Original Report

Epidemiology and Molecular Characterization of Nosocomially Transmitted Multidrug-Resistant *Klebsiella pneumoniae*

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ABSTRACT

Objectives: To describe the epidemiology, antimicrobial susceptibility, genomic profiles, and control of a nosocomial outbreak of multidrug-resistant *Klebsiella pneumoniae* (MRKP) that occurred in the pediatric oncology unit of the University of Malaya Medical Centre in Kuala Lumpur.

Materials and Methods: A prospective epidemiologic and microbiologic study was conducted of MRKP isolated from the blood and wound of a boy with necrotizing fasciitis after a 7-day course of ceftazidime and amikacin. In the following 2 weeks, phenotypically similar MRKP were isolated from the blood cultures of four other patients and rectal swabs of another three patients and two liquid soap samples located in the same ward.

Results: Antimicrobial profiles demonstrated that all the isolates were resistant to ceftazidime, sensitive to imipenem and ciprofloxacin, and confirmed to be extended-spectrum beta-lactamase producers. Plasmids of varying molecular weights were present in all isolates. In eight of these isolates, which included four from blood, there were common large molecular weight plasmids ranging from 80 kb to 100 kb. Pulsed-field gel electrophoresis analysis using *Xba*I demonstrated six different DNA profiles, A to F. Profile A was shared by two blood culture isolates and were related by 91%. Profile B was found in one rectal swab isolate and one isolate from liquid soap and were related by 94%. Profile C was shared by one blood isolate and one liquid soap isolate and showed 100% relatedness. Profiles D, E, and F each were demonstrated by one blood isolate and two rectal swab isolates, respectively. These showed only 65% relatedness.

Conclusions: The MRKP strains in this outbreak were not clonal in origin. The decline of the outbreak after 4 weeks was attributed to the reemphasis of standard infection control procedures and the implementation of a program that addressed sites of environmental contamination.

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Received: June 21, 1999; Accepted: December 1, 1999.

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Key Words: *extended-spectrum beta-lactamase, molecular epidemiology, multiresistant* Klebsiella pneumoniae, *nosocomial outbreak*

Int J Infect Dis 2000; 4:123–128.

Strains of *Klebsiella pneumoniae* that produce extended-spectrum beta-lactamases (ESBL) are becoming increasingly prevalent as nosocomial pathogens. Risk factors for the emergence of these strains include prior treatment with antimicrobials, an overall increase in the use of third-generation cephalosporins, prolonged hospital stay, and treatment in intensive care units. Hospital outbreaks of infections attributable to multidrug-resistant *K. pneumoniae* (MRKP) and ESBL-producing strains have been observed since 1984.[1,2] Most have occurred in intensive care units, oncology units, and geriatric or chronic-care facilities. Hospital colonization by ESBL-producing bacteria is complex and involves different mechanisms, such as dissemination of several epidemic strains and dissemination of plasmids and resistance genes.[1,3–7]

In the pediatric oncology unit of the University of Malaya Medical Centre, *K. pneumoniae* consistently has been the most commonly isolated gram-negative bacteria, accounting for up to 20% of blood culture isolates yearly,[8] and ceftazidime is used extensively as empirical therapy for febrile neutropenia in the unit. Strains resistant to the third-generation cephalosporins have been isolated since 1990, and there has been a steady increase to about 50% in the past 2 years. Although these strains have been isolated year-round in the unit,[9] no outbreaks of MRKP expressing ESBL activity previously have been reported.

During a 4-week period between December 1997 and January 1998, an outbreak of MRKP occurred in the 28-bed pediatric oncology unit of the University of Malaya Medical Centre, Kuala Lumpur. The index case was a 7-year-old boy with relapsed acute myelogenous leukemia who developed necrotizing fasciitis of the perineum following a testicular biopsy. Multidrug-resistant *K. pneumoniae* was isolated from his wound swabs and blood cultures. Following his admission to the ward, four other patients developed phenotypically similar MRKP sepsis, with two fatalities. This report describes the

epidemiologic studies to identify and control transmission of the *Klebsiella* strain during this outbreak.

