 35 分部（Branch），會員中有百分之三十為國際會員。

每年年會都會有超過 10000 名，來自世界各國的微生物學專家和學者躬逢其盛, 並在會場中發表超過 3500 篇的研究論文, 交換彼此的研究心得。由於我國已加入世界貿易組織，國際間各類生鮮或即食的農、漁產品皆有可能輸入國內，而且某些食品中毒原因微生物亦有可能成為生物戰劑或恐怖攻擊的武器。故此次參加本屆 ASM 年會之目的是參與國際著名的相關學會年會與其研討會，以及發表四篇壁報論文如附件一。藉此瞭解微生物學的最新發展趨勢、收集有關快速檢驗方法及參展廠商展出的最新儀器與檢驗套組之資訊以及交換彼此的檢驗研究心得，作為防範國內食品中毒原因微生物之污染及生物恐怖攻擊發生時篩檢涉嫌食品之重要參考依據，並提升微生物之檢驗研究水準及增加本局微生物研究論文在世界各地專家與會的美國微生物學會年會之能見度，以維護食品之衛生安全，保障國民之健康。

貳、 行程及紀要

今年美國微生物學會年會於美國喬治亞州亞特蘭大市的喬治亞世界會議中心（Georgia World Congress Center）舉行，本會期由六月五日起至九日止共計五天，年會會程如表一。大會於 5 日下午五時舉辦開幕典禮，包括會員大會、專題演講及頒獎。今年的大會主題是"共生之研究（Studies in Symbiosis）"，專題演講是由華盛頓大學醫學院 Jeffrey Gordon 博士主講"人類腸道中的微生物相－與數以兆萬計的朋友共餐（The Human Gut Microbiota: Dining with a Few Trillion Friends）"，他特別提到細菌和真核細胞之間的共生關係是與地球上生命有關之主要論題，群聚在我們身體表面的細菌數量估計遠超過我們體內和生殖細胞數量，大部分的細菌共生體是居住在我們腸道中，在那裡它們提供我們所欠缺的代謝特性。他認為未來若能鑑別這些宿主基因和其所表現和作用的微生物效應物質，對於易受其影響的宿主之各種感染性和非感染性疾病，將可在預防或治療上提供一些新的分子學目標和治療策略，其研究論述之精闢及深入性值得我們效法與尊敬。由於會場場地寬敞和參加的聽眾人數極多，大會於會場前面特別準備了兩個液晶大銀幕，現場上直接即時播映，使所有參加者均能清楚的看到整個演講過程。參與此次年會者有來自世界各國的微生物學者專家，雖名為美國微生物學會年會，實際上比一般國際會議有過之而無不及。會後還安排盛大的歡迎接待晚會"Atlanta on the Rise"，會中有歌曲合唱、舞會及美食，大會不但作了一次成功的城市觀光文化之宣傳，與會者也藉此互相認識和熟悉環境，共同迎接一個即將到來、為期四天的豐富知性之旅。

本次大會包括有研習會（workshops），分組演講和會議（lectures and meetings）與學術論文發表，以及參展廠商、相關政府單位和學術團體的書籍、儀器設備、檢測試劑和耗材之展覽會（exhibits）。關於本屆的美國微生物學會研習會是於年會前兩天（6 月 4 日和 5 日）舉辦的，其中有兩場是兩天研習會，有 6 場是半天的研習會，一天的研習會則共有 19 場，共計有 27 場。這些場次中有三場包含實驗室的實習，其餘只有演講不另含實習操作，演講地點是在喬治亞世界會議中心舉辦，而實習場地是移師至 Spelman college 實驗室，主要針對臨床微生物之篩選鑑別及其抗藥性之檢測，各研習會場次和主題如表二。考量時間及目前業務需要僅報名 6 月 4 日的 WS-04：應用於臨床微生物實驗室的快速循環、即時 PCR 技術（Rapid cycle, real-time PCR for the clinical microbiology laboratory）（1-DAY）以及 6 月 5 日的 WS-19：以 DNA 序列為基礎的微生物鑑定和分型：資料數據的產生和分析及結果解析（DNA sequence based identification and typing of microbes: generation and analysis of data and interpretation of results）兩場。

第一場是由梅佑醫學中心？Mayo Clinic, Rochester, Minn.？Franklin R. Cockerill 博士主持，課程包括有：快速循環即時 PCR 技術之概述、樣品收集和抽取、即時 PCR 探針、品質控制和要求準則、在病毒和寄生蟲診斷方面的應用及臨床實驗室之經驗談、在細菌診斷方面的應用及臨床實驗室之經驗談等（附件三）。首先，Cockerill 博士先引言即時定量 PCR 技術的進展及其應用，並提到 ASM 出版的臨床微生物期刊（Journal of Clinical Microbiology）之中，從 2000 年至 2003 年期間所發表與即時定量 PCR 相關的研究報告就達到 109 篇，顯見即時定量 PCR 已經是一個熱門的技術。它不僅提昇 PCR 的層次從定性大步跨躍進入定量，而且與傳統 PCR 相比，它

具有更好的特異性、能有效解決 PCR 污染問題、自動化程度高等特點，在後基因體時代的來臨，目前已廣泛利用即時定量 PCR 技術於基因表現之應用、病毒或病原菌之檢測及 SNP genotyping Assay。另外，早期的定量 PCR 技術由於無法有良好的重覆性，往往會隨不同的樣品或不同實驗室或不同次的 PCR 而有所差異，造成差異的可能因素是樣品內含有干擾物質，或實驗操作上的誤差等，導致增幅效率不同。因此在會中亦對於即時定量 PCR 技術的品質控制和要求提出許多解決方案和見解，例如每一次 PCR 實驗都應該包括適當的對照組，以確保結果的可信度，適當的對照組是指：陰性對照組、陽性對照組和內標準對照組（internal control, IC），可透過 IC 可以解決外在因子的干擾，亦即設計一段序列作為 IC，引子對亦會與此序列黏合（annealing），但增幅出來的片段長度與 Target DNA 不同，此目的乃用於檢測檢體內是否含抑制物質，以避免偽陰性的發生。

後者是由德國 Munster 大學 Dag Harmsen 教授主持，課程包括有：DNA 序列的多重比對（multiple alignments）和親緣分析（phylogenetic analysis）、利用 16S rDNA 或 *rpoB* 鑑定細菌的方法、分型方法概述、多位基因座序列（multilocus sequence typing, MLST）分型方法簡介、MLST 資料分析和結果解析、單位基因座序列（single locus sequence typing, SLST）分型方法等（附件四）。在本會場中，介紹了目前幾種常使用的分型方法，例如：保守基因序列（最普遍使用的是 16S rRNA）的比較可用於細菌基因分型鑑定，但是在某些類群，例如腸內桿菌科（*Enterobacteriaceae*），這些基因中的變異情形並不足以提供可信賴的菌種鑑定結果。*rpoB* 基因的序列（RNA polymerase beta-subunit encoding gene）比較則可視為 16S rRNA 的另一選擇，它是一種萬用型的細菌基因分型鑑定方法。有學者曾針對 14 種腸內桿

菌科菌屬進行 *rpoB* 和 16S rRNA 基因的不同菌屬間和相同菌屬間多樣性比較，發現不同菌株的 *rpoB* 序列之間所呈現的多樣性程度遠高於 16S rRNA 基因者。另外，多位基因座序列（multilocus sequence typing, MLST）分型技術在本研習會中也被著墨很多，其原理與多位基因座酵素電泳法相同（multilocus enzyme electrophoresis, MLEE），主要是利用 PCR，將一些具多形性的 housekeeping 基因放大，比較這些基因在不同菌株之間的差異而將菌株分型。每個基因座上的對偶基因是直接以核？酸序列，而非間接地由其基因產物的電泳移動性來界定。這個方法對於病原菌的分型特別有幫助，所建立的 MLST 分型資料庫也是流行病學調查的有用工具，藉此可確認環境或家畜類動物傳播至人體的可疑路徑。目前已廣泛應用於 *Bacillus cereus*、*Bordetella* spp.、*Campylobacter* spp.、*Candida tropicalis*、*Helicobacter pylori*、*Klebsiella pneumoniae*、*Neisseria* spp.、*Pseudomonas aeruginosa*、*Streptococcus* spp.、*Vibrio* spp.等菌種。而且目前亦有英國帝國學院和牛津大學分別在網際網路上架設有全球資訊網站（http://www.mlst.net/）和（http://pubmlst.org/），以提供 MLST 資料的建檔和交換以及 MLST 相關的應用工具軟體。

學術論文和專題演講於 6 月 6 日至 9 日同步進行（附件二），學術論文發表分口頭及壁報兩種方式，討論主題分為四個群組（Divisional groups）：診斷微生物學及流行病學（包括 C、F、L、U、Y 分組）、致病性與宿主反應機制（包括 A、B、D、E、G、V、Z 分組）、普通微生物學（包括 I、N、O、P、Q、R、W 分組）以及分子微生物學、生理學與病毒學（包括 H、J、K、M、S、T、X、AA 分組）（表三）。依科學興趣及研究目標再細分為 27 組（Divisions），其口頭及壁報論文篇數分別如下：A-

抗微生物化學治療（Antimicrobial Chemotherapy）3 篇和 107 篇；B-微生物的致病性（Microbial Pathogenesis）7 篇和 365 篇；C-臨床微生物學（Clinical Microbiology） 17 篇和 417 篇；D-普通醫學微生物（General Medical Microbiology）4 篇和 220 篇；E-免疫學（Immunology）4 篇和 86 篇；F-醫用黴菌學（Medical Mycology）4 篇和 89 篇；G-黴漿菌學（Mycoplasmology）4 篇和 21 篇；H-遺傳與分子生物學（Genetics and Molecular Biology）6 篇和 127 篇；I-普通微生物（General Microbiology）2 篇和 112 篇；J-超微構造與功能（Ultrastructure and Function）3 篇和 25 篇；K-微生物生理與代謝（Microbial Physiology and Metabolism）4 篇和 125 篇；L-院內感染（Nosocomial infections）1 篇和 9 篇；M-噬菌體（Bacteriophage）4 篇和 28 篇；N-微生物生態學（Microbial Ecology）4 篇和 249 篇；O-發酵與生物技術（Fermentation and Biotechnology）5 篇和 77 篇；P-食品微生物（Food Microbiology）3 篇和 97 篇；Q-環境與普通應用微生物（Environmental and General Applied Microbiology）3 篇和 427 篇；R-演化及基因體微生物學（Evolutionary and Genomic Microbiology）2 篇和 58 篇；S-去氧核糖核酸病毒（DNA Viruses）2 篇和 18 篇；T-核糖核酸病毒（RNA Viruses）1 篇和 15 篇；U-分枝桿菌學（Mycobacteriology）4 篇和 63 篇；V-臨床診斷免疫學（Clinical and Diagnostic Immunology）1 篇和 25 篇；W-微生物學教育（Microbiology Education）1 篇和 25 篇；X-真核生物的分子、細胞及普通生物學（Molecular, Cellular and General Biology of the Eukaryotes）1 篇和 20 篇；Y-公共衛生（Public Health） 4 篇和 57 篇；Z-動物健康微生物學（Animal Health Microbiology） 2 篇和 56 篇；AA-自營、共生和寄生性原生生物（Free-living, Symbiotic, and Parasitic Protists）1 篇和 11 篇，其中 Q-環境與

普通應用微生物、B-微生物的致病性、C-臨床微生物學、N-微生物生態學等組發表的壁報論文篇數最多，約佔五成。壁報論文之外，大會另外以各領域之熱門題材，邀請學者專家做專題演講或座談會。本屆年會所發表的壁報論文 2843 篇，而邀請之專題演講約有 300 場。本局今年發表的四篇壁報論文，其題目分別為「（P049）台灣地區食品中毒案分離之氣單胞菌毒素基因檢測」、「（Y035）台灣地區市售冷飲及中毒案金黃色葡萄球菌分離株凝固? 及蛋白質 A 基因聚合? 鏈反應-限制片段長度多型性分型」、「（P078）以新型多重組引子對聚合? 反應分析法偵測台灣產毒性仙人掌桿菌」及「（C386）台灣地區禽肉產品和人體中曲狀桿菌分離菌株之增殖片段長度多型性分型、血清型、致病性和毒素基因」（表四）。其中最後一篇是屬於「C-臨床微生物學」分組的「Molecular Typing, Epidemiology and Surveillance: Enteric Pathogens」子題，而其他的子題尚包括有：

