Using pulsed-field gel electrophoresis in the molecular investigation of an outbreak of Serratia marcescens infection in an intensive care unit

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ABSTRACT

Introduction: ***Serratia marcescens*** **is a well-known cause of nosocomial infections and outbreaks, particularly in immunocompromised patients with severe underlying disease. An outbreak due to** ***S. marcescens*** **infection was detected from 13 to 22 February 2001 at the intensive care unit (ICU) of our institution. We used pulsed-field gel electrophoresis (PFGE) typing to analyse the outbreak strains involved.**

Methods: A total of 25 isolates were included in this study: 12 isolates from infected patients, nine isolates from insulin solution, one isolate from sedative solution (midazolam and morphine infusion) and one isolate from frusemide solution. Two isolates from other wards which were epidemiologically-unrelated were also included.

Results: The ***S. marcescens*** **from patients, insulin solution and sedative solution showed an identical PFGE fingerprint pattern. The isolate from the frusemide solution had a closely-related PFGE pattern to the outbreak strain with one band difference. Attempts were made in the present study to identify the environmental reservoir of** ***S. marcescens*** **during the outbreak. We found that the insulin and sedative solutions used by the patients were contaminated with** ***S. marcescens*** **which was proven to be the source of the outbreak.**

Conclusion: Using PFGE, we showed that the outbreak in the ICU of our hospital was due to the clonal spread of a single strain of ***S. marcescens.***

Keywords: intensive care unit, nosocomial infection, pulsed-field gel electrophoresis, *Serratia marcescens*

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INTRODUCTION

Serratia marcescens is recognised as an aetiological agent in nosocomial infection. Outbreaks of *S. marcescens* strains are becoming more frequently reported[1-3], including urinary tract infections, wound infections, pneumonia, and bacteremia[4-6]. Environmental sources of *S. marcescens* infections in the hospital are widely described[7-9]. This micro-organism has a predilection for a moist environment. Multiple resistance of *S. marcescens* to antibiotics causes a problem in treating infected patients and controlling outbreaks[8-10]. The study of epidemiological markers is important in attempting to trace the source of contamination or to prevent dissemination of strains among patients.

Pulsed-field gel electrophoresis (PFGE) typing is highly effective in molecular epidemiological studies of bacterial isolates, and is superior to other methods in discriminating among isolates such as *Escherichia coli*, *Staphylococcus aureus*, and many other species[11]. Traditional methods are often based on phenotypic characteristics and include biotyping, serotyping[12,13], antibiogram analysis, bacteriocin typing[14], phage typing[15], and plasmid typing[16]. These traditional methods had been found to be insufficiently discriminatory, have poor reproducibility, suffer from lack of availability of specific reagents[17] and are affected by physiological factors[18]. PFGE typing can be used to evaluate the clonal relatedness among bacterial isolates and to investigate outbreaks[19]. In this study, we investigate an outbreak of *S. marcescens* infection in an intensive care unit (ICU) and to identify the source of the outbreak using combination methods of antibiogram analysis and PFGE technique.

METHODS

Twenty-five *Serratia marcescens* isolates were collected during the outbreak over the period, 10-23 February 2001. Isolates nos. 1 to 23 were from the ICU, and isolates nos. 24 and 25 were from other wards. Twelve isolates were from blood samples of eight patients, nine isolates were recovered from the insulin solution, one isolate from sedative solution, one isolate from frusemide solution and two epidemiologically-unrelated isolates were collected from other wards prior and after the outbreak period. Samples and swabs from the environment such as intravenous bottles and solutions, mechanical ventilator tubings,

Table I. Characteristics of the outbreak-related and epidemiologically-unrelated *S. marcescens* strains isolated from patients.

