Journal of Microbiology, Immunology and Infection (2019) 52, 30–34

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Original Article

Hypermucoviscosity, *rmpA*, and aerobactin are associated with community-acquired *Klebsiella pneumoniae* bacteremic isolates causing liver abscess in Singapore

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Received 15 April 2016; received in revised form 25 May 2017; accepted 5 July 2017
Available online 14 July 2017

KEYWORDS
Hepatic abscess;
String test;
Virulence factors

Abstract *Introduction:* This retrospective study investigated the clinical etiology of community-acquired bacteremic *Klebsiella pneumoniae* infections, and characterized laboratory and genetic markers which may be associated with primary liver abscess (PLA).
Methods: Community-onset *K. pneumoniae* bacteremic episodes from 2010 to 2011 were identified from the laboratory information system. Isolates were retrieved for susceptibility testing, hypermucoviscosity testing, PCR-based serotyping (K1, K2 and K5) and PCR detection of virulence genes (*rmpA*, *alls*, *kfu* and aerobactin). Clinical data collected from electronic medical records included primary and secondary diagnoses, co-existing morbidities, antibiotic therapy, and in-patient mortality.
Results: 129 bacteremic episodes were identified. The most common primary infections were pneumonia (n = 24, 18.6%), primary liver abscess (n = 21, 16.3%) and urinary tract infections (n = 21, 16.3%). Hypermucoviscosity was present in 55 isolates (42.6%). The most commonly detected virulence genes were aerobactin (n = 63, 48.8%) and *rmpA* (n = 59, 45.7%). Isolates causing liver abscess were significantly associated with a positive string test, *rmpA*, aerobactin gene, and capsular serotype K1 (all $p < 0.01$), but not with capsular serotype K2, K5, *kfu*, or *allS* genes. The absence of a positive string test, *rmpA*, or aerobactin genes had a 97.3%–100% negative predictive value for PLA. The positive predictive values of the string test, *rmpA*, aerobactin genes, and serotype K1 for PLA ranged from 31.7% to 35.6%.
Conclusion: In our study population, pneumonia and PLA were the most common sources of community-acquired bacteremia. Hypermucoviscosity, *rmpA*, aerobactin, and serotype K1

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http://dx.doi.org/10.1016/j.jmii.2017.07.003

could be useful laboratory markers to alert clinicians to arrange abdominal imaging to detect liver abscess.

Introduction

Klebsiella pneumoniae remains a significant cause of both community-acquired and healthcare associated bacteremia. Data from the 1980's reported that 25—50% of all *Klebsiella* bacteremia was from community-associated infections, with the primary portals of infection from the urinary and biliary tract.[1,2] In the last decade, an emerging syndrome of community-acquired *Klebsiella* bacteremia associated with primary liver abscess (PLA) has been reported, primarily from Taiwan,[3] but also from Asia,[4] the South Pacific,[5] Europe[6] and the United States.[7] A worldwide study of community-acquired *Klebsiella* bacteremia in 2002 demonstrated geographical variations in the etiology of infection, emphasizing the important of local epidemiology.[8]

Previous studies in Taiwan have identified several laboratory markers which identify hepato-virulent strains of *K. pneumoniae* that are associated with primary liver abscesses (PLA). A hypermucoviscous phenotype associated with a positive string test, where a viscous string is formed by touching a bacterial colony with a loop and lifting this away from the colony, is suggested to be a feature of hepato-virulent isolates.[9] A variety of virulence factors have also been linked with hepato-virulent strains of *K. pneumoniae*, including the *magA* gene (mucoviscosity associated gene A),[9] the *rmpA* gene,[10] capsular serotypes K1 and K2,[11] the *allS* gene and the *kfu* gene.[12,13]

This retrospective study investigated the clinical etiology of community-acquired *K. pneumoniae* bacteremia in Singapore, and characterized associated phenotypic and genotypic virulence factors in these isolates to determine if any microbiological marker was associated with primary liver abscess.

Materials and methods

129 *K. pneumoniae* isolates were identified over a two year period (2010—2011) from cases of community-acquired bacteremia. Community-acquired bacteremia was defined as a positive blood culture taken at or within 48 h of admission into the hospital. Identification was performed by VITEK® 2 ID-GN cards (BioMérieux, France), and disc susceptibility testing was performed using standard CLSI methodology.[14]

Clinical data was retrieved retrospectively from electronic medical records and discharge summaries and included admitting diagnosis, laboratory tests, procedure (endoscopic or surgery) and radiographic data. Initial antibiotic therapy was determined as appropriate if an antibiotic was prescribed with in-vitro activity against the subsequent *K. pneumoniae* strain isolated from blood cultures.

Bacterial isolates were retrieved from storage at −70 °C, and additional testing was performed following a minimum of two subcultures on non-selective media. The presence of hypermucoviscosity was evaluated using the string test, and a positive result was defined as formation of a viscous capsular string exceeding 10 mm in length based on our previous in-vitro work on optimization of the performance characteristics of the string test, which was performed using the same 129 *K. pneumoniae* strains in this study.[15] Capsular serotyping of isolates was performed using a multiplex PCR method which identifies K1, K2 and K5 serotypes.[16] The presence of *allS*, *kfu*, *rmpA* and aerobactin virulence genes were determined by separate PCR tests (Table 1).