- Antimicrobial Susceptibility Testing Methods - Automated Systems
- Diagnosis and Suceptibility of *Mycobacteria*
- Molecular Typing, Epidemiology and Surveillance: *Staphylococcus*
- Sexually Transmitted Diseases
- Molecular Identification of Bacteria in Specimens, Including Blood Cultures
- Manual Identification of Gram Positives
- Molecular Typing, Epidemiology and Surveillance: *Streptococcus* and *Enterococcus*
- Molecular Identification of Bacterial Isolates including Agents of BT
- Diagnostic Virology - Molecular Methods-I
- Methods for Detection and Identification of Gram Negative Rods

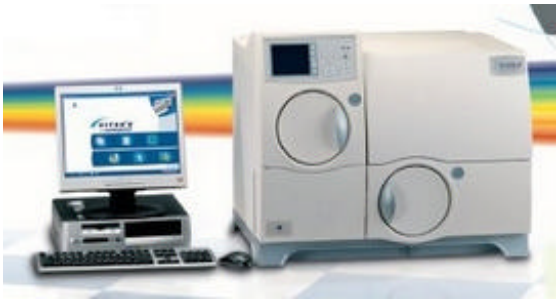
- Diagnostic Bacteriology - Automated Identification Methods
- Diagnostic Mycology and Diagnostic Parasitology
- Molecular Typing, Epidemiology and Surveillance: Other Gram Negative Pathogens
- Viral Detection and Surveillance
- Molecular Typing, Epidemiology and Surveillance: Miscellaneous Microorganisms
- Specimen Collection, Transportation and Processing
- Antimicrobial Susceptibility Testing Methods - Gram Positive Cocci
- Diagnosis and Susceptibility of *Clostridium difficile*
- Antimicrobial Susceptibility Testing Methods - Gram Negative Organisms
- Diagnostic Virology - Molecular Methods-II

由大會安排的研討會場數及參加的壁報論文篇數，即可看出目前此分組受到重視的程度與研究方向、問題為何。在壁報論文展示的第三天，剛好遇見本局科技顧問 FDA **的馮寄新博士，由於距離上次赴美國華盛頓特區的** FDA **總部拜訪馮博士至今大概有十年未再謀面，只有透過電子郵件互相聯絡，因此此番碰面有如他相遇故知，在經過短暫寒敘後，馮博士主動問及本局廖前局長、孫副局長、施組長和周組長等人的近況以及局裡目前情況，職皆一一加以簡要說明，相談之下感覺到馮博士對於有機會受邀回國訪問與專題演講，以貢獻其專業素養及研究心得甚感興趣。另外，此次在年會中以壁報論文之方式發表，頗受一些研究學者專家的關注，紛紛留下名片或電子郵件地址，要求提供完整之壁報論文報告，回國後業已分別以電子郵件一一回覆（附件一），並利用此次出國所見所聞的心得，將壁報論文**

再加以修改補實，待整理完後即會正式發表於相關的學術期刊。以下就各領域所閱覽的壁報論文擇要說明如下：

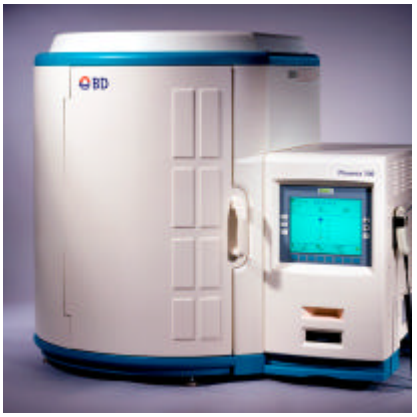
有關於應用分子生物技術於病原菌鑑定與檢測方面－應用多位基因座序列（multilocus sequence typing, MLST）分型方法進行亞洲國家致病性霍亂弧菌分離菌株的基因分型，證實MLST是一個具有高度區分能力且穩定的方法；當比較金黃色葡萄球菌分離菌株的MLST和PFGE分型能力時，PFGE區分能力優於MLST，惟PFGE的restriction patterns較MLST的數值化資料難以比對。現今，即時定量PCR技術檢測已廣泛應用於致病性腸炎弧菌、創傷弧菌（*Vibrio vulnificus*）、金黃色葡萄球菌、沙門氏桿菌、病原性大腸桿菌、曲狀桿菌、*Norovirus*、*Hepatitis* C及Enteric Viruses等。

有關於傳統檢驗技術的改良及各種微生物快速檢測試劑和自動化鑑定系統的優劣比較及評估等方面－目前對於大多數病原菌的檢驗仍然倚賴傳統的分離病原菌、生化試驗及血清學試驗等方法，許多微生物檢測試劑廠商則致力於改良原有的方法，而開發出新的檢測試劑或鑑別用培養基，例如有針對生物恐怖攻擊的快速檢測套組。在微生物自動化鑑定系統方面，本局的VITEK微生物自動鑑定儀已經使用十多年，雖然歷經幾次軟硬體升級，但由於科技進步迅速，目前新一代的微生物自動化鑑定系統已經陸續問世，此次在ASM廠商展覽會場亦有多家重量級的儀器商展示最新型的相關儀器，例如bioMérieux的VITEK® 2系統，Becton Dickinson Diagnostic System的Phoenix™系統及DADE BEHRING的MicroScan Walk-Away®系統等。VITEK®



圖一、bioMérieux 的 VITEK® 2

2系統（bioMérieux, Marcy l'Etoile, France）結合ID-GNB和ID-GPC鑑定卡利用螢光判讀（fluorimetric reading）可以在3小時內鑑定病原菌，此系統的缺點在於它的可鑑定菌株資料庫不足，尤其是*Pseudomonas* spp.和*Acinetobacter* spp.等非發酵性桿菌屬以及*Streptococcaceae*等革蘭氏陽性球菌屬。目前已有新的改良機型VITEK® 2 Advanced Colorimetry™上市，利用比色判讀（colorimetric reading）方式，並且搭配新的GP和GN鑑定卡，宣稱可以改善上述缺點。法國Lille大學醫學中心微生物-衛生實驗室Wallet等人發表一篇"比較兩種機型鑑定臨床革蘭氏陰性桿菌和革蘭氏陽性球菌的效能"（Performance of the new VITEK2 GN and GP cards for identification of clinical Gram-negative bacilli and Gram-positive cocci）（C-194）壁報論文。發現VITEK® 2 Advanced Colorimetry™的GP和GN鑑定卡分別可正確鑑定出94.4%和97.0%，皆高於VITEK® 2系統的ID-GPC和ID-GNB鑑定卡的89.1%和87.6%，惟鑑定時間也由後者原來3小時內延長至前者系統的6-8小時，不過還是可以在一天之內得到鑑別結果。美國Sellenriek等人比較VITEK® 2、Phoenix™及MicroScan Walk-Away®系統鑑定臨床革蘭氏陰性桿菌和革蘭氏陽性球菌的能力，結果三個系統中Phoenix™具有最高的正確率，VITEK® 2有最低的錯誤鑑定率，而無法完全鑑定的比率則偏高，另外若改用VITEK® 2 Advanced Colorimetry™則可提高其正確率。



圖二、Becton Dickinson Diagnostic System的Phoenix™

VITEK® 2系統與本局目前使用的VITEK系統比較，具有下列特點：

☞ VITEK® 2系統鑑定卡的資料庫比早期的VITEK系統擴充許多。

- ☞ VITEK® 2**系統完全是以條碼輸入取代過去在鑑定卡上手寫？體編號的方式，容易追蹤建檔。**
- ☞ VITEK® 2**系統的鑑定卡封口和判讀部分是位於同一個密閉隔間內，比早期的**VITEK**系統更為便利。**
- ☞ VITEK® 2**系統會自動剔除已判讀完之鑑定卡於廢棄卡片專用的收集桶內，然後一起滅菌處理。如此可避免鑑定卡一直留存於判讀機內而造成卡槽壽命降低或菌液洩漏之缺點。**

在微生物快速檢測試劑套組方面－目前有 Tetracore BioThreat Alert® Test Strips（Anthrax、Botulinum toxin、Plague (*Y. pestis*)、Staphylococcal enterotoxin、Ricin、*Brucella*、Tularemia、Orthopox)、RIDASCREEN® ELISA **檢驗套組（***Campylobacter* spp.、*Clostridium difficile* toxin A/B、*Clostridium perfringens* enterotoxin、Verotoxin、*Adenoviruses*、*Astrovirus*、*Norovirus*、*Rotavirus*)、TECRA UNIQUE PLUS ELISA **檢驗系統（***Campylobacter* spp.、*Listeria spp.*、*Salmonella* spp.、*Staphylococcus aureus*、Staphylococcal enterotoxin)、VIDAS® ELISA **檢驗系統（***Campylobacter* spp.、*Listeria spp.*、*Salmonella* spp.、*Staphylococcus aureus*、Staphylococcal enterotoxin)，**後兩者是一種在自動化免疫分析儀上進行** ELISA **的方法，亦是目前研究最多的** ELISA **檢測方法，敏感性很高，與傳統** ELISA **法有很好的一致性。**

在傳統分離培養基的開發或改良方面－有英國 Nottingham Trent **大學的** Iversen **等人開發出** DFI（Druggan-Forsythe-Iversen）**增菌培養液和** DFI **分離培養基，不但可使整個阪崎腸桿菌（***Enterobacter sakazakii***）的檢驗時程較傳統方法縮短兩天，並且可提高該菌的檢出率。澳洲** Vienna **醫學院的** Manafi **和** Lang**則比較三種阪崎腸桿菌分離培養基(**DFI agar, Oxoid, UK; ESIA agar,

AES, France; CES agar, Merck, Germany）的效能，發現三者在 45℃培養 24 小時抑制雜菌的效果皆優於 37℃培養。

另外在會場展示攤位尚包括相關專業領域之圖書出版公司，因此可收集到最新出版的專業書籍資料，尤其有些書籍在會場上直接訂購會有折扣優惠，若是會員折扣更多價錢更低，蠻划算的。ASM 也發行了 11 種期刊，均為世界有名且 SCI Impact 極高的優良期刊，其攤位現場也準備其發行的近期期刊供人翻閱，有需要亦可免費贈閱，至於 ASM 所出版的 170 幾種專業書籍（附件四），對於相關的研究極具參考價值，有很多是微生物專業領域的經典之作。除了參展廠商之外，很多與微生物有關之研究機構亦設有攤位，例如美國國家生物科技資料中心（National Center for Biotechnology Information, NCBI）、美國菌株保存中心（American Type Culture Collection, ATCC）、美國農業部食品安全研究資訊機構（USDA, National Food Safety Research Information Office, FSRIO）、美國疾病管制局（Centers for Disease Control and Prevention, US-CDC）、美國食品藥物管理署（Food and Drug Administration, FDA）、美國國家衛生研究院（National Institute of Health, NIH）、美國國家科學院（National Academy of Science, NAS）等，均在展覽會場設有攤位，使參觀者對其有所瞭解。

藉此瞭解微生物學的最新發展趨勢、收集有關快速檢驗方法及參展廠商展出的最新儀器與檢驗套組之資訊以及交換彼此的檢驗研究心得，作為防範國內食品中毒原因微生物之污染及生物恐怖攻擊發生時篩檢涉嫌食品之重要參考依據，並提升微生物之檢驗研究水準及增加本局微生物研究論文在世界各地專家與會的美國微生物學會年會之能見度，以維護食品之衛生安全，保障國民之健康。第 106 屆美國微生物學會年會將於 2006 年 5 月

21 **日至** 25 **日，在佛羅里達州（**Florida**）的奧蘭多（**Orlando**）橘郡會議中心（**Orange County Convention Center**）舉行。**