MATERIALS AND METHODS

Epidemiologic Investigations

Apart from the four patients with positive blood cultures, screening for MRKP from "potential reservoirs" among other patients and in the environment was carried out. Rectal swabs were taken from 12 patients who were in the ward for febrile neutropenia or to receive chemotherapy, were in the unit at the time of admission of the index case, and had remained in the ward during the 2-week outbreak period. Short-staying patients (e.g., those admitted for blood transfusions) were not screened. Multidrug-resistant *K. pneumoniae* was isolated from three of these patients.

Two 500-mL glass bottles of heparinized saline, that were prepared daily on the ward for common use were sampled, as was a 2-L bottle of sodium bicarbonate solution that was dispensed in small quantities to all patients for gargling. Liquid soap containing detergent but no antibacterial substance, in four dispensing flasks located in various parts of the ward and used for handwashing also were examined for MRKP. This solution was supplied from the central hospital store in sealed 5-L stock bottles direct from the manufacturer. Flasks were filled by the ward staff on a twice-weekly basis. The liquid soap when initially opened was sterile.

Bacteriologic Methods

Specimen Processing

Blood cultures were processed using an automated Bactec 9240 continuous monitoring system (Becton-Dickinson Microbiology Systems, Sparks, MD, USA) following a 5-day protocol. Environmental and patient screening specimens (wound, rectal swabs, liquid soap samples, heparinized saline, and sodium bicarbonate solution) were either enriched in nutrient broth prior to subculture or directly cultured on standard bacteriologic media. Isolates were identified by standard bacteriologic methods.

Bacterial Strains

Nine strains of MRKP were isolated and were subjected to antimicrobial and molecular studies: four from blood cultures of four patients hospitalized for sepsis (samples B1 to B4), three from rectal swabs of three long-term patients on the ward screened (samples RS1 to RS3), and two from diluted liquid soap solution (samples Env1 and Env2). The strains from the soap solutions were isolated from two of the four liquid soap flasks used in the ward on a single day in the second week of the outbreak. The details are shown in Table 1. The isolates from the index case were not available for molecular characterization.

Table 1. Source and Plasmid Profiles of Multidrug-Resistant *K. pneumoniae* Isolated during the Outbreak

*Strain**	*Date of Isolation*	*Source*	*Molecular Weight of Plasmids (kb)*
B1	Dec 16, 1997	Blood	100
B2	Dec 18, 1997	Blood	100, 4
B3	Dec 20, 1997	Blood	80, 3
B4	Dec 23, 1997	Blood	80
RS1	Dec 30, 1997	Rectal swab	100, 3, 1.8
RS2	Dec 30, 1997	Rectal swab	34, 4
RS3	Dec 30, 1997	Rectal swab	100, 3, 1.8
Env1	Jan 4, 1998	Liquid soap	150, 80, 60, 34, 2.5, 2, 1.5
Env2	Jan 4, 1998	Liquid soap	150, 80, 60, 34, 2.5, 2, 1.5

B = blood sample from patients hospitalized for sepsis; RS = rectal swab from 3 of 12 long-term patients; Env = environmental from 2 of 4 liquid soap solutions used in the ward, collected on a single day in the second week of the outbreak.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities were determined using the National Committee for Clinical Standards (NCCLS) disk diffusion method on Mueller-Hinton agar and interpreted according to NCCLS guidelines. *Escherichia coli* ATCC 25922 was used as the control strain.[10] Antibiotics tested were ampicillin, trimethoprim-sulfamethoxazole, gentamicin, amikacin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, imipenem, and ciprofloxacin. Resistance to ceftazidime was used as a marker of resistance to all third-generation cephalosporins. Ceftazidime resistance was defined as a zone of inhibition of less than 15 mm. Production of ESBL was inferred on the basis of a positive synergy test between ceftazidime and amoxicillin-clavulanate on double-disk diffusion testing. Criteria for synergy between amoxicillin-clavulanate and ceftazidime was as described by Murray.[11] E-test ESBL screen strips (AB Biodisk, Solna, Sweden) impregnated with ceftazidime alone at one end and ceftazidime-clavulanate on the other were used to confirm ESBL production. A greater than fourfold logarithm reduction in the ceftazidime minimum inhibitory concentration (MIC) in the presence of clavulanate was taken as positive for ESBL production. The outbreak strain was defined as any *K. pneumoniae* resistant to all antibiotics except imipenem and ciprofloxacin and exhibiting ESBL activity.