Isolate no.	Date of isolation (day/month/year)	Patient no.	Underlying disease*	Source	Ward	PFGE type	Outcome
1	13/2/01	1	Gullian Barre syndrome	Blood	ICU	A	
2	16/2/01			Blood		A	Recovered
3	20/2/01			Blood		A	
4	18/2/01			Blood		A	
5	13/2/01	2	Severe pancreatitis with bronchopneumoniae	Blood	ICU	A	Died on 15/2/01
6	13/2/01	3	Craniopharyngioma	Blood	ICU	A	Died on 17/2/01
7	15/2/01	4	Acute exacerbation of COAD with pneumonia	Blood	ICU	A	Died on 15/2/01
8	14/2/01	5	MVA with flail chest	Blood	ICU	A	Died on 21/2/01
9	15/2/01	6	Diabetic nephropathy with PUO	Blood	ICU	A	Died on 15/2/01
10	16/2/01	7	Uncontrolled diabetes mellitus	Blood	ICU	A	Died on 24/2/01
11	22/2/01		with septicaemia	Blood		A	
12	18/2/01	8	MVA with intracerebral bleeding	Blood	ICU	A	Died on 23/2/01
X	10/2/01	9	Respiratory infection	Sputum	Medical	B	Recovered
Y	23/2/01	10	Alleged MVA	Swab left foot	Trauma	C	Recovered

*COAD: chronic obstructive airway disease; PUO: pyrexia of unknown origin; MVA: motor vehicle accident.

Table II. Characteristics of the outbreak-related *S. Marcescens* strains isolated from environmental sources.

Isolate no.	Date of isolation (day/month/year)	Source	Samples from patients no.	Ward	PFGE type
13	17/2/01	Insulin sol.	3	ICU	A
14	16/2/01	Insulin sol. (i/v line)	4	ICU	A
15	16/2/01	Sedative sol.	5	ICU	A
16	16/2/01	Insulin sol.			A
17	17/2/01	Insulin sol. (syringe)			A
18	20/2/01	Insulin sol.			A
19	20/2/01	Insulin sol. (i/v line)	7	ICU	A
20	22/2/01	Frusemide sol.			A1
21	22/2/01	Insulin sol. (i/v line)			A
22	17/2/01	Insulin sol. (i/v line)	8	ICU	A
23	20/2/01	Insulin sol. (i/v line)			A

** sol.: solution; i/v: intravenous.

Table III. Antimicrobial susceptibility pattern of *S. marcescens* isolates.

Isolate no.	Antimicrobial susceptibility pattern*											
	Am	Ctx	Cxm	Chl	Cip	Gn	An	Cfp	Imp	Net	Caz	Amc
1-23	R	R	R	R	R	S	S	R	S	S	S	R
X	R	S	S	S	S							
Y	R	S	R	S	S							

* AM: ampicillin; Ctx: cefotaxime; Cxm: cefuroxime; Chl: chloramphenicol; Cip: ciprofloxacin; Gn: gentamicin; An: amikacin; Cfp: cefoperazone; Imp: imipenem; Net: netilmicin; Caz: ceftazidime and Amc: amoxicillin-clavulanic acid.

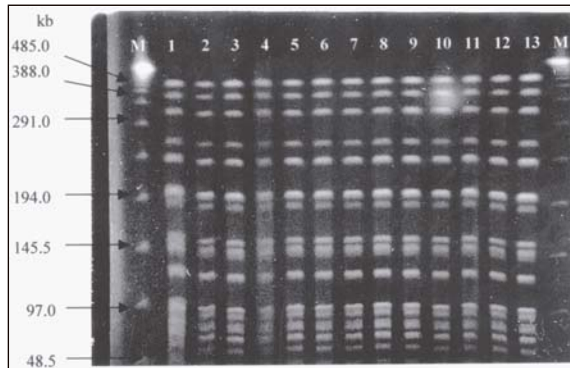

Fig. 1 PFGE fingerprint of *S. marcescens* strains isolated during the outbreak. (M: Lambda DNA marker).

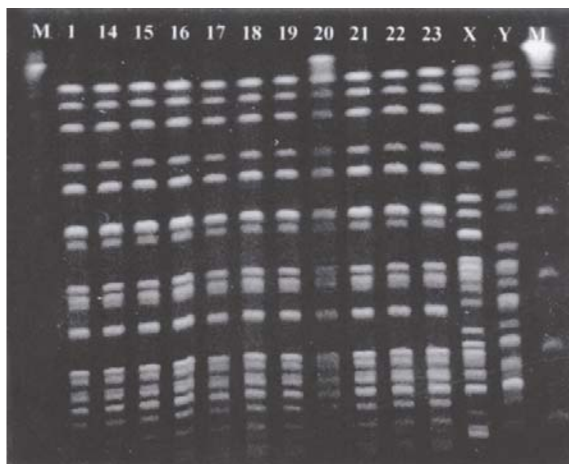

Fig. 2 PFGE fingerprint of *S. marcescens* strains and epidemiologically-unrelated strains (isolate X and Y) isolated during the outbreak. (M: Lambda DNA marker).

water catches, humidifiers and drugs (heparial 1,000) were sent for microbiological testing for isolation of *S. marcescens*.