參、 心得與建議

美國微生物學會歷史悠久，會員規模龐大，分佈於世界各地。一年一度的年會是 ASM 的重頭戲，因為微生物學上最新發展都將透過此盛會發表，而全球的微生物學者專家以及提供相關檢測研究軟硬體廠商也會齊聚一堂，所以在這場全球矚目的盛會上，將可目睹著名的微生物學家和了解微生物學技術的重大趨勢。另外，從每年年會的會前線上（on line）報名繳費、住宿旅館線上預約訂房、會程安排、學術論文發表、研習會課程和專題演講議題、會場場地設施等方面切入觀察，亦可深刻體會到大會安排之細緻完善與設想之周到，真不愧是大型國際會議的典範，舉凡為方便參觀者索取資料，大會為每位參加者所準備的參觀卡（ExpoCard）（只要告知個人需求，廠商會在其電腦上刷此卡，將個人的姓名、住址、工作單位及工作性質等基本資料輸入電腦中，日後廠商即可據此寄相關資料給參觀者）；亦可利用此卡在 E-Central 地點上網搜尋會程及會議地點、參展廠商和產品訊息和接收電子郵件；設置可以隨時無線上網的專區；幼兒託寄中心等等都是鮮明的例子。

本局已經連續三年派員參加 ASM 舉辦的年會，此次本組將有關食品中毒病原菌的研究成果整理成四篇論文於年會中發表，非但有助於增加本局研究論文在國際著名之學術團體 ASM 年會之能見度和展現本局專業方面的技術與能力，而且論文的被接受發表，也直接鼓舞了研究團隊的士氣及提昇個人工作成就感，更是達到國際學術交流的目的，例如加拿大公共衛生署（Public Health Agency of Canada）食因性人畜共通疾病實驗室（Laboratory for Foodborne Zoonoses）的 Catherine Yoshida 和美國中西部研

究所佛羅里達分部的 Susan Ditty 皆透過所留下的電子郵件地址索取壁報論文資料，而職亦分別留下名片給聖路易斯兒童醫院（St. Louis Children's Hospital）微生物免疫部門的 Patricia Sellenriek、美國 FDA 獸醫中心（Center for Veterinary Medicine）動物與食品微生物分部的 Linda English 與美國 FDA 灣岸海產實驗室（Gulf Coast Seafood Laboratory）的 Jessica Nordstrom 索取其壁報論文（附件一），結果都慨然回信獲得所需的資料。除了參與學術論文的發表之外，各專業領域的分組會議及專題討論也值得鼓勵參與，因為可以與各國專家學者討論及分享心得，發表意見的機會較多，影響力也較直接，又可趁機結交活躍於國際各專業領域的專家學者，有機會亦可邀請他們（包括本局的科技顧問）至本局訪問、專題演講及講習，此舉也不失為出國研習開會獲取研究新知和分享心得另闢一可行蹊徑。

本次代表本局參加研習課程及會議，除了交換彼此的檢驗研究心得之外，所收集之內容亦十分豐富，舉凡與本組業務或研究內容相關的資料皆有所得，例如防範國內食品中毒原因微生物之污染及生物恐怖攻擊發生時篩檢涉嫌食品之相關的快速檢驗方法、參展廠商展出的自動化檢測儀器與檢測套組之最新發展趨勢資訊、即時定量 PCR 技術的品質控制和要求（陰性對照組、陽性對照組和內標準對照組）等。此外，於出國前即先與同仁討論其所需資料，以便在眾多研習會及會議中把握時間迅速掌握相關的資料，以便回國後將所得資料交予組內同仁，俾使此次出國獲得最大之效益，希冀達到雖然只有一人出國，但卻能與各位同仁一起共享成果之目標。

參與國際級學術性組織年會或研討會活動，進行面對面交流溝通確實是促進學術交流和提升專業知識之一種捷徑，惟除此之外，更應針對相關食品安全和食品保全（Food Security）議題，建立相關領域學者專家之資料

庫與聯絡管道，以備未來不時之需；甚至更應有效地利用無遠弗屆的網際網路與電子郵件，藉以迅速收到問題諮詢的即時解答及研究心得的交流之效，如此才能使本局完成維護食品衛生安全和保障國民健康之使命。

表一、美國微生物學會第 105 **屆年會會程摘要表**

6**月** 4**日** 8:30 am-4:30 pm	**研習會研習**
6**月** 5**日** 7:30 am	ASM **的年度慈善募款迷你馬拉松比賽**
6**月** 5**日** 8:30 am-4:30 pm	**研習會研習**
12:00 noon-5:00 pm	**與會者註冊**
5:00 am-7:30 pm	**大會開幕典禮，包括會員大會、專題演講及頒獎。專題演講由華盛頓大學醫學院** Jeffrey Gordon **博士主講**”Studies in Symbiosis”
6**月** 6**日**-9**日**	**研討會、學術論文發表、分組演講和會議、廠商展示**

表二、各研習會場次、主題及日程表

WS-01	**Introductory Clinical Mycology: Help for the Beginner (2-Day Workshop)** June 4-5, 2005. Length: 14 hours Organizer: Dr. Glenn Roberts, Mayo Clinic, Rochester, MN
WS-02	**An Introduction to GMP Microbiology (2-Day Workshop)** June 4-5, 2005. Length: 14 hours Organizer: Dr. Donald Singer, GlaxoSmithKline, Collegeville, PA
WS-04	**Rapid Cycle, Real-Time PCR for the Clinical Microbiology Laboratory** June 4, 2005. Length: 7 hours (Note: WS-03, the two day version of this workshop – with laboratory – is fully subscribed) Organizer: Dr. Franklin R. Cockerill, III, Mayo Clinic, Rochester, MN
WS-05	**Susceptibility Testing Update for the Clinical Microbiology Laboratory** June 4, 2005. Length: 7 hours Organizer: E. Kent Korgenski, Primary Children' s Medical Center, Salt Lake City, UT
WS-06	**Rapid, Cost-Effective Identification of Gram-Negative Rods (Laboratory)** June 4, 2005. Length: 7 hours Organizer: Dr. Mary K. York, MKY Microbiology Consulting, Walnut Creek, CA
WS-07	**The Laboratory Information System (LIS): Maximizing Its Value in the Clinical Microbiology Laboratory** June 4, 2005. Length: 7 hours. Organizer: Dr. Joan Barenfanger, Memorial Medical Center, Springfield, IL
WS-08	**Verification, Validation and Accreditation** June 4, 2005. Length: 7 hours Organizer: Dr. Michael Brodsky, Brodsky Consultants, Thornhill, ON, Canada
WS-09	**A Short Course in Applied Statistics for the Researcher** June 4, 2005. Length: 7 hours. Organizer: Dr. Daryl Paulson, Bioscience Laboratories, Inc., Bozeman, MT
WS-10	**Clinical Mycobacteriology: What is Old, Still Used and New?** June 4, 2005. Length: 7 hours. Organizer: Melodie Beard, Laboratory Corporation of America, Burlington, NC
WS-11	**Transfer and Spread of Pathogens in Food and Healthcare Environments** June 4, 2005. Length: 7 hours Organizer: Dr. Ewen C. Todd, Michigan State Univ., East Lansing, MI
WS-12	**The Gram-Positive Challenge: Clinical Importance of Aerobic Catalase-Negative Gram-Positive Cocci (Laboratory)** June 4, 2005. Length: 3.5 hours Organizer: Dr. Claudia J. Hinnebusch, Univ. of California Clinical Labs, Los Angeles, CA
WS-13	**Modern Molecular Microbiology** June 5, 2005. Length: 7 hours Organizer: Dr. Danny L Wiedbrauk, Warde Medical Laboratory, Ann Arbor, MI

WS-14	**Anaerobic Bacteriology for the Clinical Laboratory** June 5, 2005. Length: 7 hours Organizer: Dr. Diane M. Citron, R.M. Alden Research Lab, Santa Monica, CA
WS-15	**DNA Microarrays and Gene Expression** June 5, 2005. Length: 7 hours Organizer: Dr. Virgil Rhodius, Univ. of California, San Francisco, CA
WS-16	**Microbial Source Tracking Using Indicator Organisms** June 5, 2005. Length 7 hours Organizer: Dr. Jill Stewart, NOAA, Charleston, SC
WS-17	**A Practical Introduction to Predictive Microbiology** June 5, 2005. Length: 7 hours Organizer: Dr. Mark L. Tamplin, USDA/ARRS/ERRC, Wyndmoor, PA
WS-18	**Conducting Research with Select Agents in an Academic Environment** June 5, 2005. Length: 7 hours Organizer: Dr. Eric A. Johnson, Univ. of Wisconsin, Madison, WI
WS-19	**DNA Sequence Based Identification and Typing of Microbes: Generation and Analysis of Data and Interpretation of Results.** June 5, 2005. Length: 7 hours Organizer: Dr. Dag Harmsen, Universitaet Muenster, Muenster, Germany
WS-20	**Streamlining Microbiology – Your Laboratory Holds the Key** June 5, 2005. Length: 7 hours Organizer: Dr. Sandra Chakonas, Loyola Univ. Medical Center, Maywood, IL
WS-21	**Gram-Positive Rods in the 21st Century: An Updated Look at Bacillus, Listeria, Corynebacteria and other Related Aerobic Gram-Positive Rods** June 5, 2005. Length: 7 hours Organizer: Dr. Claudia J. Hinnebusch, Univ. of California Clinical Labs, Los Angeles, CA
WS-22	**In Vitro an In Vivo Bacterial and Viral Test Methods Used to Assess the Efficacy of Topical Antimicrobial Products** June 5, 2005. Length 7 hours Organizer: Dr. Joyce Graf, The Cosmetic, Toiletry, and Fragrance Association, Washington, DC
WS-23	**Microbial Applications of RNAi Technolgies** June 5, 2005. Length 3.5 hours Organizer: Brenda A. Wilson, Univ. of Illinois, Urbana, IL
WS-24	**Staphylococcal Small Colony Variants** June 5, 2005. Length: 3.5 hours Organizer: Dr. Richard A. Proctor, Univ. of Wisconsin Medical School, Madison, WI
WS-25	**Oligonucleotide Fingerprinting of Ribosomal RNA Genes** June 5, 2005. Length: 3.5 hours Organizer: Dr. James Borneman, Univ. of California, Riverside, CA
WS-26	**Modern Phenotypic Testing: Approaches to Complement Genomics** June 5, 2005. Length: 3.5 hours Organizer: Dr. Barry R. Bochner, Biolog, Inc., Hayward, CA
WS -27	**Anti-Fungal Agents and How to Detect Resistance to Them** June 5, 2005. Length: 3.5 hours

表三、美國微生物學會依專長領域或興趣分組(A-AA)

Division A	Antimicrobial Chemistry
Division B	Microbial Pathogenesis
Division C	Clinical Microbiology
Division D	General Medical Microbiology
Division E	Immunology
Division F	Medical Mycology
Division G	Mycoplasmology
Division H	Genetics and Molecular Biology
Division I	General Microbiology
Division J	Ultrastructure and Function
Division K	Microbial Physiology and Metabolism
Division L	Nosocomial Infections
Division M	Bacteriophage
Division N	Microbial Ecology
Division O	Fermentation and Biotechnology
Division P	Food Microbiology
Division Q	Environmental and General Applied Microbiology
Division R	Evolutionary and Genomic Microbiology
Division S	DNA Viruses
Division T	RNA Viruses
Division U	Mycobacteriology
Division V	Clinical and Diagnostic Immunology
Division W	Microbiology Education
Division X	Molecular, Cellular and General Biology of the Eukaryotes
Division Y	Public Health
Division Z	Animal Health Microbiology
Division AA	Free-Living, Symbiotic and Parasitic Protists

表四、美國微生物學會年會中所發表的四篇壁報論文

展示日期與時間	壁報編號	題　目	作　者
06/07 9:00	P049	台灣地區食品中毒案分離之氣單胞菌毒素基因檢測（Detection of Virulence Gene of Motile Aeromonads Isolated from Food-Borne Outbreaks in Taiwan）	張育彰、王貞懿、施養志、
06/07 9:00	Y035	台灣地區市售冷飲及中毒案金黃色葡萄球菌分離株凝固? 及蛋白質 A 基因聚合? 鏈反應-限制片段長度多型性分型（Subtyping of *Staphylococcus aureus* Strains Isolated from Marketed Cold Drinks and Food Poisoning Outbreaks in Taiwan by PCR-RFLP Methods of Coagulase and Protein A Gene）	黃翠萍、施養志、王貞懿、張育彰、賴韻如、黃蘊璞
06/08 13:00	P078	以新型多重組引子對聚合? 反應分析法偵測台灣產毒性仙人掌桿菌（A Novel Multiplex PCR Assay to Detect Toxigenic *Bacillus cereus* Strains in Taiwan）	楊怡真、黃翠萍、黃蘊璞、王貞懿、施養志
06/09 9:00	C386	台灣地區禽肉產品和人體中曲狀桿菌分離菌株之增殖片段長度多型性分型、血清型、致病性和毒素基因（Amplified Fragment Length Polymorphism, Serotyping, Quinolone Resistance, Virulence and Toxin Genes of *Campylobacter jejuni* Strains from Poultry and Humans in Taiwan）	方紹威、楊晴如、施養志、周正俊、游若?

