

Population-based surveillance for hypermucoviscosity *Klebsiella pneumoniae* causing community-acquired bacteremia in Calgary, Alberta

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The characteristics of hypermucoviscosity isolates among *Klebsiella pneumoniae* causing community-acquired bacteremia were investigated. The hypermucoviscous phenotype was present in 8.2% of *K pneumoniae* isolates, and was associated with *rmpA* and the K2 serotype; liver abscesses were the most common clinical presentation. The present analysis represents the first population-based surveillance study of hypermucoviscosity among *K pneumoniae* causing bacteremia.

Key Words: Bacteremia; Community acquired; Hypermucoviscous; *Klebsiella pneumoniae*; Population-based surveillance

Klebsiella pneumoniae is among the most important causes of serious hospital-acquired and community-onset bacterial infections in humans (1). During the past decade, community-acquired infections due to hypermucoviscous *K pneumoniae*, consisting of primary liver abscess, meningitis and endophthalmitis, were first recognized in Southeast Asia. These phenotypes of *K pneumoniae* have also been associated with bacteremia (2) and pneumonia (3,4), especially in patients with comorbid conditions such as diabetes mellitus (1). Hypermucoviscous isolates of *K pneumoniae* appear to be common in Taiwan and South Africa (5) but rare in Western Europe and North America (5,6).

In Canada, recent reports from the Canadian Hospital Ward Antibiotic Resistance Surveillance (CANWARD) program showed that *K pneumoniae* was, overall, the fifth most common bacteria isolated during 2006 to 2009 (7), the third most common bacteria among urinary tract isolates during 2007 to 2009 (8) and the fourth most common among bloodstream isolates during 2007 to 2009 (9). A previous study from Calgary (Alberta) showed that *K pneumoniae* was the second most common cause of Gram-negative bloodstream infection in this centralized North American region (10). Very limited data are available regarding the prevalence and characteristics of hypermucoviscous *K pneumoniae* in large geographical areas. The present study was designed to determine the prevalence, and clinical and molecular characteristics of hypermucoviscosity isolates among community-acquired bacteremia due to *K pneumoniae* in Calgary from 2001 to 2007.

METHODS

Study population

In Calgary, the Calgary Zone (formerly known as the Calgary Health Region [CHR]) provides all publicly funded health care services to the 1.2 million people residing in the cities of Calgary and Airdrie and numerous adjacent communities covering an area of 37,000 km². Acute care is provided principally through one pediatric and three

La surveillance en population de l'hypermucoviscosité en présence de *Klebsiella pneumoniae* responsable de bactériémie d'origine non nosocomiale à Calgary, en Alberta

Les chercheurs ont examiné les caractéristiques des isolats d'hypermucoviscosité en cas de *Klebsiella pneumoniae* responsable de bactériémie d'origine non nosocomiale. Ils ont constaté la présence du phénotype hypermucovisqueux dans 8,2 % des isolats de *K pneumoniae*, qui s'associait au *rmpA* et au sérotype K2. Les abcès hépatiques en étaient la présentation clinique la plus courante. La présente analyse est la première étude de surveillance en population de l'hypermucoviscosité en cas de *K pneumoniae* responsable d'une bactériémie.

large adult acute care centres. A centralized laboratory (Calgary Laboratory Services) performs the routine clinical microbiology services for general practitioners, medical specialists, community clinics and acute care centres within the Calgary Zone. The Conjoint Health Research Ethics Board at the University of Calgary and CHR approved the study.