Susceptibility of the micro-organisms to the following antibiotics were determined by the disk diffusion method on Muller-Hinton agar according to the procedures suggested by the National Committee for Clinical Laboratory Standard[20]: ampicillin 10µg, cefotaxime 30µg, cefuroxime 30µg, chloramphenicol 30µg, ciprofloxacin 5µg, gentamicin 10µg, amikacin 30µg, cefoperazone 75µg, imipenem 10µg, netilmicin 30µg, ceftazidime 30µg and amoxicillin-clavulanic acid 20/10µg.

PFGE analysis

Preparation of genomic DNA for PFGE was performed as previously described[21] with in-house modification. A well-isolated colony of each isolate was inoculated into 5ml of tryptic soy broth and incubated overnight at 37ºC with shaking. The broth culture was adjusted to a concentration of 1 x 10^9 bacterial per ml. The culture was harvested by centrifugation. The cells were washed in TE buffer (10 mM Tris-HCl, 50 mM EDTA; pH 7.5), and then resuspended in the same buffer. This bacterial suspension was then mixed with an equal volume of 2% low-melting point agarose (Sigma, USA), and was allowed to solidify in a 100µl plug mold (Bio-Rad, USA). Plugs were removed and incubated in 2ml of ES buffer (1% N-laurylsarcosine in 0.5M EDTA, pH 8.0) containing 50µg of ribonuclease A (Qiagen, Germany) per ml and 2mg/ml lysozyme (Sigma, USA) overnight at 37ºC with gentle shaking.

The buffer was then replaced with 2ml ES buffer containing 1mg/ml proteinase K (Sigma, USA), and the plugs were incubated at 50ºC overnight with shaking. The plugs were then washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) at 4ºC. For restriction endonuclease digestion, plug slices were equilibrated in 100µl of the appropriate restriction buffer at room temperature, after which 40U of *XbaI* enzyme (New England Biolabs, USA) were added and incubated according to the manufacturer's instructions. Plug slices were then loaded into 1.2% PFGE agarose (Sigma, USA) in 0.5x TBE buffer. PFGE was performed with a CHEF-DRIII system (Bio-Rad, USA) using the technique developed by Chu et al[22] for 24 hours with pulse times of 5 to 20 seconds. The gels were stained with ethidium bromide and photographed under ultraviolet light using GelDoc 2000 (Bio-Rad, USA) equipment.

Differences between isolates were determined by visual comparison of DNA fragments. Based on the criterion of Tenover et al[19], isolates were considered to be identical if their PFGE patterns were identical. An isolate was considered to be a closely-related strain if the PFGE pattern differed from the outbreak pattern by one to three bands. An isolate was considered to be possibly related if its PFGE pattern differs from the outbreak pattern by four to six bands. An isolate was considered distinct if the isolate differed from the outbreak pattern by seven or more bands[19].

RESULTS

Table I lists the characteristics of the 12 outbreak-related *S. marcescens* isolated from patients and two epidemiologically-unrelated isolates (isolate X and isolate Y). Table II show the characteristics of outbreak-related *S. marcescens* isolated from environmental sources. Antimicrobial susceptibility testing (Table III) show that all the isolates from the outbreak strains have the same pattern of susceptibility. These isolates were resistant to ampicillin, cefotaxime, cefuroxime, chloramphenicol, ciprofloxacin, cefoperazone and amoxicillin-clavulanic acid, and were susceptible to gentamicin, amikacin,

imipenem, netilmicin and ceftazidime. All the isolates were non-ESBL producers. The two unrelated isolates had different susceptibility patterns.