P-049

Detection of virulence gene of motile aeromonads isolated from food-borne outbreaks in Taiwan

Yu-Chang Chang, Jan-Yi Wang, Daniel Yang-Chih Shih

Food Microbiology Division, Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, Taiwan, R.O.C.

161-2, Kuan Yang St., Nankang
Taipei, 11513, Taiwan, R.O.C.
yuchang@mail.fda.gov.tw

Abstract

Among various *Aeromonas* species, motile aeromonads are most common cause of human infections such as septicemia and gastroenteritis. The pathogenesis of *Aeromonas* infection are complex and multifactorial, with the involvement of a number of virulence factors. So far, many extracellular enzymes have been implicated in the pathogenesis of those micro-organisms. Besides the enterotoxins, there are over thirty categories of extracellular proteins and toxins secreted by some motile aeromonads which includes hemolysin, protease, cytotoxins and lipase. The involvement of several genes on the pathogenesis of motile aeromonads has been demonstrated. The present research was carried out in order to test the presence of some of these virulence genes by PCR. Six of isolates were carrying all of the six virulence genes, and 4 of isolates no virulence gene were detected.

Introduction

Aeromonas spp. comprises mesophilic motile and psychrophilic nonmotile gram-negative ubiquitous bacteria. The species principally associated with gastroenteritis and extra-intestinal infections are motile *Aeromonas*. The role of motile *Aeromonas* as human enteric pathogens is not fully clarified, but there is strong evidence that some strains which harboring virulence genes can cause gastroenteritis. A significant number of *Aeromonas* virulence genes have been described, including the hemolysin genes (*hly*), enterotoxin genes (*ent*), glycerophospholipid-cholesterol acyltransferase gene (*satA*), elastase gene (*ela*), lipase gene (*lip*), serine protease gene (*ser*), and so on. Our aim is to determine any differences in the distribution of those virulence genes at foodborne outbreak isolates.

Materials and methods

Bacterial strains: A total of 68 Aeromonas strains were collected from 32 food-borne outbreaks, as listed on Table1.

Table 1. Distribution of various *Aeromonas* spp from food- borne outbreak samples

Source	*Aeromonas* spp.	No. of isolates (%) positive for			
		A. hydrophila	*A. caviae*	*A. sobria*	*A. encheleia*
Sea food & processed	21	7	8	4	2
Grain & processed	11	5	5	-	1
Meat & processed	5	2	3	-	-
Vegetable & processed	4	1	1	2	-
Beverage	3	-	1	2	-
Complex food	2	1	1	-	-
Swab	22	4	16	1	1
Total	68	20(29.4)	35(51.5)	9(13.2)	4(5.9)

Oligonucleotide primers and PCR conduction: The oligonucleotide primers showed on Table 2. DNA samples (5 ng per reaction mixture) were amplified in a 25-μl reaction mixture consisting of 50 mM potassium chloride; 10 mM Tris chloride (pH 8.3); 1.25 mM magnesium chloride; 200 μM dNTP; 2.0 μM dATP, dTTP, dCTP, and dGTP; 2.0 μM each test primers, and 1.5 U of FastStart Taq DNA polymerase. The amplification procedure consisted of an initial denaturation step at 94 ℃ / 5 min followed by 30 cycles with denaturation at 95 ℃ / 30 sec, annealing at 60 ℃ / 1 min, and extension at 72℃ / 1min. A final extension step was done at 72 ℃ / 7 min. Ten microliters of the reaction mixture was then analyzed by submarine gel electrophoresis in 2.0 % agarose at 5 V / cm, and the reaction products were visualized with UV light after staining with ethidium bromide.

Phenotypic expression of virulence factors assay: Fig 1.

Table 2. Primer pairs used for PCR amplification.

Name	Sequence (5' to 3')	PCR product(bp)	Reference
ser1	CAC CGA AGT ATT GGG TCA GG	350	Chacon et. al., 2003
ser2	GGC TCA TGC GTA ACT CTG GT		
GCATf	CTCCTGGAATCCCAAGTATCAG	237	Chacon et. al., 2003
GCATr	GGCAGGTTGAACAGCAGTATCT		
lip1	CA(C/T) CTG GT(G/T) CCG CTC AAG	247	Chacon et. al., 2003
lip2	GT(A/G) CCG AAC CAG TCG GAG AA		
hly3	GTGACCACCAAGAACAAGT	406	This work
hly5	TCCAGTCCCACCACTTCACTT		
ela1	AGCCGTCCCTTTGTTGTGA	263	This work
ela3	TTGATCTCCTTGACCGTGT		
ent12	ATGATGGGGCAGTGGCAAA	612	This work
ent41	CTTGTTCTTGGTGGAGACCT		

Blastase activity test Protease activity test Hemolysis activity test

Fig 1. The enzyme activity test.

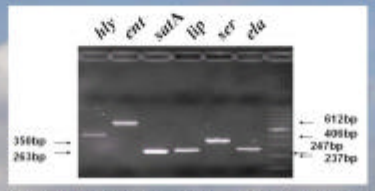


Fig 2. Detection of virulence genes in *Aeromonas* spp. by PCR.

Results and discussion

- Sixty-eight *Aeromonas* strains were isolated from 32 food- borne outbreaks with 264 samples in Taiwan. Among the 68 isolated strains four different species are represented. *A caviae* is the dominating species (35/68) while *A. hydrophila* is the second most frequently isolated species (20/68). Nine strains of *A. sobria* were found while 4 *A. encheleia* were isolated.
- The sizes of the amplification products obtained by the PCR were identical to those predicted from the design of the primers (Fig 2; Table 2).
- The result of detection virulence genes: 66.3 % of these strains were positive for enterotoxin gene, 26.1 % for hemolysin gene, 39.7 % for elastase gene, 21.0 % for serine protease gene, 80.3 % for glycerophospholipid:cholesterol acyltransferase gene and 81.1 % for lipase gene, and the virulence factors: 54.2 % for protease activity, 31.6 % for hemolysis activity and 20.5 % for elastase activity. The results showed on Fig 3.

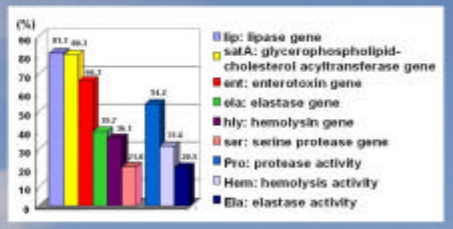
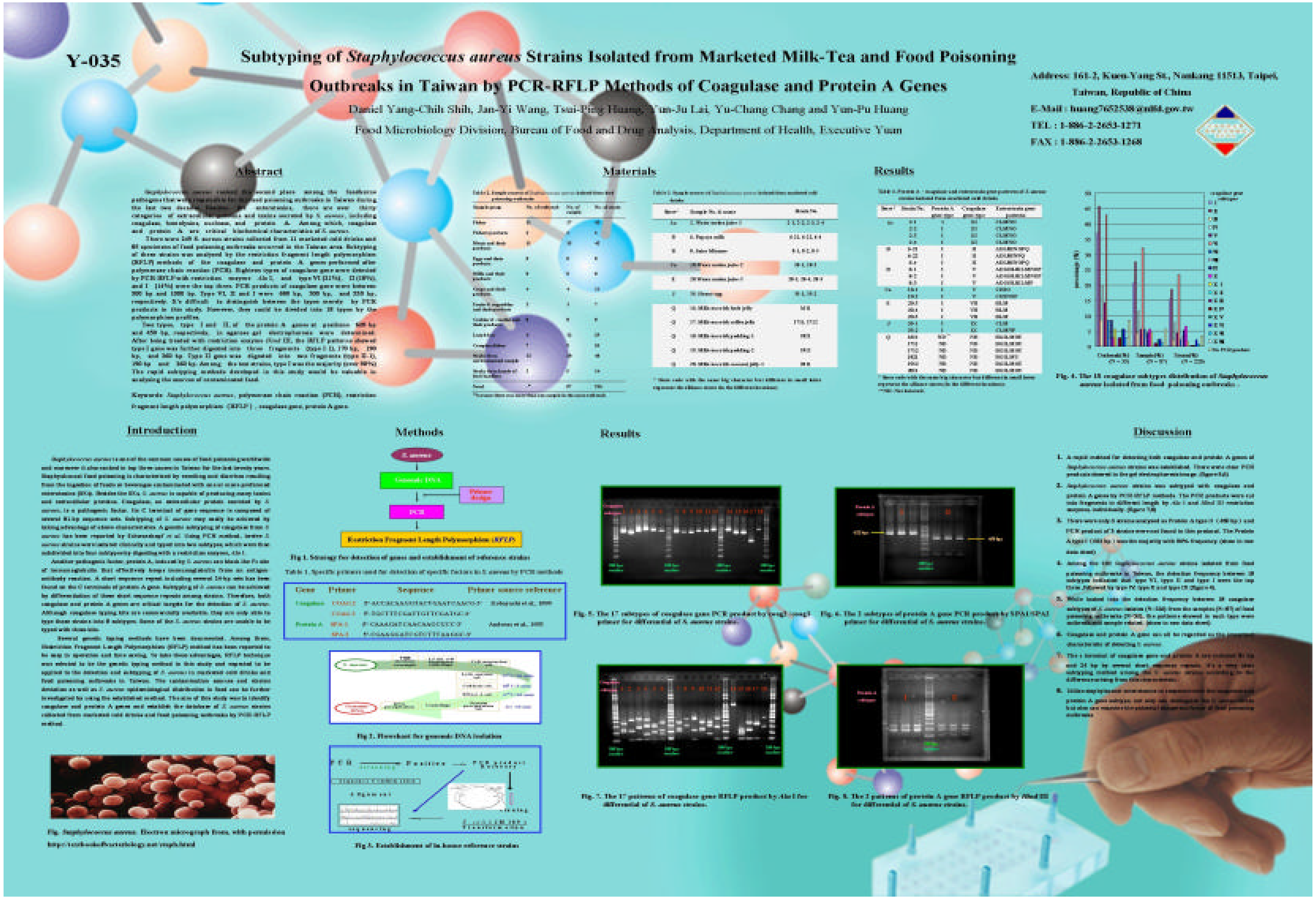


Fig 3. Result of distribution of virulence genes and virulence factors test.

- Six isolates carry all of the six virulence genes, and 4 isolates which have no virulence gene were detected. All of the elastase activity assay in which having positive strains were PCR positive for *ela* gene.
- In conclusion, this PCR-based method is rapid, sensitive, and specific for the detection of potential virulence of motile aeromonads.