Patient information

All Calgary and surrounding area residents (population 1.2 million) with community-acquired bacteremia due to *K pneumoniae* from January 2001 to December 2007 (n=134) were included in the present study. An active, retrospective, population-based surveillance cohort design was used. Surveillance for bacteremic *K pneumoniae* infections was conducted by Calgary Laboratory Services, a regional laboratory system that receives more than 95% of all blood samples submitted for culture from hospitals, nursing homes and clinics in the CHR. Additional clinical and outcome details were obtained for all patients admitted to any of the four major acute care hospitals (representing ≥95% CHR admissions) using data available from the regional corporate data warehouse. Hospital-acquired cases were classified as patients who developed infections 48 h after admission to a health care centre. Community-onset cases were classified as patients who visited community-based collection sites or lived in nursing homes, or those within the first two days of admission to an acute care facility. The community-onset cases were further classified as having either community-acquired or health care-associated community-onset infections (11). Health care-associated community-onset cases were those that occurred among nursing home residents or hemodialysis patients, or individuals who were either admitted to a hospital for at least two days in the preceding 90 days or received care through a hospital-based clinic in the preceding 30 days. For the purpose of the present study, only patients who presented with community-acquired bacteremia due to *K pneumoniae* were included.

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TABLE 1
Characteristics of patients with *Klebsiella pneumoniae* bacteremia

Variable	String test result		P
	Negative	Positive	
Isolates, n	124	10	
Mean age, years	66	63	ns
Admitted to hospital A	26.6	100	<0.001
Admission, days, mean	12.2	18.3	ns
Male sex	52.4	60	ns
<i>mpA</i>	0.8	80	<0.001
Case fatality rate	7.3	10	ns
Comorbidities			
Any	48.4	50	ns
Malignancy	4.8	0	ns
Diabetes	21.8	30	ns
Renal disease	8.9	10	ns
Transplantation	1.6	0	ns
Hepatitis B	0	10	ns
Hepatitis C	0	0	ns
Liver disease	8.1	10	ns
HIV	0	0	ns
Rheumatoid arthritis	0.8	0	ns
Systemic lupus erythematosus	0	0	ns
Cerebrovascular accident	0.8	0	ns
COPD	3.2	0	ns
Asthma	0	0	ns
Inflammatory bowel disease	2.4	0	ns
Ethanol abuse	3.2	0	ns
Heart disease	17.7	0	ns
Multiple sclerosis	3.2	0	ns
Infectious diagnosis			
Primary bacteremia	12.1	0	ns
Pneumonia	5.6	0	ns
Urinary tract infection	33.9	10	ns
Biliary	33.9	20	ns
Intra-abdominal abscess	8.9	10	ns
Pancreatitis	3.2	0	ns
Liver abscess	2.4	40	0.001
Central nervous system infection	0	20	0.01

Data presented as % unless otherwise indicated. COPD Chronic obstructive pulmonary disease; ns Not statistically significant

String test for hypermucoviscosity

The string test was performed on the *K pneumoniae* isolates as previously described (12) by touching a colony with a loop and pulling up. A positive string test resulted in the formation of an elongated mucoviscous string (≥ 5 mm in length), which indicated a hypermucoviscosity phenotype.

Detection of hypermucoviscosity-associated genes and K serotypes

Amplification of the *mpA* and *magA* genes and the K1, K2 and K5 serotypes was performed by polymerase chain reaction in all isolates as previously described (13,14).

Pulsed-field gel electrophoresis

Genetic relatedness of string test-positive strains was compared with a randomly selected negative group. Strains were typed by pulsed-field gel electrophoresis (PFGE) on a CHEF Mapper apparatus (Bio-Rad Laboratories, USA) following *Xba*I digestion (15). PFGE banding patterns were analyzed using BioNumerics software (Applied Maths, Belgium), and relatedness was calculated using the UPGMA algorithm with similarity of bands using the Dice coefficient. Cluster designation was based on isolates showing approximately 80% or greater relatedness, which corresponds to the "possibly related (4-6 bands difference)" criteria of Tenover et al (16).

Statistical analysis

Differences in proportions were compared using Fisher's exact test for pairwise comparison. The *t* test was used for the comparisons of means.

RESULTS

Clinical characteristics of patients with community-acquired bacteremia due to *K pneumoniae*

During 2001 to 2007, 134 Calgary residents with incident community-acquired bloodstream infections due to *K pneumoniae* were identified; the mean age of the patients was 66 years, 65 (52%) were male and the case fatality rate was 7.3% (Table 1). The majority of infections (68%) involved the urinary and biliary tracts. Nearly one-half of the patients (ie, 48%) had underlying comorbid conditions, with diabetes mellitus and heart diseases being the most common (Table 1).