Plasmid Analysis

Plasmid DNA was extracted by a modification of the technique of Kado and Liu and described by Threlfall et al.[12,13] A single colony of each strain of *K. pneumoniae* was inoculated into 5 mL of Luria-Bertani (LB) broth, and following overnight incubation with aeration at 37°C, cells were harvested by centrifugation at 13,000 rpm. The pellet was resuspended in 200 μL of snail acetone solution for 15 minutes at room temperature, followed by

centrifugation at 13,000 rpm, and the cells were resuspended in 20 μL of Tris-EDTA. The cells were then lysed by the addition of 100 μL of Tris-sodium dodecylsulfate (SDS) to the suspension and heating at 55°C for 30 minutes. Chromosomal DNA, protein-SDS complexes, RNA, and cell debris were removed by extraction with phenol-chloroform and centrifugation, and the aqueous phase, containing the plasmid DNA, was harvested. Bromophenol blue was added to plasmid DNA solution, and the plasmids were analyzed by electrophoresis in horizontal 0.7% agarose gels (Sigma Type 2, Sigma Chemical, St. Louis, MO, USA), submerged in Tris-borate-EDTA (TBE) buffer at 100 V for 2 hours, and stained with ethidium bromide. Plasmid DNA was visualized by transillumination with short-wave ultraviolet light. The molecular weights of the plasmids were determined in relation to the mobility of four reference plasmids carried in a strain of *E.coli* 39R861.[14]

Pulsed-Field Gel Electrophoresis

Bacterial DNA was immobilized in agarose plugs as described by Kaufmann and Pitt and digested for 18 hours at 37°C, using the restriction endonuclease *Xba*I (20 units, Promega, Madison, WI, USA).[15] The digested DNA fragments were separated on a 1.2% agarose gel by pulsed-field gel electrophoresis (PFGE) in a CHEF-DR II system (Contour Clamped Homogenous Electric Field, Bio-Rad Laboratories, Richmond, CA, USA) in 0.5× TBE buffer at 200 V for 33 hours, with pulsing times of 2 to 8 seconds for 11 hours, 10 to 20 seconds for 11 hours, and 15 to 40 seconds for 11 hours. The separated DNA was stained with ethidium bromide and visualized under ultraviolet transillumination. Lambda DNA ladder standard (Bio-Rad) was used as the molecular size marker. DNA banding patterns were analyzed for similarity using Dice coefficient with the aid of the BioImage Whole Band Analyzer software (BI Systems Corporation, Ann Arbor, MI, USA).

RESULTS

Environmental and Patient Screening to Determine the Source of Outbreak

In addition to the four isolates from the blood of four patients with septicemia, MRKP was also isolated from rectal swab samples of three of the four long-staying patients on the ward and from two of four liquid soap samples examined. Susceptible strains of *Klebsiella* species and *Citrobacter* species also were isolated from the liquid soap samples but, not being epidemiologically linked to the outbreak, were not subjected to further evaluation. The samples of heparinized saline were found to be sterile, and the sodium bicarbonate solution grew only *Bacillus* species. Direct questioning and observation revealed that ward staff failed to clean the liquid soap dispensing flasks prior to topping-up; adding new soap when 20 to 30 mL of "old" liquid soap remained in the flasks. It also was noted that ward staff and doctors' hands touched the spout of the flasks when taking the liquid soap to wash their hands after examining the patients. This was postulated to be the source of contamination of the soap and also may be the mode of cross-contamination of staff, with subsequent transfer to patients and other liquid soap flasks. Inadequate cleaning and refilling of half-filled flasks allowed the outbreak strain to remain in the environment.

