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Hypervirulent Klebsiella pneumoniae: prevalence, clinical presentation and antibiotic resistance in a French tertiary care hospital.

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Introduction

Klebsiella pneumoniae (KP) is a Gram-negative bacillus of enteric origin responsible primarily for healthcare-associated infections and infections in debilitated settings (1,2). The hypervirulent pathotype of KP (hvKP) has characteristics such as hypermucoviscosity, unique serotypes with high pathogenicity mainly mediated by virulence plasmids (3). Previous molecular biology studies have demonstrated the polyclonal and non-subtype associated nature of hvKP infections (4).

Objective

To determine the prevalence in clinical isolates of the different virulence genes defining hvKp in association with clinical presentation and antibiotic resistance

Methods



Figure 1. Analysis protocol. The strains studied (n=207) were identified consecutively from December 2021 to April 2022 in documented infections (samples received at the bacteriology laboratory of the infectious agent department of the University Hospital of Poitiers). Klebsiella pneumoniae were cultured on blood agar before extraction by heat shock (95°C 5 min). Extracts were then assayed/diluted (100 ng per reaction) before amplification and separation of PCR products on agarose gel.

Gene (n;%)	Eye E (n=1)	Blood B (n=22)	Pneumonia P (n=16)	Abdomen A (n=17)	Cutaneous C (n=5)	Urinary U (n=146)	Multiple (n=21)
<i>magA</i> (n=113; 54,6%)	1 (100%)	13 (59,1%)	10 (62,5%)	8 (47,1%)	4 (80.0%)	77 (52,7%)	14 (66,7%)
<i>iutA</i> (n=80; 38,6%)	0	9 (40,9%)	11 (68,8%)	5 (29,4%)	1 (20.0%)	54 (37.0%)	6 (28,6%)
<i>allS</i> (n=100; 48,3%)	0	11 (50.0%)	7 (43,8%)	9 (52,9%)	3 (60.0%)	70 (47,9%)	7 (33,3%)
<i>kfu</i> (n=102; 49,3%)	0	11 (50.0%)	8 (50.0%)	9 (52,9%)	2 (40.0%)	72 (49,3%)	10 (47,6%)
<i>K2</i> (n=105 ; 50,7%)	0	14 (63,6%)	10 (62,5%)	7 (41,2%)	2 (40.0%)	72 (49,3%)	9 (42,9%)
<i>rmpA</i> (n=49; 23,7%)	0	8 (36,4%)	4 (25.0%)	7 (41,2%)	1 (20.0%)	29 (19,9%)	3 (14,3%)
<i>entB</i> (n=189; 91,3%)	1 (100%)	16 (72,7%)	15 (93,8%)	17 (100%)	5 (100%)	135 (92,5%)	19 (90,5%)
<i>mrkD</i> (n=198 ; 95,7%)	1 (100%)	22 (100%)	16 (100%)	16 (94,1%)	5 (100%)	138 (94,5%)	20 (95,2%)
<i>ybtS</i> (n=65; 31,4%)	0	8 (36,4%)	6 (37,5%)	5 (29,4%)	2 (40.0%)	44 (30,1%)	4 (19.0%)
Number of gene per bacteria mean (SEM)	3 (-)	5,09 (0,33)	5,38 (0,31)	4,88 (0,44)	5.0 (0,71)	4,73 (0,12)	4,38 (0,26)

Table 3. Distribution of virulence genes according to the nature of the clinical isolation specimen. Strains isolated from respiratory specimens had a significantly higher number of virulence genes compared to strains isolated from multiple sites (p<0.05) or urine (p<0.1).