Reference

Chacon MR, Figueras MJ, Castro-Escarpulli G, Soler L, Guarro J: Distribution of virulence genes in clinical and environmental isolates of *Aeromonas* spp. Antonie Van Leeuwenhoek. 2003; 84(4):269-78


Y-035
Subtyping of Staphylococcus aureus Strains Isolated from Marketed Milk-Tea and Food Poisoning Outbreaks in Taiwan by PCR-RFLP Methods of Coagulase and Protein A Genes
Daniel Yang-Chih Shih, Jan-Yi Wang, Tsui-Ping Huang, Yun-Ju Lai, Yu-Chang Chang and Yun-Pu Huang
Food Microbiology Division, Bureau of Food and Drug Analysis, Department of Health, Executive Yuan
Address: 161-2, Kuen-Yang St., Nankang 11513, Taipei, Taiwan, Republic of China
TEL : 1-886-2-2653-1271
FAX : 1-886-2-2653-1268
Abstract
Materials
Results
Introduction
Methods
Results
Discussion
S. aureus
Genomic DNA
Primer design
PCR
Restriction Fragment Length Polymorphism (RFLP)
Fig 1. Strategy for detection of genes and establishment of reference strains
Fig 2. Flowchart for genomic DNA isolation
Fig 3. Establishment of in-house reference strains

P-078

A Novel Multiplex PCR Assay to Detect Toxigenic *Bacillus cereus* Strains in Taiwan

I. C. Yang, T. P. Huang, Y. P. Huang, J. Y. Wang, D. Y. C. Shih
Bureau of Food and Drug Analysis, Taipei, TAIWAN, R.O.C

Add: 161-2, Kuen-Yang st., Nankang, Taipei, 11513, Taiwan, R.O.C
Tel: 886-2-2653-1271
E-mail: yangichen@nlfd.gov.tw

ABSTRACT

B. cereus (BC) is responsible for diarrheal and emetic types of food poisoning. 5 different diarrheal enterotoxins have been characterized. The emetic poisoning is caused by dodecadepsipeptide and the specific DNA sequence of emetic strain was found. The objective of the present work was to establish a multiplex PCR assay to detect the enterotoxin genes and emetic-specific sequence of BC strains and analyze the BC strains in Taiwan. 143 strains of food-poisoning and food-borne BC strains were analyzed. 8 primer sets were designed to detect *hblC*, *hblD*, *hblA*, *nheA*, *nheB*, *nheC*, *bceT*, and *entFM*. 2 primer sets were to detect *cytK* because of sequence variation and the PCR products were at the same size. 1 primer set specific to emetic strain was used. 1 primer set designed from 16S-23S rRNA ITS was used as an internal control. The multiplex PCR assay could simultaneously amplify PCR products of 11 different sizes. All of the 143 strains belong to 8 different patterns of toxin profile. The percentage of different patterns was 14.0%, 20.3%, 4.9%, 0.7%, 2.8%, 22.4%, 33.6% and 1.4% respectively. The prevalence was 100% of *nheA*, *nheB*, *nheC*, and *entFM*. 39.9% strains carried *hblC*, *hblD*, *hblA*. 59.4% and 17.5% strains had *cytK* and *entFM* respectively. 1.4% was emetic strains. All of the other bacterial strains were negative in specificity test. The sensitivity is 0.1 ng. The novel multiplex PCR assay could detect all of the enterotoxic and emetic-specific genes/sequence of BC simultaneously and was applied to analyze the strains in Taiwan.

INTRODUCTION

In Taiwan for the period 1991-2003, 139 outbreaks (11.6%) were due to *B. cereus* exceeded only by *Vibrio parahaemolyticus* and *Staphylococcus aureus*.

B. cereus is responsible for diarrheal and emetic types of food poisoning. Diarrheal poisoning is caused by heat-labile enterotoxins produced during vegetative growth of *B. cereus* in the small intestine. Five different enterotoxins have been characterized: two protein complexes, hemolysin BL (HBL) and nonhemolytic enterotoxin (NHE), and three enterotoxic proteins, enterotoxin T (bc-D-ENT), cytotoxin K (CytK), and enterotoxin FM (EntFM). HBL consists of three protein components: L2, L1 and B transcribed from the genes *hblC*, *hblD*, and *hblA*. NHE consists of three protein moieties: B, L2, and L1 encoded by the three genes *nheA*, *nheB*, and *nheC*. The emetic poisoning is caused by a heat-stable depsipeptide cereulide.

To our knowledge, no method was available to detect the toxin genes and emetic-strain specific sequence of the species in the *B. cereus* group simultaneously. The objective of the present work was to establish a multiplex PCR assay method to detect the enterotoxin and emetic-strain specific sequence of *B. cereus* and to analyze the distribution of enterotoxin genes and emetic-specific sequences in food strains of various origins isolated in Taiwan.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Reference strains *B. cereus* BCRC 10603 (diarrheagenic) and *B. cereus* BCRC 17039 (emetic) were used for evaluation of multiplex PCR method.

TABLE 1. Bacterial strains used in this study

Species	Strain number[a]	Total number
B. cereus	BCRC 10603 BCRC 17039 141 food-poisoning and food-borne strains [illegible]	143
Other *Bacillus* spp.	*Bacillus circulans* BFDA [illegible], *B. licheniformis* BFDA [illegible], *B. megaterium* BFDA [illegible], *B. pumilus* BFDA [illegible], *B. sphaericus* BFDA [illegible], *B. subtilis* BFDA [illegible]	6
Non-*Bacillus* spp.	*Aeromonas hydrophila* BCRC [illegible], *Campylobacter coli* BFDA [illegible], *Campylobacter jejuni* BFDA [illegible], *Clostridium perfringens* BCRC [illegible], [illegible] BCRC [illegible], [illegible] BCRC [illegible], *Escherichia coli* BCRC [illegible], *Listeria monocytogenes* BCRC [illegible], *Pseudomonas aeruginosa* ATCC [illegible], *Salmonella* [illegible] BCRC [illegible], *Staphylococcus aureus* BCRC [illegible], *Staphylococcus* [illegible] BCRC [illegible], *Staphylococcus* [illegible] BCRC [illegible], *Staphylococcus* [illegible] BCRC [illegible], *Staphylococcus* [illegible] BCRC [illegible], *Staphylococcus* [illegible] ATCC [illegible], *Vibrio parahaemolyticus* BCRC [illegible], *Yersinia enterocolitica* BFDA [illegible]	18

[a]ATCC, American Type Culture Collection, Rockville, Md., USA; BCRC, Bioresource Collection and Research Center, Hsinchu, Taiwan; BFDA, Bureau of Food and Drug Analysis, Taipei, Taiwan.

Primers. The primers used for the detection are presented in Table 2. When designing, the sequences of all of the 12 primer pairs were chosen carefully to obtain appropriate melting temperature and a common terminal sequence T at the 3' end to avoid the formation of stable overlaps.

DNA extraction and PCR. For DNA extraction, bacteria were placed in BHI and incubated overnight at 30℃. One milliliter of cells was removed and centrifuged. The pellet was resuspended, boiled for 10 min and centrifuged again. The supernatant was stored at -20 ℃ before use.

The final reaction mixture (50 μL) of PCR contained 200 μM concentrations of each dNTP, 1 × PCR buffer, 2 U of FastStart Taq DNA Polymerase, 40 to 350 nM of primers (Table 2), and 1 μL of template DNA. Amplification consisted of initial denaturation at 95 ℃ for 5 min, followed by 30 cycles of 95 ℃ for 30 s, 60 ℃ for 30 s, and 72 ℃ for 45 s. A final 7-min 72 ℃ extension was followed. PCR products were analyzed on a 2% (wt/vol) agarose gel and electrophoresis was carried out for 40 min at 100 V.

Sensitivity and specificity test. To determine the sensitivity of the assay, a serial 10-fold dilution of template DNA was amplified. Twenty-four bacterial strains other than the species in the *B. cereus* group were used in specificity test, including 6 strains of other *Bacillus* spp. and 18 non-*Bacillus* spp (Table 1).

TABLE 2. Primers used for multiplex PCR in this study

Primer name	Sequence (5'→3')	Targeted gene	Product size (bp)	Amount (each primer)[a]	Primer design
[illegible]	[illegible]	*hblA*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*hblC*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*hblD*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*nheA*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*nheB*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*nheC*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*entFM*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*bceT*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*cytK*	[illegible]	[illegible]	This study
[illegible]	[illegible]	*cytK*	[illegible]	[illegible]	This study
[illegible]	[illegible]	Emetic strain-specific sequence	[illegible]	[illegible]	Ehling-Schulz et al. (2004)
[illegible]	[illegible]	[illegible] ITS	[illegible]	[illegible]	This study

[a]The amount of each primer used in multiplex PCR reaction (μL). The initial concentration is 100 nM.

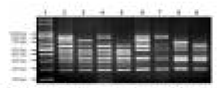


FIGURE 1. The agarose gel of the multiplex PCR products with DNA from the strains of different toxigenic patterns. Lanes 1 and 12, 100 bp ladder marker; lane 2, *B. cereus* BCRC 10603 (pattern I); lane 3, *B. cereus* BCF [illegible] (pattern IV); lane 4, *B. cereus* BCF [illegible] (pattern [illegible]); lane 5, *B. cereus* BCF [illegible] (pattern [illegible]); lane 6, *B. cereus* BCF [illegible] (pattern V); lane 7, *B. cereus* BCF [illegible] (pattern VI); lane 8, *B. cereus* BCRC 17039 (pattern VIII); lane 9, *B. cereus* BCF [illegible] (pattern VII).

TABLE 3. Different toxigenic patterns of strains used in this study

Pattern	*hblC*	*hblD*	*hblA*	*nheA*	*nheB*	*nheC*	*entFM*	*bceT*	*cytK*	ESP[a]	ITS[b]	Number of positive strains (%)
I	+	+	+	+	+	+	+	+	+	−	+	14.0%
II	+	+	+	+	+	+	+	−	+	−	+	20.3%
III	+	+	+	+	+	+	+	−	−	−	+	4.9%
IV	+	+	+	+	+	+	+	+	−	−	+	0.7%
V	−	−	−	+	+	+	+	+	+	−	+	2.8%
VI	−	−	−	+	+	+	+	−	+	−	+	22.4%
VII	−	−	−	+	+	+	+	−	−	−	+	33.6%
VIII	−	−	−	+	+	+	+	−	−	+	+	1.4%

[a]Emetic-specific sequence
[b]16S-23S rRNA internal transcribed spacer

RESULTS

The multiplex PCR assay. *B. cereus* BCRC 10603 lacks the emetic-specific sequence but carries all of the other enterotoxin genes. Ten PCR products of *B. cereus* BCRC 10603 at different sizes could be amplified simultaneously by using multiplex PCR established in this study (Figure 1). *B. cereus* BCRC 17039 carries *nheA*, *nheB*, *nheC*, *entFM* and emetic-specific sequence. Six PCR products, including the product of ITS-F1/R1, could be obtained by using this multiplex PCR assay method (Figure 1).

The toxigenic patterns. According to the presence of enterotoxin genes and emetic-specific sequence, all of the 143 *B. cereus* and *B. mycoides* strains collected in this study belonged to 8 different toxigenic patterns from pattern I to VIII (Table 3). These patterns were all confirmed in each case by PCR using single primer pairs.

The distribution of enterotoxin gene and emetic-specific sequence. The prevalence was 100% of *nheA*, *nheB*, *nheC*, and *entFM*. 39.9% strains carried *hblC*, *hblD*, *hblA*. 59.4% and 17.5% strains had *cytK* and *entFM* respectively. 1.4% was emetic strains.

Sensitivity and specificity test. The sensitivity of the multiplex PCR assay was approximately 100 pg. The specificity was 100%.

DISCUSSION

Multiplex PCR offers a significant time and cost saving advantage. The principal challenge in developing a multiplex PCR system is overcoming primer dimer formation. By choosing a common terminal sequence at the 3' ends of all primers, the formation of stable overlaps can be avoided. A hot start Taq polymerase was also used to overcome primer dimer formation. To avoid preferential amplification of the shorter amplicons, we optimized the concentration of each primer and the extension time of PCR cycles. Both of them were important factors to affect a multiplex PCR assay according to our experience. The results of replica multiplex PCR to test reproducibility were also satisfactory.