Antimicrobial Susceptibility and Extended-Spectrum Beta-Lactamase Production

All of the strains isolated from the liquid soap, rectal swabs, and blood cultures showed the same susceptibility pattern. Among all strains, resistance to ampicillin, gentamicin, amikacin, trimethoprim-sulfamethoxazole, cefuroxime, cefotaxime, ceftriaxone, cefoperazone, and ceftazidime was noted, but susceptibility to imipenem and ciprofloxacin was evident. Screening for ESBL production by the double-disk synergy test was positive for all isolates. The MIC of all nine strains to ceftazidime was greater than 32 μg/mL, and the MIC value with the ceftazidime-clavulanate combination was 0.5 μg/mL in four strains and 1.0 μg/mL in five strains. A reduction of more than 32-fold in the MIC of ceftazidime in the presence of clavulanate was seen in all the epidemic strains, confirming ESBL production.

Plasmid Analysis

Plasmid analysis showed that all strains carried one or more plasmids of varying molecular weights (MW) from 1.5 kb to 150 kb in size; but all of them, including four from blood, had at least one high-MW plasmid with a molecular size of 150, 100, 80, 60, and 34 kb (Figure 1; see Table 1). Large MW plasmids of 80 kb to 100 kb commonly were present in all strains except RS2, isolated from a rectal swab.

Plasmid profile patterns were identical for the two environmental strains from liquid soap (Env1 and Env2). Both strains carried a 34-kb plasmid that was also present in rectal swab isolate RS2. Two other strains from rectal swab samples (RS1 and RS3) showed identical profiles. Two blood isolates (B1 and B2) had a common 100-kb plasmid that also was present in rectal isolates RS1 and RS3. An 80-kb plasmid was common to the remaining two blood isolates (B3 and B4), and this plasmid also was found in both environmental isolates Env1 and Env2.

Pulsed-Field Gel Electrophoresis

Six major DNA profiles designated A to F were identified (Figure 2). Dendrogram analysis of the PFGE profiles demonstrated three major clusters with an overall relatedness of 65% among the nine strains (Figure 3). Two

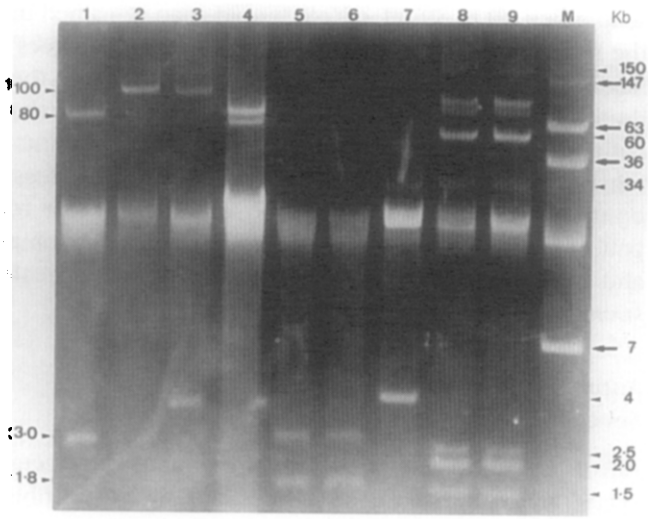

Figure 1. Plasmid profiles of *K. pneumoniae* strains. Lane 1, B3; lane 2, B1; lane 3, B2; lane 4, B4; lane 5, RS3; lane 6, RS1; lane 7, RS2; lane 8, Env1; lane 9, Env2; lane M, *E. coli* 39R861 as molecular-weight standard.