Results

Patient characteristics

There was a preponderance of male patients (n = 73, 56.7%), with an average age was 69 years (range 22—100 years). Three clinical conditions accounted for half of all community-acquired infections: pneumonia (n = 24, 18.6%), primary liver abscess (n = 21, 16.3%) and urinary tract infections (n = 21, 16.3%) (Table 2). When classified by organ system, infections originating from the gastrointestinal tract accounted for most infections (n = 55, 42.6%), followed by infections from the urinary tract (n = 21, 16.3%). On average, patients had two documented pre-existing co-morbidities (range 0—6). The most commonly documented pre-existing conditions were hypertension (n = 71, 55.0%), diabetes mellitus (n = 57, 44.2%), hyperlipidemia (n = 53, 41.1%) and ischemic heart disease (n = 23, 17.8%). In-hospital mortality was 25.6% for all bacteremic episodes. Mortality was significantly higher for patients with a diagnosis of pneumonia (Risk ratio 3.6, 95% CI 2.2—6.1), but lower in patients with primary liver abscess (Risk ratio 0.2, 95% CI 0.02—1.1). Appropriate initial antibiotic therapy was associated with a lower mortality rate, but this did not reach statistical significance (Risk ratio 0.5, 95% CI 0.3—1.1). For patients with primary liver abscess, metastatic infective spread was documented in one patient (5%).

Microbiological characteristics

K. pneumoniae isolates were mostly susceptible to amoxicillin—clavulanate (88%), ceftriaxone (94%), piperacillin—tazobactam (94%), trimethoprim—sulfamethoxazole (96%) and gentamicin (96%), with lower susceptibilities to ciprofloxacin (84%) and cefuroxime (60%). Extended-

Table 1 Primers used for determination of capsular serotype and virulence factors.

Target gene	Primer name	Primer sequence	Product size (bp)	Reference
allS	1416R	5′-CCG TTA GGC AAT CCA GAC-3′	1090	12
	336F2	5′-TCT GAT TTA (A/T)CC CAC ATT-3′		
Aerobactin	Aerobactin-F	5′-GCA TAG GCG GAT ACG AAC AT-3′	556	21
	Aerobactin-R	5′-CAC AGG GCA ATT GCT TAC CT-3′		
kfuBC	kfuB-F1179	5′-GAA GTG ACG CTG TTT CTG GC-3′	797	13
	kfuC-R649	5′-TTT CGT GTG GCC AGT GAC TC-3′		
K1	MagAF1w	5′-GGT GCT CTT TAC ATC ATT GC-3′	1283	16
	MagAR1	5′-GCA ATG GCC ATT TGC GTT AG-3′		
K2	Wzy-F1	5′-GAC CCG ATA TTC ATA CTT GAC AGA G-3′	641	16
	Wzy-R1	5′-CCT GAA GTA AAA TCG TAA ATA GAT GGC-3′		
K5	K5wzxF360	5′-TGG TAG TGA TGC TCG CGA -3′	280	16
	K5wzxR639	5′-CCT GAA CCC ACC CCA ATC-3′		
rmpA	*rmpA*-F	5′-ACT GGG CTA CCT CTG CTT CA-3′	535	22
	rmpA-R	5′-CTT GCA TGA GCC ATC TTT CA-3′		

spectrum beta-lactamases were present in five isolates (3.9%). Based on the PCR serotyping method, the most common serotypes were K1 (n = 23, 17.8%) and K2 (n = 23, 17.8%) with a smaller number of K5 serotypes (n = 7, 5.4%). Seventy-six isolates were not typeable by PCR. Based on the string test, 55 isolates (42.6%) were positive for hypermucoviscosity. The most prevalent virulence gene present was aerobactin (n = 63, 48.8%), followed by *rmpA* (n = 59, 45.7%), *kfu* (n = 52, 40.3%), and *alls* (n = 22, 17.0%) respectively.

Correlation of microbiological markers with primary liver abscess

The following microbiological features were significantly associated ($p < 0.01$) with *K. pneumoniae* isolates causing liver abscess: a positive string test, *rmpA* gene and aerobactin gene (Table 3). When used as a marker for identifying bacteremia isolates that were associated with PLA, a positive string test had a sensitivity and specificity of 90.4% and 66.6%, respectively. In our test population, the string test had a positive predictive value (PPV) of 34.5%, and a very high negative predictive value (NPV) of 97.3%. The sensitivities, specificities, PPV and NPV of the tested virulence factors for identifying bacteremic *K. pneumoniae* strains associated with primary liver abscess were as follows: *rmpA* (100%, 64.8%, PPV 35.6%, NPV 100%), aerobactin (95.2%, 60.2%, PPV 31.8%, NPV 98.5%), *kfu* (52.3%, 62.0%, PPV 21.2%, NPV 87.0%), *alls* (28.6%, 85.2%, PPV 27.2%, NPV 86.0%) and serotype K1 (38.1%, 86.1%, PPV 34.8%, NPV 87.7%). The most common serotype associated with primary liver abscess was K1 (n = 8, 38.1%) ($p < 0.01$) but K2 (n = 2, 9.5%), K5 (n = 3, 14.3%) and non-K1/K2/K5 (n = 8, 38.1%) serotypes were also present.