All of the *B. cereus* strains carried genes coded for enterotoxins NHE and EntFM. It may be speculated that NHE and EntFM play important roles in relation to food poisoning in Taiwan because of their high prevalence. The relative low-prevalent enterotoxins may also contribute to the virulence of *B. cereus* strains. The number of different enterotoxins produced by a specific strain may be the important determinants for the variation of *B. cereus* virulence. Different patterns of toxin genes possessed by different *B. cereus* strains also provide a useful epidemiological tool to trace the contaminant source. Among all the tested strains in this study, only 2 were emetic type *B. cereus*. It showed that emetic type *B. cereus* were not predominant in Taiwan. All of the emetic type *B. cereus*, including *B. cereus* BCRC 17039, belonged to the same toxigenic pattern (pattern VIII). These strains carried emetic-specific sequence and also carried enterotoxin genes coded for the enterotoxins of NHE and EntFM. It appeared that emetic toxic *B. cereus* strains may also have the ability to produce enterotoxin. This result explained the symptoms of emetic type *B. cereus* food poisoning when vomiting was sometimes followed by diarrhea. All the *B. cereus* strains tested in this study carried at least one toxin gene. It suggested that all strains of *B. cereus* might be treated as potential toxic strains before more evidence was found.

CONCLUSION

The established multiplex PCR assay method could rapidly detect the enterotoxin genes and emetic-specific sequence of *B. cereus* simultaneously and was applied to analyze the *B. cereus* strains collected from food samples in Taiwan. All of the 143 strains belonged to 8 different toxigenic patterns. The distribution of enterotoxin genes and emetic-specific sequence were also studied. The prevalence was 100% of *nheA*, *nheB*, *nheC*, and *entFM*. 39.9% strains carried *hblC*, *hblD*, *hblA*. 59.4% and 17.5% strains had *cytK* and *entFM* respectively. 1.4% was emetic strains. The genes From these data, it may be speculated that NHE and EntFM play important roles in relation to food poisoning in Taiwan because of their high prevalence. They could also be the target markers for screening toxigenic *B. cereus* strains in food. The relative low-prevalent enterotoxins may also contribute to the virulence of *B. cereus* strains. The number of different enterotoxins produced by a specific strain may be the important determinants for the variation of *B. cereus* virulence. Different patterns of toxin genes possessed by different *B. cereus* strains also provide a useful epidemiological tool to trace the contaminant source. Among all the tested strains in this study, only 2 were emetic type *B. cereus*. It showed that emetic type *B. cereus* were not predominant in Taiwan. All of the emetic type *B. cereus* belonged to the same toxigenic pattern. These strains carried emetic-specific sequence and also carried enterotoxin genes coded for the enterotoxins of NHE and EntFM. It appeared that emetic toxic *B. cereus* strains may also have the ability to produce enterotoxin. This result explained the symptoms of emetic type *B. cereus* food poisoning when vomiting was sometimes followed by diarrhea. All the *B. cereus* strains tested in this study carried at least one toxin gene. It suggested that all strains of *B. cereus* might be treated as potential toxic strains before more evidence was found.

C-386

AFLP, Serotyping, Quinolone Resistance, Virulence and Toxin Genes of *Campylobacter jejuni* Strains from Poultry and Humans in Taiwan

Address: Division of Food Microbiology
Bureau of Food & Drug Analysis
161-2, Kuen-Yang Street,
Taipei 115, Taiwan,
Republic of China
Phone: 886-2-2653-1271
Fax: 886-2-2653-1268
E-Mail: ufpfang@nlfd.gov.tw

S. W. Fang,[1]* C. J. Yang,[1] D. Y. C. Shih,[1] C. C. Chou,[2] and R. C. Yu[2]

[1]Bureau of Food and Drug Analysis, Taiwan, ROC and [2]National Taiwan University, Taiwan, ROC

Abstract

The high-resolution genotyping method of AFLP analysis was used to study the genetic relationships between *Campylobacter jejuni* strains isolated from chicken-related samples (n = 32) and humans (n = 27) between 1994 and 2003 in Taiwan. All *C. jejuni* strains showed highly heterogeneous fingerprints, and no fingerprints exclusive for chicken strains or for human strains were obtained. All *C. jejuni* strains were separated in two distinct genetic clusters at 40% genetic similarity and 42 different AFLP types at 90% similarity. In some AFLP types of *C. jejuni* strains, AFLP fingerprints of chicken-related strains were identical to those of strains from humans with gastroenteritis. The predominant serotypes in *C. jejuni* strains were B:2 and Y:37. All strains belonging to serotype O:19 grouped into one single AFLP type. One chicken sample harbored simultaneously a ciprofloxacin-resistant and a ciprofloxacin-sensitive *C. jejuni* strains. Furthermore, *C. jejuni* strains were found to occur simultaneously in another chicken and have the characteristics of quinolone resistance. The *flaA*, *ceuE* and *cadF* were found in 100%, 98.4% and 96.8% of isolates, respectively. The *cdtB*, *cdt* genes, *cdtA* and *cdtC* were detected from 100.0%, 98.4%, 90.5% and 82.5% of isolates, respectively.

Introduction

Molecular methods have increasingly been used as alternatives to serologic methods to type *Campylobacter* strains. Amplified fragment length polymorphism (AFLP) is a new technique used for *Campylobacter* typing. Increasing antimicrobial resistance in *Campylobacter* is a recognized problem, and quinolone resistance is most common in human isolates of *C. jejuni*. In Taiwan, cross-resistance between nalidixic acid and ciprofloxacin was found in 66% of human clinical *C. jejuni* isolates. The purposes of this study were to (i) establish the distributions of common AFLP types of *C. jejuni* isolated from chickens and humans in Taiwan, (ii) identify the most common serotypes of *C. jejuni* strains in Taiwan, and (iii) investigate the relationships among AFLP types, serotypes, quinolone resistance, virulence and toxin genes of *C. jejuni*.

Methods

Serotyping and Quinolone Resistance of *Campylobacter jejuni*

25 g Sample+100 mL Preston broth

↓ 42℃ 24 hrs, microaerobic

mCCDA (Oxoid) [illegible]

↓ 42℃ 48 hrs, microaerobic

Heart infusion agar (Difco)

↓ 42℃ 48 hrs, microaerobic

Gram Stain, Biochemical Characterization Test & Serotyping based on heat-stable Penner's antigens by passive hemagglutination using a serotyping set including 25 antisera (Campylobacter Antisera Set, Denka Seiken Co., Tokyo, Japan)

↓

Antimicrobial Susceptibility determinations were made by the E-test (AB-Biodisc, Solna, Sweden)

List of steps, reagents and PCR conditions of AFLP in this study

Steps	Reagents	Conditions
Restriction enzyme digestion	[illegible]	[illegible]
Adaptor ligation	[illegible]	[illegible]
Pre-selective PCR	[illegible]	[illegible]
Selective PCR	[illegible]	Touchdown PCR

List of primers, amplicon sizes and PCR conditions of toxin genes in this study

Target / primer	Sequences (amplicon sizes)	PCR conditions (Bang et al., 2001)
[illegible]	[illegible] (Amplicon: 165 bp)	94℃ 1 min 42℃ 2 min 30 cycles 72℃ 3 min
[illegible]	[illegible] (Amplicon: 495 bp)	
[illegible]	[illegible] (Amplicon: 555 bp)	

List of primers, amplicon sizes and multiplex PCR conditions in this study

Target / primer	Sequences (amplicon sizes)	PCR conditions
[illegible]	[illegible] (Amplicon: 794 bp) [illegible] (Amplicon: 400 bp)	94℃ 1 min 44℃ 1 min 30 cycles 72℃ 3 min
[illegible]	[illegible] (Amplicon: 2213 bp) [illegible]	94℃ 1 min 47℃ 2 min 30 cycles 72℃ 3 min

Results

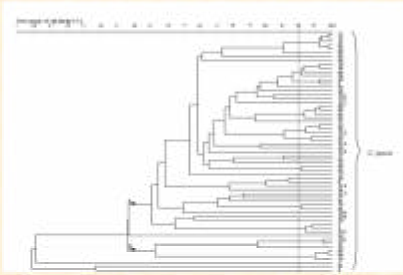
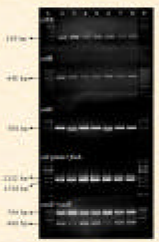


Fig. 1 The dendrogram of AFLP patterns of 63 *C. jejuni*. Cluster analysis was performed with Bionumerics by using UPGMA and the Pearson product-moment correlation coefficient. At 90% similarity were considered to be closely related.

Table 1 PCR detection of putative virulence and toxin genes in 63 *C. jejuni* strains isolated from different sources

Species	Sources				No. of PCR positive with						
	Chicken	Human	Water	Other	*cadF*	*ceuE*	*flaA*	Cdt genes	*cdtA*	*cdtB*	*cdtC*
C. jejuni	32				30	32	32	32	27	32	24
		27			27	26	27	26	27	27	26
			3		3	3	3	3	2	3	2
				1	1	1	1	1	1	1	0
Total percentage	50.8%	42.9%	4.8%	1.6%	96.8%	98.4%	100.0%	98.4%	90.5%	100.0%	82.5%

Fig. 2 Agarose gel electrophoresis of PCR products obtained with primers for the *cdtA*, *cdtB*, *cdtC*, *cdt* genes+*flaA* and *ceuE*+*cadF* specific for *C. jejuni*, detection of different virulence and toxin genes by PCR. Lane 2 to lane 8 are *C. jejuni* strains. Lane 1: 100 bp ladder markers except the *cdt* genes+*flaA* genes (using 1 kb marker), lane 2: 8-1, lane 3: S14-1, lane 3: 16AE-4, lane 4: 20E-1, lane 5: 21E-6, lane 6: 5-2-5, and lane 7: MMH91032006.



1. Most of *C. jejuni* isolates (74.6%) were resistant to Quinolone (Ciprofloxacin and Nalidixic acid).
2. A total of 33 *C. jejuni* isolates were found to react with the antisera against 9 serotypes, and 30 strains (47.6%) remained untypeable.
3. B:2 (14) and Y:37 (7) were the predominant serotypes and comprised 64% (21/33) of the isolates with serotype.
4. Forty-two different AFLP types were observed in all 63 *C. jejuni* isolates, and 11 AFLP types were present in more than one isolate (Fig.1). The predominant AFLP type 1 was observed in 5 (7.9%) isolates, and type 5 and 12 in 4 (6.3%) isolates, respectively (Fig.1). Cluster analysis revealed two subgroups (A and B) with 40% genetic similarity (Fig. 1).
5. The four serotypes (B:2; J:11; P:21 and Y:37) among *C. jejuni* isolates were found in human case and chicken-related samples, and these types constitute the most numerous indistinguishable groups of isolates. In addition, AFLP fingerprints of chicken-related strains were identical to strains from humans with gastroenteritis in AFLP types 1, 5, 12, 14 and 31.
6. Some chicken samples yielded multiple strains of *Campylobacter*, harboring simultaneously a quinolone-resistant and a quinolone-sensitive strains attributed to the same species.
7. The *flaA*, *ceuE* and *cadF* were found in 100%, 98.4% and 96.8% of isolates, respectively. The *cdtB*, *cdt* genes, *cdtA* and *cdtC* were detected from 100.0%, 98.4%, 90.5% and 82.5% of isolates, respectively (Table 1).
8. The frequency of *cdtC* gene among *C. jejuni* isolates from human were higher than that among *C. jejuni* isolates from chicken.

Discussion and Conclusion

1. These data support the conclusions from previous epidemiological studies that chicken-related samples, obtained from chicken food processing plant and slaughter plant could act as a vehicle for human sporadic cases.
2. Most identical serotypes were distributed among different genotypes, suggesting that serotyping alone cannot be used for strain identification.
3. Antibiotic-resistant *Campylobacter* with toxin or virulence genes may be more 'virulent' than antibiotic-susceptible isolates without those genes.

附件一

國外研究學者索取壁報論文後回函致謝的郵件

寄件者: "Catherine Yoshida" <Catherine_Yoshida@phac-aspc.gc.ca>
收件者: <_$BJ}>R0R_ (B <ufpfang@nlfd.gov.tw>)>
傳送日期: 2005年6月21日下午07:38
主旨: Re: ASM

Hello Shao-Wei,

Thank you very much for your ASM poster.

Catherine

Catherine Yoshida, M.Sc.