By PFGE typing, the outbreak strains (except isolate no. 20) had an identical PFGE fingerprint designated as type A (Figs. 1 & 2). Strains isolated from the insulin solution (isolate nos. 13, 14, 16, 17, 18, 19, 21, 22 and 23) from the infected patients also had the same PFGE pattern. However, the strain isolated from intravenous frusemide solution (isolate no. 20) from patient no. 7 showed one-fragment difference compared to the strains with PFGE type A. The strain had one extra band at molecular weight more than 485 kb. This strain was considered as being closely-related to the strain with PFGE type A, and was designated as subtype A1. The PFGE fingerprint of the two epidemiologically-unrelated strains (isolate X and Y) demonstrated two distinct patterns (types B and C, respectively). Isolate X was isolated prior to the outbreak period on 10 February 2001, and isolate Y was isolated after the outbreak period on 23 February 2001.

DISCUSSION

Severe illness due to *Serratia marcescens* is generally seen in immunocompromised patients[8]. In the present article, all patients had various severe underlying diseases and they were treated with imipenem/cilastin. These patients are susceptible to *S. marcescens* infection. Seven out of eight bacteremic patients died. An effective typing method is a prerequisite for identifying and controlling bacterial outbreaks. Phenotypic methods such as antibiotic testing help guide the microbiologist to monitor an outbreak. However, these techniques could not confirm the relationship among outbreak strains. The development of PFGE typing methods based on fingerprinting of bacterial genome has given microbiologists tools to detect the origins and route of hospital outbreaks and epidemics and a step toward control and elimination of infection[19].

PFGE typing revealed that all twelve *S. marcescens* strains isolated from the patients' blood were genetically identical (PFGE type A), and that this clone was responsible for the outbreak in the ICU. PFGE was able to distinguish two other strains in which distinct PFGE patterns that were distinct from the outbreak strain were epidemiologically-unrelated. Extensive investigation and cultures of samples from environment started on 16 February 2001 when four patients (patient nos. 1, 2, 3 and 4) in the ICU had *S. marcescens* bacteremia. From this environmental investigation, we identified that the insulin, sedative and frusemide solutions that were used by the patients were contaminated with *S. marcescens* with an identical PFGE pattern. However, one isolate (isolate no. 20) had minor genomic differences designated as subtype A1 and was isolated from frusemide solution at the end of the outbreak period. This strain had an extra DNA fragment at molecular weight more than 485 kb, compared to the outbreak strain. The fragment may have been introduced to the *S. marcescens* strain during the course of the outbreak, because bacterial populations may alter in genetic structure due to the stress of survival in hostile environments. This is reflected in the appearance of the genetic rearrangement of the bacteria.

The mode of transmission of nosocomial bacterial infections has rarely been identified. In the present study, no attempt was made to identify the carrier of *S. marcescens* among hospital personnel, doctors and nurses in the ICU. However, it is argued that cross-transmission occurs mainly from the contaminated hands of hospital staff. Hand to hand spread by hospital personnel is the most important factor in horizontal transmission[6,23,24]. During the outbreak, a number of infection control measures were introduced. Strict hand washing and wearing of gloves by personnel between their handling of patients was stressed. This study is in agreement with others that stressed the importance of environmental sources of *S. marcescens* epidemics[25,26]. The usage of multi-vial insulin solutions and sharing of drugs in the early management of the patients contribute to the spread of *S. marcescens* among patients in the ICU.

To our knowledge, this is the first report of applying PFGE typing to the study of an outbreak caused by *S. marcescens* infection in our country. *S. marcescens* strains recovered during the course of the small outbreak in the ICU exhibited indistinguishable genomic patterns. This report revealed that one cluster of hospital-acquired bacteremia associated with *S. marcescens* appeared to be a single outbreak due to the genetic homogeneity of the clinical isolates.

In conclusion, PFGE typing was found to be highly discriminatory and reproducible for the epidemiological investigation of *S. marcescens* infection. This typing method can facilitate the reliable evaluation of the clonal relationship of *S. marcescens* isolates and the identification of the common sources of outbreaks. It is important to type suspected epidemic strains in order to understand the natural history of *S. marcescens* outbreaks and to educate the staff regarding cross-transmission, to avoid on-going spread of the organism. The results and outcome from this study clearly shows how clinical evaluation and microbiological testing can help identify outbreaks. The knowledge gained from this outbreak led us to change our ICU policy pertaining to multi-vial drug usage and sharing of

drugs in the ICU. We further stepped up our surveillance in ICU infection control compliance among staff and to date, there have been no other outbreak in the ICU.

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