<i>entB</i> (n=189; 91,3%)	Pos	Neg	B/A	B/U	<i>rmpA</i> (n=49; 23,7%)		Pos	Neg	B/U	A/U	
Eye E (n=1)	1 (0,5)	0(-)			Eye E (n=1)		0 (-)	1 (0,6)			
Blood B (n=22)	16 (8,5)	6 (33,3)					ood B (n=22)	8 (16,3)	14 (8,9)		
Pneumonia P (n=16)	15 (7,9)	1 (5,6)	0,0267		Pneumonia P (n=16)		4 (8,2)	12 (7,6)			
Abdomen A (n=17)	17 (9,0)	0(-)			0,012	Abdo	omen A (n=17)	7 (14,3)	10 (6,3)	0,0987	
Cutaneous C (n=5)	5 (2,6)	0(-)			Cutaneous C (n=5)		1 (2,0)	4 (2,5)		0,0619	
Urinary U (n=146)	135 (71,4)	11 (61,1)			Urir	ary U (n=146)	29 (59,2)	117 (74,1)			
Multiple (n=21)	19 (10,1)	2 (11,1)			Multiple (n=21)		3 (6,1)	18 (11,4)			
<i>iutA</i> (n=80; 38,6%)	Pos	Neg	P/A	P/U	M/P	Table 4	I		· · · · ·		
Eye E (n=1)	0 (-)	1 (0,8)				and association of virulence					
Blood B (n=22)	9 (11,3)	13 (10,23)									
Decumenta D (n-16)	11/12 0)	E (2 0)								-	

	Cure	death	p-
	(n=184)	(n=23)	value
Number of gene per bacteria (mean; SEM)	4,76 (0,11)	5,13 (0,30)	0,26
Gene name (n)			
magA (n=113; 54,6%)	99 (53,8%)	14 (60,8%)	0,66
<i>iutA</i> (n=80; 38,6%)	73 (39,7%)	7 (30,4%)	0,5
<i>allS</i> (n=100; 48,3%)	88 (47,8%)	12 (52,2%)	0,83
<i>kfu</i> (n=103 ; 49,8%)	89 (48,4%)	13 (56,5%)	0,51
<i>k2</i> (n=106; 51,2%)	91 (49,5%)	14 (60,9%)	0,38
<i>rmpA</i> (n=49;23,7%)	42 (22,8%)	7 (30,4%)	0,44
<i>entB</i> (n=189; 91,3%)	169 (91,8%)	20 (87.0%)	0,43
<i>mrkD</i> (n=199; 96,1%)	175 (95,1%)	23 (100%)	0,6
<i>ybtS</i> (n=65 ; 31,4%)	55 (29,9%)	10 (43,5%)	0,23

Results

dePoitier

Primer			EMBL	Amplicon
name	DNA sequence (5' to 3')	Target gene product/function	accession no.	size (bp)
ybtS_for	GACGGAAACAGCACGGTAAA	Siderophore	AB298504	242
ybtS_rev	GAGCATAATAAGGCGAAAGA			
mrkD_for	AAGCTATCGCTGTACTTCCGGCA	Adhesin type 3 fimbriae	EU682505	340
mrkD_rev	GGCGTTGGCGCTCAGATAGG			
entB_for	GTCAACTGGGCCTTTGAGCCGTC	Siderophore	CP000647	400
entB_rev	TATGGGCGTAAACGCCGGTGAT			
rmpA_for	CATAAGAGTATTGGTTGACAG	Regulator of mucoid phenotype A	X17518	461
rmpA_rev	CTTGCATGAGCCATCTTTCA			
K2_for	CAACCATGGTGGTCGATTAG	Capsular serotype K2 and	EF221827	531
K2_rev	TGGTAGCCATATCCCTTTGG	hypermucoviscosity phenotype		
kfu_for	GGCCTTTGTCCAGAGCTACG	Iron transport and phosphotransferase	AB115591	638
kfu_rev	GGGTCTGGCGCAGAGTATGC	function		
allS_for	CATTACGCACCTTTGTCAGC	Allantoin metabolism	AB115590	764
allS_rev	GAATGTGTCGGCGATCAGCTT			
iutA_for	GGGAAAGGCTTCTCTGCCAT	Siderophore	AY378100	920
iutA_rev	TTATTCGCCACCACGCTCTT			
magA_for	GGTGCTCTTTACATCATTGC	Capsular serotype K1 and	AY762939	1,283
magA_rev	GCAATGGCCATTTGCGTTAG	hypermucoviscosity phenotype		

Table 1. Multiplexed PCR protocol. Amplifications were enabled by primers published by Compain F. targeting **nine genes** at the same time (1). Verification on agarose gels have been performed at the same time. The gels were read blindly by two operators, with the advice of a third operator in case of discordance to allow classification.