Research Technician
Laboratory for Foodborne Zoonoses
Public Health Agency of Canada
110 Stone Road West, Guelph, ON, N1G 3W4
Telephone: 519.822.3300 x246
Facsimile: 519.822.2280

國外研究學者索取壁報論文的郵件

寄件者: "Ditty, Susan" <sditty@mriresearch.org>
收件者: <ufpfang@nlfd.gov.tw>
傳送日期: 2005年6月20日下午11:22
主旨: Request for ASM poster C-386

Dear Dr. Fang,

I would be very interested in receiving a copy of the poster you presented at ASM in Atlanta. Please do not send as a ZIP file – our email security doesn't let those files through.

Thank you very much.

Susan

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Susan Ditty
Senior Scientist
Midwest Research Institute-Florida Division
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Palm Bay, FL 32909
sditty@mriresearch.org
321-723-4547, ext. 340
321-722-2514, Fax

向國外研究學者索取其壁報論文所收到的郵件

寄件者: "Patricia Sellenriek" <pxs3395@bjc.org>
收件者: <ufpfang@nlfd.gov.tw>; <jsagurton@ormc.org>; <sovernma@pop.uky.edu>; <granilg@questdiagnostics.com>; <fern.parisian@sickkids.edu>; <lisa.onischuk@state.nm.us>; <lstutzman@trekds.com>; <kwait@unch.unc.edu>; <paula.morge@us.gambro.com>
傳送日期: 2005年6月11日上午06:10
附加檔案: 2005 Poster22 .ppt; SUSCEPTIBILITY LIMITATIONS3doc.doc
主旨: ASM Poster C-203
Please find attach a copy of my poster C-203 (Comparison of automated instruments for ID/SUS) from ASM.

Regards,

Patricia Sellenriek M (ASCP) SM
Microbiology/ Immunology/ Serology Supervisor
St. Louis Children's Hospital
(314) 454-6115
psellenriek@bjc.org

寄件者: "English, Linda L" <LENGLISH@CVM.FDA.GOV>
收件者: <ADELA_RAMOS@agriculture.senate.gov>; <ufpfang@nlfd.gov.tw>
傳送日期: 2005年6月12日下午11:58
附加檔案: ASM poster (small) 060305 rev.ppt
主旨: request for poster A066 2005 ASM mtg
Hello,
I hope you had a good trip to Atlanta and returned home safely. I've attached a power point file of my poster, A-066, that you requested at the ASM meeting last week.

Best regards,

Linda English

寄件者: "Nordstrom, Jessica L" <Jessica.Nordstrom@cfsan.fda.gov>
收件者: <Stephen.bell@noaa.gov>; <kverville2@washcoll.edu>; <ufpfang@nlfd.gov.tw>
傳送日期: 2005年6月15日上午03:35
附加檔案: ASM 05 Nordstrom.ppt
主旨: ASM Poster

Thank you all for your interest in my poster at the ASM meeting. As per your requests, I have attached a copy of the poster. Please let me know if you have any questions or would like additional information.

Best regards,
Jessica

Jessica Nordstrom
Microbiologist
US FDA / GCSL
Dauphin Island, AL
251-694-4480 x 238

C-203

COMPARISON OF MicroScan Walk-Away®, Phoenix™ AND VITEK-TWO® MICROBIOLOGY SYSTEMS USED IN THE IDENTIFICATION AND SUSCEPTIBILITY TESTING OF BACTERIA

Patricia Sellenriek[1], Jennifer Holmes[1], Rhonda Ferrett[1], Russell Drury[2] and Gregory A. Storch[1,3]

St. Louis Children's Hospital[1], Missouri Department of Health and Senior Services[2] & Department of Pediatrics, Washington University School of Medicine[3]

Washington University in St. Louis School of Medicine

Children's Hospital • St. Louis

ABSTRACT (Revised)

We compared three automated microbiology systems: MicroScan Walk-Away® (DADE BEHRING INC., West Sacramento, CA), Phoenix™ [P] (Becton Dickinson Diagnostic System, Sparks, MD) and VITEK TWO® [V] (bioMérieux, Durham, NC). Two different panels were used with the MicroScan system: Rapid [MR] and conventional [MC]. Organisms tested included clinical isolates from cultures obtained from a variety of body sites from patients at SLCH and a subset of challenge organisms. The comparison standard was identification by a reference lab (Missouri Department of Health or CFF B. cepacia Research Laboratory, Ann Arbor, MI) or, when not available, consensus defined as two or more systems with matched results. To date, 363 isolates have been tested of which 347 have reference lab or consensus identification to species level.

Identification Summary

	No. tested	No. (%) correctly identified MR	MC	P	V
Gram negative	224	184 (82%)	176 (79%)	208 (93%)	172 (77%)
Gram positive	123	102 (83%)	94 (76%)	110 (89%)	99 (80%)

Note: Gram negative isolates were analyzed and compared at the species level when possible. When species level identification was not possible comparison was at the genus level. Gram positive isolates were always analyzed and compared at the species level.

Antimicrobial Susceptibility Test (AST) Summary

For susceptibility testing, 296 isolates were tested with a total of 2723 antimicrobials. The number of major errors and very major errors per system: MC 4/2, P 3/0 and V 1/3. Each system was unable to perform susceptibility for such reasons as no organism identification provided by the system or the identified organism not in system's AST database. The number of susceptibility results that were not available per system: M 469/2723 (17%), P 342/2723 (13%) and V 667/2723 (24%).

Time Study

Time to perform daily maintenance and panel set up times were analyzed for each system. The time to perform daily maintenance of each system (minutes): M 3.4, P 1.25 and V 0.5. The time to set up one panel per system (minutes): M 3.53, P 3.4 and V 3.19.

INTRODUCTION

In most clinical laboratories a quick and accurate identification of organisms is important for successful treatment of patients with bacterial infections. Restraints placed on busy clinical laboratories in today's healthcare setting mandate efficient work effort. Repeat testing or additional off-line testing delay results. In addition, susceptibility rules require accurate organism identification in order to apply identification-based algorithms.

In this study we compared automated systems from 3 different manufacturers, Becton Dickinson (Phoenix), BioMérieux (VITEK 2) and DADE BEHRING (MicroScan). DADE BEHRING provided two different systems for our study (Rapid Identification and Conventional ID/AST combination).

METHODOLOGY

Three automated identification and AST systems were compared using organisms from clinical patient samples along with a subset of challenge organisms. All inocula for testing were prepared from the same 18-24 h subculture from a tryptic soy agar with sheep blood that was incubated in O_2 for Gram negative and CO_2 for Gram positive organisms (except MicroScan Rapid Negative). For the MicroScan Rapid Negative panel, we used an 18-24 h subculture from a MacConkey agar incubated in O_2.

Comparison Standard for Identification

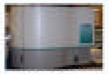
The identification results from all systems were compared for species agreement. Off-line testing to speciate or discriminate was not used.

1. If two or three manufacturers' systems obtained the same result, that result would be the comparison standard.

2. If all systems disagreed, the organism was sent to the Missouri State Public Health Laboratory for identification or the CFF B. cepacia Research Laboratory. The State or CFF B. cepacia Research Lab identification was used as the comparison standard. If neither identify the organism, the isolate was removed from the study. 16 isolates were excluded for this reason.

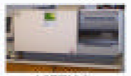
Comparison Standard for Antimicrobial Susceptibility Testing (AST)

AST results were compared using isolates that had a consensus identification. Comparison was limited to antibiotics that were tested by all three systems. Any disagreements between the systems that involved S to R or R to S were further analyzed using a MIC broth microdilution reference method as described in the National Committee for Clinical Laboratory Standards (NCCLS) 2003. "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved standard M2-A6. Major error or very major error definitions were those of the U.S. Department of Health and Human Services, Food and Drug Administration, 2003. Class II Special Control guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA. A major error was defined as Reference susceptible, test resistant. A very major error was defined as Reference resistant, test susceptible. Any isolate that fell into the major error or very major error category was repeated by the instrument yielding the discrepant result. Minor errors were not analyzed in this study. Only reproducible discrepancies were counted for analysis.



MicroScan

Phoenix



VITEK 2

	MicroScan	Phoenix	VITEK 2
Time to perform daily maintenance	3.40 min	1.25 min	0.5 min
Time to set up one panel	3.53 min (with prompt**)	3.40 min	3.19 min
Size: Height/Width/Depth (inches)	34/39/28.5	41.7/44.3/25.5	26.3/39.6/27.7
Weight (lbs)	400	333	240
Average time to identification	2.5 h (Rapid)* 6 - 18 h (Conventional)*	4.3 h*	3 h 5.7 h (Colorimetric)
Average time to AST	20 h	12 h	9 h

*provided by company

**Prompt Inoculation System-D provides the microbiologist with a method for obtaining standardized inocula while eliminating the need for turbidity adjustment.

Identification Summary

Organism	# Orgs Tested	MicroScan Rapid: Match to Species Level	Match to Genus Level	Wrong ID	No ID	Failure to fully ID	MicroScan Conventional: Match to Species Level	Match to Genus Level	Wrong ID	No ID	Failure to fully ID	Phoenix: Match to Species Level	Match to Genus Level	Wrong ID	No ID	Failure to fully ID	VITEK 2: Match to Species Level	Match to Genus Level	Wrong ID	No ID	Failure to fully ID
Enteric GNB	133	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Non-Enteric GNB	91	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Staphylococcus	61	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]
Streptococcus	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]
Enterococcus	11	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	11	*	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]
Other GPC	2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total	347	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
%		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Staphylococcus, Streptococcus and Enterococcus were not evaluated for genus level only identification

Failure to fully ID: isolates that were included as a possible identification with a low probability. Further testing needed to differentiate.

Identification Summary (Continued): Including Testing with VITEK 2 Colorimetric Card

Organism	# Tested	MicroScan Rapid: Match to Species	Match to Genus	Wrong ID	No ID	Failure to fully ID	MicroScan Conventional: Match to Species	Match to Genus	Wrong ID	No ID	Failure to fully ID	Phoenix: Match to Species	Match to Genus	Wrong ID	No ID	Failure to fully ID
Enteric GNB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Non-enteric GNB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Staphylococcus	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Streptococcus	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Organism	# Tested	VITEK 2: Match to Species	Match to Genus	Wrong ID	No ID	Failure to fully ID	VITEK 2 Colorimetric: Match to Species	Match to Genus	Wrong ID	No ID	Failure to fully ID
Enteric GNB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Non-enteric GNB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Staphylococcus	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Streptococcus	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Antimicrobial Susceptibility Testing Summary

Organism	No. Orgs Tested	Total # abx tested	MicroScan: Very Major Error	Major Error	Not Avail	Phoenix: Very Major Error	Major Error	Not Avail	VITEK 2: Very Major Error	Major Error	Not Avail
Enteric GNB	133	1580	1		107		1	61	1		229
Non-enteric GNB	91	611	1		282			249	1		374
Staphylococcus	61	488		4	72		2	32	1	1	64
Enterococcus	11	44			8						
TOTAL	296	2723	2	4	469	0	3	342	3	1	667

Not Avail: No susceptibility results available due to either no organism identification from a system or organism identification not in systems database

MicroScan, Phoenix and VITEK 2 had 107, 133 and 25 susceptibility results respectively available but with no other system to compare

Summary of Major and Very Major Errors

Study #	Antibiotic	Organism	Reference MIC**	MicroScan	Phoenix	VITEK 2
135	Cephalothin	K. oxytoca	R	R	I	S
198	Clindamycin	S. epidermidis	S	R	R	S
63	Gentamicin	S. aureus	R	I	R	S
196	Levofloxacin	S. epidermidis	S	R	R	S
198	Levofloxacin	S. haemolyticus	S	R	S	S
302	Oxacillin	S. lugdunensis	S	R*	S	
220	Penicillin	S. saprophyticus	S	S		R
244	Ticarcillin	C. koseri	S	S	R	S
33	Ticarcillin	E. coli	R	S	R	I
65	Trimethoprim Sulfa	S. maltophilia	R	S	R	
192	Trimethoprim Sulfa	S. maltophilia	R	R	R	S
			Major errors	4	3	1
			Very Major errors	2	0	3

* MicroScan actual MIC value same as reference value, interpretation different

** Reference broth microdilutions performed at Clinical Microbiology Institute, Wilsonville, OR 97070

CONCLUSIONS

Identification:

- All 4 systems provided a high level of accurate identification
- Phoenix had the highest number of correct identifications
- VITEK 2 Fluorimetric method had the fewest number of wrong identifications
- VITEK 2 Fluorimetric method had the largest combined number of no identifications and organisms that failed to fully identify of, whereas Phoenix had the fewest number
- Phoenix performed better for non-enteric GNB than the other systems
- MicroScan Conventional Panel had the most organisms that failed to fully identify
- In a limited evaluation, VITEK 2 Colorimetric card provided improved identifications compared to VITEK 2 Fluorimetric card

Susceptibility:

- Major and Very Major Errors were infrequent in all systems
- Each system has limitations of testing drug/bug combinations that could pose problems for clinical laboratories. Phoenix has the greatest number of limitations.