Table 2 Primary source of bacteremia.

Primary infection	N (% of total)	In-hospital mortality (%)	Average number of co-morbidities (range)
Respiratory tract			
Pneumonia	24 (19%)	15 (62.5%)	2.0 (0–5)
Urinary tract			
Pyelonephritis	15 (12%)	1 (6.7%)	2.1 (0–4)
Urinary tract infection	21 (16%)	5 (23.8%)	3.2 (0–6)
Gastro-intestinal tract			
Cholangitis	18 (14%)	1 (5.6%)	1.8 (0–4)
Cholecystitis	5 (4%)	0 (0%)	1.8 (0–4)
Intra-abdominal sepsis (various)	7 (5%)	1 (14.3%)	1.3 (0–3)
Primary Liver abscess	21 (16%)	1 (4.8%)	1.6 (0–4)
Pancreatitis	2 (2%)	2 (100%)	3.5 (3–4)
Peritonitis	2 (2%)	2 (100%)	2.5 (1–4)
Others			
Infective epiglottitis	1 (1%)	0 (0%)	3
Meningitis	1 (1%)	1 (100%)	1
No primary source identified	8 (6%)	4 (100%)	2.3 (1–4)
Soft tissue infection	3 (2%)	0 (0%)	3 (2–4)
Vascular-line infection	1 (1%)	0 (0%)	4

Table 3 Microbiological markers and primary liver abscess.

Microbiological marker	Number of isolates positive (% of total) where		p value
	Primary infection other than liver abscess (n = 108)	Infection due to primary liver abscess (n = 21)	
Aerobactin	43 (39.8%)	20 (95.2%)	<0.01
alls	16 (14.8%)	6 (28.6%)	0.15
kfu	41 (40.0%)	11 (52.4%)	0.23
rmpA	38 (35.2%)	21 (100.0%)	<0.01
Positive string test	36 (33.3%)	19 (90.5%)	<0.01
Serotype K1	15 (13.9%)	8 (38.1%)	<0.01
Serotype K2	21 (19.4%)	2 (9.5%)	0.3
Serotype K5	4 (3.7%)	3 (14.3%)	0.1

Discussion

In our patient population, community-acquired pneumonia, primary liver abscess and complicated urinary tract infections were the major infections leading to bacteremia with *K. pneumoniae*. Bacteremic pneumonia was associated with a high mortality. The most comprehensive evaluation of community-acquired *K. pneumoniae* bacteremia was published in 2002, which identified significant differences in bacterial etiology based on geographic locality.[8] Bacteremia in Taiwan and South Africa were most likely to be associated with pneumonia, while urinary tract infections were the leading cause of bacteremia in other geographic localities. The relative importance of K. *pneumoniae* as a cause of community-acquired pneumonia in Asian countries was also supported by a recently published review.[17]

Klebsiella bacteremia secondary to PLA is an emerging phenomenon, and this may be associated with an invasive syndrome, leading to secondary metastatic infections. Reports suggest that the K1 and K2 serotype are significantly associated with PLA,[18] however, in our study, over half of strains associated with PLA were non-K1 or K2 serotypes. Laboratory features that have been reported in hepatovirulent *K. pneumoniae* include the presence of hypermucoviscosity, demonstrated by a positive "string test" which can be performed by all microbiology laboratories. Other potential virulence markers include genes responsible for extracellular polysaccharide synthesis (*rmpA*), a siderophore component (aerobactin), allantoin metabolism (*allS*) and uptake of ferric iron (*kfu*).[19] In our test population, a negative "string test" or absence of the aerobactin or *rmpA* gene reliably excluded the presence of PLA.

There are some limitations to this study. The data was derived exclusively in a single hospital, and the results from this study would need to be evaluated in the context of local epidemiology. The evaluation of molecular markers and capsular serotypes was not comprehensive: for example, a recently published assay included the *entB*, *ybtS*, *iutA* and *mrkD* genes,[19] and there are at least six liver abscess-associated capsular serotypes: K1, K2, K5, K20, K54, and K57.[20] Finally, we did not perform additional ST typing to identify the clonal complexes which might be attributed to hepatic virulence and PLA. However, the study primary aim of this study was not epidemiological in nature, but to evaluate the diagnostic potential of phenotypic or genotypic markers in *K. pneumoniae* to potentially identify patients with PLA.

In summary, our study demonstrated that three infections constituted the majority of community-acquired *K. pneumoniae* bacteremia. A positive string test, *rmpA* gene and aerobactin gene were significantly associated with bacteremia *K. pneumoniae* strains causing PLA. The presence of hypermucoviscosity, *rmpA*, aerobactin, and serotype K1 could be useful laboratory markers to alert clinicians to arrange abdominal imaging to detect PLA.

Conflicts of interest

None to declare.

Acknowledgements and funding

There was no external or internal funding source for this study.

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