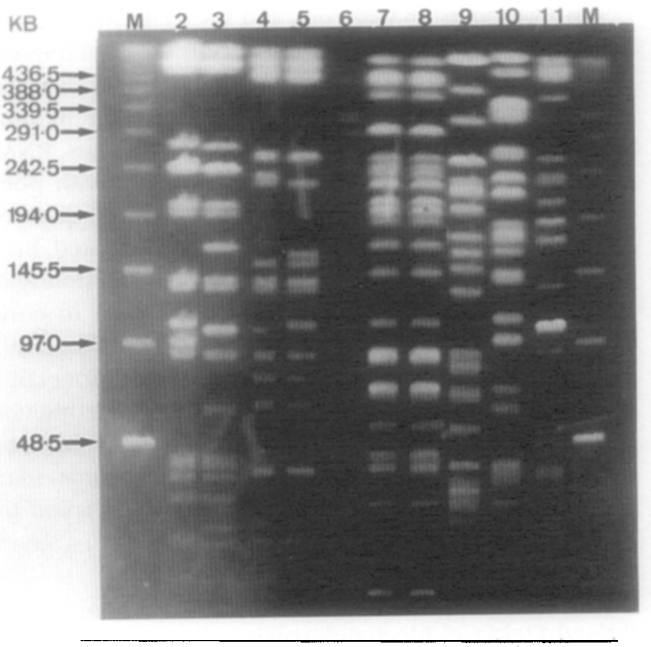

Figure 2. DNA macrorestriction profiles of *K. pneumoniae* strains using *Xba*I. Lane M, phage lambda DNA (48.5 kb multimers) marker. Lanes 2 and 3, profile A, B1 and B4; lanes 4 and 5, profile B, RS1, and Env1; lane 6, *Not*I-digested DNA of *E. coli* MG1655 as marker; lanes 7 and 8, profile C, B3 and Env2; lane 9, profile D, B2; lane 10, profile E, RS2; lane 11, profile F, RS3.

blood isolates (B1 and B4) belonged to profile A and were related by 91%. Profile B was shared by one rectal swab isolate (RS1) and one liquid soap isolate (Env1) and these were related by 94%. The two isolates, B3 from blood and Env2 from liquid soap, of profile C were found to have identical banding patterns and showed 100% relatedness. Profiles D, E, and F were demonstrated by one blood isolate (B2), one rectal swab isolate (RS2), and another rectal swab isolate RS3, respectively.

DISCUSSION

Outbreak Control

Control of the outbreak was achieved with strategies specifically aimed at sources of contamination and re-

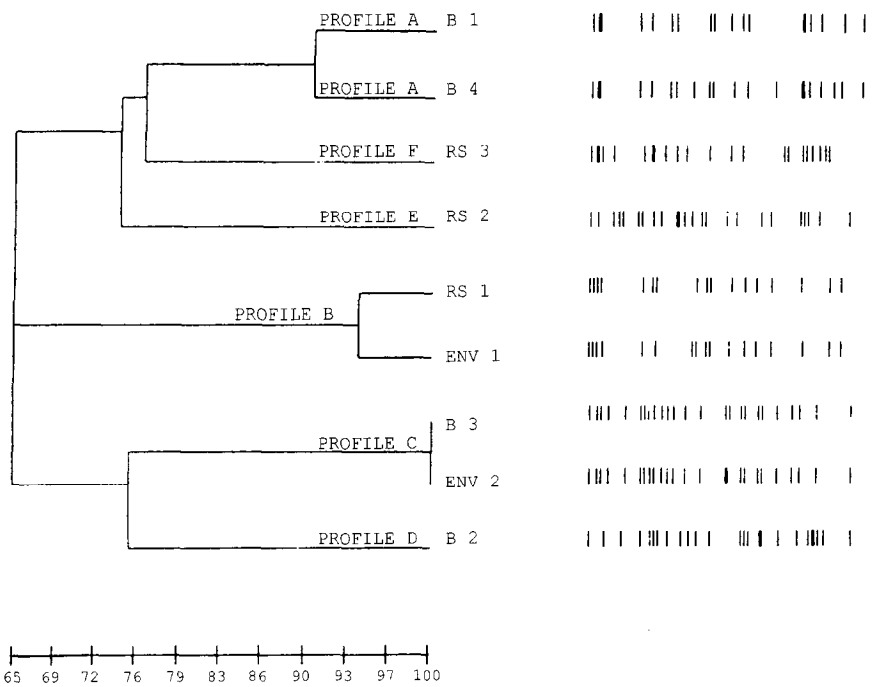

Figure 3. Dendrogram of relatedness of DNA macrorestriction profiles of *K. pneumoniae* strains.