Time Study:

- Time to identification was longest for MicroScan Conventional Panel
- Time to AST was longest for MicroScan Conventional Panel
- Time to set up per test was similar for all systems

Acknowledgement: Our special thanks to the Missouri Department of Health and Human Services (Jefferson City, MO) for their help with isolate identifications. Also, we thank Maria Traczewski and Steven Brown, Ph.D. at Clinical Microbiology Institute (Wilsonville, OR) for their help with providing the MIC broth dilutions used in our AST arbitration.

A-006

Prevalence and Antimicrobial Susceptibility of *Campylobacter* Isolated from Retail Meat and Poultry in the United States, NARMS 2003

L.L. English, D.G. White, E. Hall-Robinson, S.L. Ayers, S.K. Hubert, R.D. Walker, and the NARMS Working Group
Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine,
Food and Drug Administration, Laurel, MD 20708

ABSTRACT

Background – To better understand the contribution of the food supply to antibiotic resistance among enteric bacteria, including Campylobacter, the National Antimicrobial Resistance Monitoring System (NARMS) was expanded in 2002 to include surveillance of retail meat. In 2003, this component of NARMS was further enhanced by including more sampling sites and a greater number of retail meat and poultry samples. **Methods** – In 2003, Campylobacter were recovered from a monthly sampling of chicken breasts, ground turkey, ground beef, and pork chops purchased from grocery stores in eight participating FoodNet sites (CA, CT, GA, MD, MN, NY, OR, and TN). Isolates were speciated using a PCR technique and tested for susceptibility to five antimicrobials using the CLSI/NCCLS agar dilution procedure. **Results** – Final data indicate that 13.6% of 3523 retail meat samples were contaminated with Campylobacter. A total of 479 isolates were recovered from 52.3% (469/897) of chicken breast; 0.6% (5/857) of ground turkey; 0.4% (4/889) of pork chop; and 0.1% (1/880) ground beef samples. *C. jejuni* was the predominant species recovered (69.7%), followed by *C.coli* (29.9%). Of the 334 *C. jejuni* isolates, 14.1% (47) had an MIC ≥ 4 µg/mL for ciprofloxacin, 23.1% (77) had an MIC ≥ 16 µg/mL for doxycycline, and all were susceptible to erythromycin. Of the 143 *C. coli* isolates, 14.0% (20) had an MIC ≥ 4 µg/mL for ciprofloxacin, 46.2% (66) had an MIC ≥ 16 µg/mL for doxycycline, and 11.2% (16) had an MIC ≥ 8 µg/mL for erythromycin. All Campylobacter isolates were susceptible to meropenem, and with the exception of one *C. jejuni* isolate, all were susceptible to gentamicin. Twenty-nine isolates showed resistance to multiple antimicrobials. **Conclusions** – Campylobacter, including antimicrobial resistant strains, are present in retail chicken breasts and can serve as a reservoir of resistant strains in the food processing chain.

INTRODUCTION

The retail meat component of NARMS is a cooperative effort of FDA's Center for Veterinary Medicine, CDC, and FoodNet laboratories. This poster presents final data on the prevalence and antimicrobial susceptibility of *Campylobacter* recovered from 897 samples of chicken breasts, 889 samples of pork chops, 880 samples of ground beef, and 857 samples of ground turkey collected at retail throughout 2003 by eight FoodNet laboratories. Initial isolation and identification of *Campylobacter* were done by the FoodNet labs; isolates were then frozen and shipped to CVM for final identification and antimicrobial susceptibility testing.

METHOD

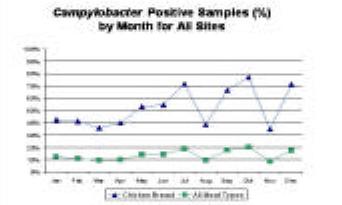
- **Sampling:**
  Monthly Jan-Dec 2003
  8 FoodNet Sites
  10 Packages each:
  Chicken Breasts (CB),
  Pork Chops (PC),
  Ground Beef (GB), &
  Ground Turkey (GT)
- **Isolation:**
  Campy Cefex Agar (CCA)
  Purification on Blood Agar
- **Speciation:**
  PCR [1,2,3,4,5]
- **Sample Preparation:**
  One CB or PC; 25g GB or GT
  Rinsed with 250mL BPW
  50mL Rinse + 50mL 2X Bolton Broth
- **Incubation:**
  42C 24-48 hrs
  5% O2 10% CO2 85% N2
- **Identification:**
  Gram stain with
  Catalase, Oxidase, Hippurate, or
  Wet Mount for Motility
- **Antimicrobial Susceptibility Testing:**
  CLSI (formerly NCCLS) Agar Dilution [6]
  Ciprofloxacin (Cip), Doxycycline (Dox), Erythromycin (Ery), Gentamicin (Gen), Meropenem (Mer)

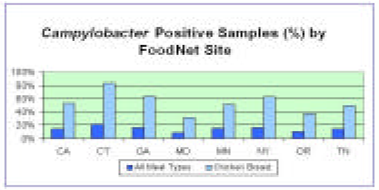
RESULTS

Campylobacter Positive Samples

Meat Type	Total # Samples	*C. jejuni* #	*C. jejuni* %	*C. coli* #	*C. coli* %	*Campylobacter* #	*Campylobacter* %
Chicken Breast	897	329	(36.7)	138	(15.4)	469[†]	(52.3)
Ground Turkey	857	4	(0.5)	1	(0.1)	5	(0.6)
Pork Chops	889	0	(0.0)	4	(0.4)	4	(0.4)
Ground Beef	880	1	(0.1)	0	(0.0)	1	(0.1)
All Meats	3523	334[a]	(9.3)	143[b]	(3.9)	479[†]	(13.6)

[a] C. jejuni = 69.7% of isolates [b] C. coli = 29.9% of isolates
[†] Includes two chicken breast samples positive for C. lari





Multiple Drug Resistance Phenotypes

	C. jejuni #	*C. jejuni* %	*C. coli* #	*C. coli* %	All Campy #	All Campy %
CIP-DOX	12	(3.6)	7	(4.9)	19	(4.0)
DOX-ERY	0	(0.0)	5	(3.5)	5	(1.0)
CIP-DOX-ERY	0	(0.0)	5	(3.5)	5	(1.0)
Total MDR	12	(3.6)	17	(11.9)	29	(6.1)

% Resistance and % Distribution of MICs among *Campylobacter* Isolates from All Meats

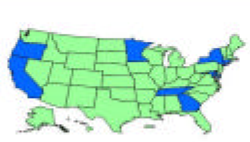
MICs (µg/mL) [c,d]

	Campy [a]	%R	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
CIP	All [b]	14.4				1.9	48.6	26.7	9.1		0.2	0.4	1.9	5.0	6.7	0.4	
	C. jejuni	14.1				2.1	57.2	21.9	4.5		0.3	0.6	2.4	6.1	4.8	0.3	
	C. coli	14.0				1.4	29.4	38.5	16.8				0.7	1.4	11.2	0.7	
DOX	All	29.9				17.1	23.4	6.4	1.7	1.5	2.5	4.4	14.2	16.5	11.5		
	C. jejuni	23.1				23.1	21.9	3.9	1.5	0.6	2.7	6.0	17.4	16.8	6.3		
	C. coli	46.2				3.5	27.3	7.7	2.1	3.5	2.1	0.7	7.0	16.1	23.8		
ERY	All	3.3						2.3	16.1	43.6	23.0	11.7	0.4	0.2			
	C. jejuni	0.0						0.9	18.3	55.1	21.0	4.8					
	C. coli	11.2						5.6	11.2	17.5	28.0	28.6	1.4	0.7			
GEN	All	0.2					1.0	21.5	62.4	14.2	0.6				0.2		
	C. jejuni	0.3					0.9	15.0	69.0	15.9					0.3		
	C. coli	0.0					1.4	37.1	50.3	10.5	0.7						
MER	All	0.0		21.9	50.1	15.8	10.0	0.6	1.0	0.2	0.2						
	C. jejuni	0.0		29.0	62.3	6.0	2.7										
	C. coli	0.0		4.2	22.4	39.2	27.3	2.1	3.5	0.7	0.7						

[a] For All Campy, N=479; for *C. jejuni*, N=334; for *C. coli*, N=143.
[c] ▭ Denotes concentrations tested. For MER, 0.001 (µg/mL) was the lowest concentration tested.
[b] Includes two *C. lari*, both resistant to ciprofloxacin.
[d] | Denotes resistant breakpoint used. Currently, there are no CLSI interpretive criteria for Campylobacter.

CONCLUSIONS

- Campylobacter were recovered from approximately half of the chicken breast samples but infrequently from the other meats.
- *C. jejuni* was isolated 2-3 times more often than *C. coli*.
- The percent positive samples varied among the FoodNet sites.
- There was no clear seasonality in the rate of Campylobacter isolation, though there were spikes in July, Sept-Oct, and December.
- Campylobacter were generally susceptible to erythromycin, gentamicin, and meropenem, though *C. coli* showed some resistance to erythromycin.
- 29.9% of Campylobacter were resistant to doxycycline with *C. coli* accounting for about 2/3 of this resistance.
- 14.4% of Campylobacter were resistant to ciprofloxacin with *C. jejuni* and *C. coli* exhibiting similar proportions.
- Some multiple drug resistance was observed, again more often in *C. coli* than in *C. jejuni*.



Acknowledgements

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New York State Department of Health David Axelrod Institute
Oregon Public Health Laboratory
Tennessee Department of Health Laboratory Services
CDC National Center for Infectious Diseases – Foodborne and Diarrheal Diseases Branch

References

Real Time PCR and Standard Culture Methods for Estimating Total and Pathogenic *Vibrio parahaemolyticus*-MPN in Alaskan Oysters

Q-349

Jessica Nordstrom[1], Shelley Murray[2], Christine Allison[2], Collette Bentz[2], Sara Watt[2] Joseph McLaughlin[3], Eric Thompson[3], Elizabeth Sachs[4], Calley Vandegrift[4], George Blackstone[1], and Angelo DePaola[1]
[1]FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528; [2]Alaska Dept. of Environmental Conservation, Palmer, AK 99645; [3]Alaska Dept. of Health and Social Services, Anchorage, AK 99503; [4]Washington Dept. of Health, Seattle, WA 98155

ABSTRACT

INTRODUCTION

RESULTS AND DISCUSSION

Figure 1.

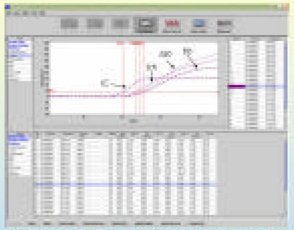

Figure 2.

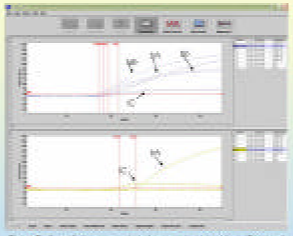

Figure 3.

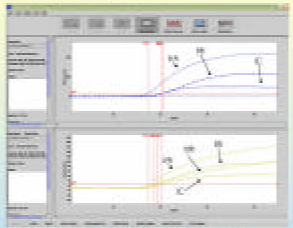

Figure 4.

MATERIALS AND METHODS

CONCLUSIONS