The hypermucoviscous phenotype was detected in 10 of 134 (8.2%) isolates of *K pneumoniae*. The clinical characteristics of patients infected with the hypermucoviscous isolates are shown in Table 2. The mean age was 63 years, six (60%) of the patients were male and five had underlying comorbid illnesses (Table 2). The most common clinical syndrome was liver abscesses (n=4), followed by infections of the biliary tract (n=2). The remainder of patients (n=4) presented with subdural empyema, meningitis, urinary tract infection and nonhepatic intra-abdominal sepsis (Table 2).

The hypermucoviscous phenotype was more common at one of the acute care centres (nine of 43 compared with one of 91; $P<0.05$). There was no association with age, underlying medical condition or mortality with the hypermucoviscous phenotype (Table 1). There was no apparent annual increase in the numbers of hypermucoviscous isolates during the seven years of study. Statistical analysis showed that the hypermucoviscous phenotype was significantly associated with *mpA* positivity, and the presence of liver and central nervous infections, but not with underlying illness or clinical outcomes (Table 1).

Characterization of hypermucoviscosity *K pneumoniae* phenotype and associated genes

The molecular characterization of these 10 isolates were as follows: eight of 10 was positive for *mpA*, four of 10 were positive for K2 serotype and one of 10 for K5 serotype, while the remaining five were negative for K1, K2 and K5 serotypes (Table 2). PFGE typing results showed no genetic relationship among hypermucoviscous isolates.

Polymerase chain reaction for serotype K1 revealed an additional four isolates that tested positive for the K1 serotype but negative with the string test (Table 3). These isolates also tested positive for *magA* while one also tested positive for *mpA* (Table 3). PFGE was performed on 10 invasive string test-positive isolates (Table 2) as well as an additional four invasive isolates that were string test negative but positive for K1 and *magA* (Table 3). PFGE typing results showed no genetic relationship among them (Figure 1). The clinical characteristics of the patients infected with these isolates are presented in Table 3.

DISCUSSION

Recently, hypermucoviscous *K pneumoniae* had also emerged as a cause of community-acquired pyogenic liver abscesses in some non-Asian countries including the United States and Canada, especially among immigrants originating from Asia (1,2,4). We conducted a population-based surveillance study that investigated the prevalence, and clinical and molecular characteristics of hypermucoviscosity isolates among *K pneumoniae* responsible for community-acquired bacteremia in a centralized North American region. Our results showed that these isolates were present in 8.2% of bloodstream infections due to *K pneumoniae* in Calgary over a seven-year period (approximately 1.2 cases per million per annum), with liver abscesses being the most common underlying source for the bacteremia. These clinical features are similar to those previously reported from Asia and North America (6,15,17). We were unable to find cases of pneumonia as previously described in South Africa (3,5).

TABLE 2
Characterization of hypermucoviscosity *Klebsiella pneumoniae* (Kp)

Isolate	Sex	Age, years	Primary disease	String test	Mucoviscosity		K serotype*	Comorbid illness	Admit days	Hospital	Year
					genes						
Kp 01	Male	40	Liver abscess	Positive	<i>rmpA</i>		K2	None	13	A	2001
Kp 14	Male	48	Liver abscess	Positive	<i>rmpA</i>		K2	None	12	A	2001
Kp 23	Male	81	Acute cholangitis	Positive	–		NT	None	10	A	2002
Kp 32	Female	58	meningitis	Positive	<i>rmpA</i>		NT	None	57	A	2002
Kp 50	Female	76	Acute cholangitis	Positive	<i>rmpA</i>		K2	CLD	16	A	2003
Kp 57	Male	53	Liver abscess	Positive	<i>rmpA</i>		K5	HBV hepatitis	12	A	2004
Kp 64	Female	74	Urinary tract infection	Positive	<i>rmpA</i>		NT	DM	4	A	2004
Kp 121	Female	84	Liver abscess	Positive	<i>rmpA</i>		NT	DM, CKD	35	A	2007
Kp 123	Male	68	Subdural empyema	Positive	<i>rmpA</i>		K2	DM	12	A	2007
Kp 125	Male	54	Intra-abdominal sepsis	Positive	–		NT	None	14	A	2007