	Eye E (n=1)	Blood B (n=22)	Pneumonia P (n=16)	Abdomen A (n=17)	Cutaneous C (n=5)	Urinary U (n=146)	Multiple (n=21)
Age (mean ; SEM)	65 (31)	65,9 (4,6)	57,4 (3,76)	60,1 (5,17)	60,6 (3,02)	64,1 (1,98)	69,8 (3,48)
SexRatio (F ; M)	1/0	8/14	4/12	4/13	2/3	106/40	14/7

Table 2. Distribution of strains analyzed according to the nature of the clinical specimen (n=207).

	()-/	0 (0)01	0 0201		
Abdomen A (n=17)	5 (6,3)	12 (9,4)	0,0381	0.017	
Cutaneous C (n=5)	1 (1,3)	4 (3,1)		0,017	0,022
Urinary U (n=146)	54 (67,5)	92 (72,4)			
Multiple (n=21)	6 (7,5)	15 (11,8)			

genes according to the nature of the clinical isolation specimen.

> Table 5. Distribution of virulence genes
> according to lethality.

> > Amoxicillin-Clavulanic

cotrimoxazole (p < 0.1).

and

Acid

mrkD was significantly more associated with *iutA* and *magA* (p<0.05 and p<0.1). entB and iutA were significantly more frequently found in strains isolated from blood or respiratory sources (p<0.05). *rmpA* tended to be found in strains isolated from abdominal or urinary samples (p < 0.1).

	Community-	Healthcare-		
	acquired	associated	p-value	
	infection (n=160)	infection (n=47)		
lumber of gene per bacteria	4 71 (0 12)	E 22 (0 20)	0.0262	
mean; SEM)	4,71(0,12)	5,25 (0,20)	0,0362	
Gene name (n)				
nagA (n=113;54,6%)	84 (52,5%)	29 (61,7%)	0,32	
<i>utA</i> (n=80; 38,6%)	62 (38,8%)	18 (38,3%)	1	
<i>IIIS</i> (n=100; 48,3%)	80 (50.0%)	20 (42,6%)	0,41	
<i>fu</i> (n=103 ; 49,8%)	77 (48,1%)	24 (51,1%)	0,74	
2 (n=106;51,2%)	76 (47,5%)	29 (61,7%)	0.0986	
<i>mpA</i> (n=49; 23,7%)	33 (20,6%)	16 (34.0%)	0.0778	
ntB (n=189; 91,3%)	145 (90,6%)	44 (93,6%)	0,77	
nrkD (n=199; 96,1%)	152 (95.0%)	47 (100%)	0,2	
<i>btS</i> (n=65 ; 31,4%)	45 (28,1%)	20 (42,6%)	0,0741	

Table 6. Distribution of virulence genes according to the nosocomial or non-nosocomial origin of the strain studied. Strains involved in community-acquired infections had significantly fewer virulence genes than healthcare-associated infection strains (p<0.05). The k^2 , *rmpA* and *ybtS* genes tended to be associated with nosocomial origin (p < 0.1).