emphasis of standard infection control procedures. Barrier precautions for infected and colonized patients were instituted. All patients with proven MRKP sepsis were treated with a 14-day course of imipenem, whereas colonized patients did not receive any antibiotic therapy and were sent home as soon as their clinical condition permitted. Carriage of MRKP apparently was transient, because repeat screening of these patients yielded negative results. The ward was closed to all new admissions during the outbreak and rational use of antibiotics with restricted use of ceftazidime was reemphasized to all doctors. Staff education activities on proper handwashing techniques, including the use of elbows to dispense soap from flasks of liquid soap and to turn on taps were carried out. It was mandatory for all liquid soap flasks to be emptied and soaked in hypochlorite solution on a weekly basis prior to being refilled. Hand disinfection with Hibiscrub™ or Hibisol™ (Zeneca Ltd., Macclesfield, Chesire, UK) containing chlorhexidine was instituted as an interim measure. The last case of MRKP occurred 4 weeks after the index case; no additional cases of MRKP sepsis occurred during the subsequent 3-month period.

Molecular Typing and Mode of Transmission

Outbreaks of ceftazidime-resistant *K. pneumoniae* have been attributed to the clonal spread of resistant strains and to the emergence of several resistant strains.[2,7,16-19] In the latter, resistance often is coded on large, multiresistance plasmids that, in some cases, have evolved in different ways within the same institution.[16,20] The results of plasmid analysis of the isolates responsible for these outbreaks often have suggested that several strains of *K. pneumoniae* as well as other *Enterobacteriaceae* are involved.[16]

The epidemic strains involved in this study had identical antibiotic profiles but were not clonal in origin. In contrast to the present outbreak, where two pairs of strains had identical plasmid profiles, plasmid analyses of 27 sporadic blood culture isolates of ceftazidime-resistant *K. pneumoniae* from patients in this hospital between March 1995 and August 1996 showed different plasmid profiles (data not shown). Shared plasmids (100 kb, 80 kb) were seen in environmental and patient strains in this outbreak. This suggests that transfer of plasmids between strains from the environment and between patients could have occurred. On the other hand, plasmids of similar sizes (147 kb, 91 kb, 60 kb) also occurred in several sporadic isolates, including eight isolates that were recovered in the pediatric oncology unit. Restriction enzyme digests with EcoR1 of plasmid DNA from eight transconjugants derived from the sporadic strains and carrying a single plasmid encoding for ceftazidime resistance showed different RE profiles (data not shown). Two of the donor strains were from the pediatric oncology unit. The molecular weight of the transferred plasmids varied between 60 and 100 kb. Thus, it is likely that, in the present outbreak, the large 80- to 100-kb plasmids that were present in all but one of the outbreak strains were carrying the genes encoding ESBL production in addition to genes encoding resistance to other antibiotics.

Pulsed-field gel electrophoresis analysis demonstrated the presence of six major DNA profiles, indicating that a few genotypes of *K. pneumoniae* were present in the unit. Although all strains had plasmids of 150 kb and below, these did not interfere with the PFGE patterns because the profiles were unique above 150 kb.

Several modes of transmission of ESBL-producing *K. pneumoniae* have been reported in previous outbreaks. It has been suggested that the bacteria are transmitted via the hands of hospital personnel and via medical devices (e.g., endoscopes and catheters).[21-24] Environmental contamination also plays a major role in outbreaks of this organism. Sources that have been implicated as reservoirs for MRKP include the condensate in ventilator expiratory water traps, bedsheets, sinks, and mops.[25,26] Contamination of the liquid soap may have been a possible source of infection for two of the patients, as indicated by profile B and C. Topping-up of liquid soap flasks or refilling without cleaning was identified as the possible mode of cross-contamination of staff hands and subsequent transfer to patients and other liquid soap flasks. However, the study was somewhat limited by the restricted environmental sampling, consisting of solutions prepared for common use among patients on the ward and of the liquid soap solutions for handwashing. More extensive sampling of the environment may have revealed additional contaminated sources. These outbreaks can be controlled or prevented by the institution or reemphasis of standard infection control procedures and by identifying and eliminating specific reservoirs of environmental contamination.

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