*NT: Not belonging to serotypes K1, K2 or K5. CKD Chronic kidney disease; CLD Chronic liver disease; DM Diabetes mellitus; HBV Hepatitis B virus

TABLE 3
Characterization of K1- and *magA*-positive *Klebsiella pneumoniae* (Kp)

Isolate	Sex	Age, years	Primary disease	String test	Mucoviscosity		K serotype	Comorbid illness	Admit days	Hospital	Year
					gene(s)						
Kp 17	Male	83	Primary bacteremia	Negative	<i>magA</i>		K1	None	8	C	2001
Kp 49	Female	72	Intra-abdominal sepsis	Negative	<i>rmpA</i> , <i>magA</i>		K1	DM	79	B	2003
Kp 126	Female	74	Urinary tract infection	Negative	<i>magA</i>		K1	None	9	B	2007
Kp 137	Male	71	Primary bacteremia	Negative	<i>magA</i>		K1	Carcinoma	5	A	2007

DM Diabetes mellitus

K pneumoniae can produce prominent polysaccharide capsules that are associated with increased virulence by protecting the bacteria from phagocytosis and thereby preventing destruction from bactericidal serum factors (5). The string test is most often used to detect the hypermucoviscous phenotype (12). Capsular serotypes K1 and K2 have been reported as the major virulence determinants for hypermucoviscous *K pneumoniae* (13). In addition, the mucoviscosity-associated gene *magA*, which encodes for a structural outer membrane protein of the K1 serotype, as well as a regulator gene *rmpA*, have also been proposed as virulence factors. The hypermucoviscosity phenotype is related to the presence of *magA* and/or regulator of mucoid phenotype (*rmpA*) genes in *K pneumoniae* (18), and were predominant in isolates associated with pneumonia, secondary bacteremia and purulent disease (3).

The molecular characterization of the hypermucoviscosity isolates from our study showed that the majority were positive for the *rmpA* gene and K2 was the most common serotype (Table 2). Of particular interest was that we were able to detect four isolates that tested positive for the K1 serotype and *rmpA* gene but were string test negative, even after repeat testing (Table 3). These bacteria were responsible for bloodstream infections in elderly patients who presented with primary bacteremia (two cases), urosepsis and intra-abdominal sepsis. To our knowledge, the present report is the first of nonhypermucoviscosity *K pneumoniae* isolates that belong to the K1 serotype.

SUMMARY

We described the first population-based surveillance study of hypermucoviscosity isolates among community-acquired bacteremic *K pneumoniae* isolates and found that these isolates were relatively rare in Calgary, and were associated with liver and intracranial abscesses. While all the cases appeared to originate in one area of the city, the distribution over time and the varying PFGE types excludes a single source of acquisition. Our data did not include ethnicity, diet or travel history. The relationship of invasive cases in North America to these factors and the appearance of this phenotype require further investigation that was not addressed in the present study.

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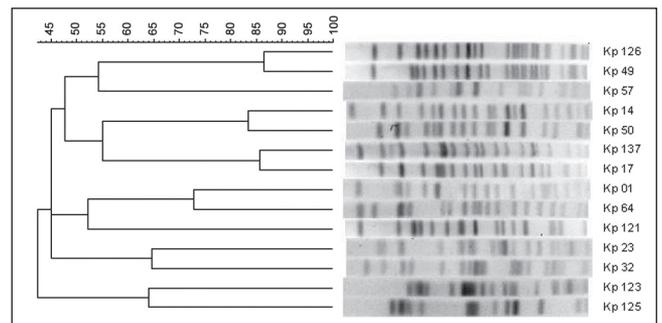


Figure 1) Pulsed-field gel electrophoresis patterns of the 10 invasive isolates that tested positive with the string test and the four invasive isolates that were string test negative but positive for serotype K1 and *magA*. Kp *Klebsiella pneumoniae*

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