	CoAmoxiClav	R (n=59)	S (n=148)	pvalue	Cephalosporin	R (n=34)	S (n=175)	pvalue
Je	Number of gene per bacteria (mean; SEM)	4,69 (0,20)	4,88 (0,12)	0,41	Number of gene per bacteria (mean; SEM)	4,82 (0,30)	4,83 (0,11)	0,97
	Gene name (n)			1	Gene name (n)			
	magA (n=113; 54,6%)	37 (62,7%)	76 (51,4%)	0,17	<i>magA</i> (n=113; 54,6%)	21 (61,8)	92 (52,6)	0,35
52	<i>iutA</i> (n=80; 38,6%)	14 (23,7%)	66 (44,6%)	0.0069	<i>iutA</i> (n=80; 38,6%)	8 (23,5)	72 (41,1)	0,0565
	allS (n=100; 48,3%)	26 (44,1%)	74 (50%)	0,53	<i>allS</i> (n=100; 48,3%)	15 (44,1)	85 (48,6)	0,71
	<i>kfu</i> (n=102; 49,3%)	23 (39.0%)	80 (54,1%)	0.0644	<i>kfu</i> (n=102 ; 49,3%)	13 (38,2)	89 (50,9)	0,19
32	<i>k2</i> (n=105; 50,7%)	28 (47,5%)	78 (52,7%)	0,54	<i>k2</i> (n=105 ; 50,7%)	19 (55,9)	86 (49,1)	0,57
52	<i>rmpA</i> (n=49; 23,7%)	13 (22%)	36 (24,3%)	0,86	<i>rmpA</i> (n=49; 23,7%)	6 (17,6)	43 (24,6)	0,51
1	entB (n=189; 91,3%)	57 (96,6%)	132 (89,1%)	0,11	<i>entB</i> (n=189; 91,3%)	33 (97,1)	156 (89,1)	0,21
41	mrkD (n=198; 95,7%)	58 (98,3%)	141 (95,3%)	0,44	<i>mrkD</i> (n=198; 95,7%)	32 (94,1)	166 (94,9)	0,7
74	<i>ybtS</i> (n=65; 31,4%)	21 (35,6%)	44 (29,7%)	0,41	<i>ybtS</i> (n=65 ; 31,4%)	12 (35,3)	53 (30,3)	0,55
	Cotrimoxazole F	R (n=37)	S (n=169)	pvalue			1-1:	-1
86 78	Number of gene per bacteria (mean; SEM)	,51 (0,29)	4,89 (0,11)	0,16	antibiotic resis	resen stance	based	or d on
77	Gene name (n)	I			the presence of	Evirula		noc
	magA (n=113; 54,6%) 2	5 (67,6%)	88 (52,1%)	0,1	the presence of		ince ge	e2.
), Z	<i>iutA</i> (n=80; 38,6%) 7	7 (18,9%)	73 (43,2%)	0.0084	<i>iutA</i> was signif	icantly	assoc	iated
<mark>41</mark>	allS (n=100; 48,3%) 10	6 (43,2%)	84 (49,7%)	0,59	with resistance	e to	Amoxi	cillin-
0	<i>kfu</i> (n=102 ; 49,3%) 13	3 (35,1%)	90 (53,3%)	0.0685				
U	<i>k2</i> (n=105; 50,7%) 18	8 (48,6%)	88 (52,1%)	0,72			JUIIIIOXa	azoie
n	<i>rmpA</i> (n=49; 23,7%) 5	5 (13,5%)	44 (26%)	0,14	(p<0.05). <i>iutA</i>	tend	ed to	be
d	<i>entB</i> (n=189; 91,3%) 3	5 (94,6%)	154 (91,1%)	0,74	associated wi	th ca	nhaloe	norin
5	<i>mrkD</i> (n=198; 95,7%) 3	7 (100%)	162 (95,9%)	0,36				
I I	<i>ybtS</i> (n=65 ; 31,4%) 12	1 (29,7%)	54 (32%)	0,85	j susceptibility (p<	:(0.1). <i>K</i>	<i>tu</i> tend	ed to
2,					be associated	with re	esistanc	e to



Among the virulence arsenal of KP, two genes have been initially associated with invasive infections ie. magA (mucosity-associated gene A) and rmpA (regulator of mucoid phenotype A). (5). Epidemiological studies have shown that hypervirulent KP have iron acquisition systems encoded by entB whose receptors are encoded by iutA, are distinct from Yersiniabactin (encoded by ybtS) (6, 7, 8). allS (allantoin metabolism) is associated with strains involved in hepatic abscesses (16). mrkD is involved in adhesion to extracellular matrices (9)

Conclusion

This cohort performed on all strains consecutively isolated in a medical laboratory brings a new light on the genomic arsenal of virulence of KP. The high prevalence of certain genes (mrkD) and entB) (already suggested in the literature) raises the need for new studies to deepen our knowledge of their pathophysiology and to allow an appropriate diagnosis.

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