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

Jean-Philippe Martellosio[1,2], Thais Villers[1,2], Nabil Gastli[3], Gael Rambault[1,2,4], Lauranne Broutin[2], Julie Cremniter[1,2,4]
Anthony Michaud[2], Chloé Plouzeau-Jayle[2], France Roblot[1,2,4], Christophe Burucoa[1,2,4], Maxime Pichon [1,2,4]

[1] University of Poitiers , Medicine and Pharmacy faculty, Poitiers, France
[2] University Hospital of Poitiers, Poitiers, France
[3] Bacteriology Laboratory, hôpital Cochin, AP-HP. CUP, Paris, France
[4] INSERM U1070, PHAR2 Pharmacology of Antimicrobial Agents and Antiibiotic resistance, Poitiers, France

Contact : maxime.pichon@univ-poitiers.fr

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

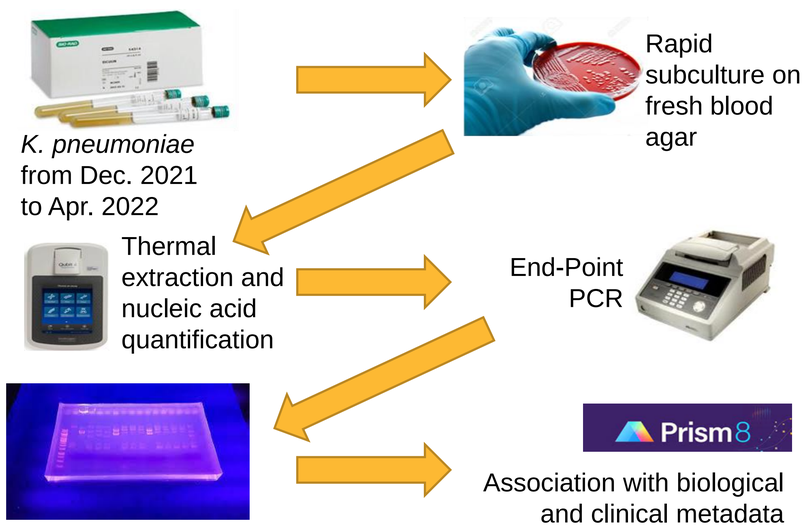

Figure 1. Analysis protocol. The strains studied (n=207) were identified consecutively from December 2021 to April 2022 in documented infections (samples received at the bacteriology laboratory of the infectious agent department of the University Hospital of Poitiers). *Klebsiella pneumoniae* were cultured 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.

Primer name	DNA sequence (5′ to 3′)	Target gene product/function	EMBL accession no.	Amplicon 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 hypermucoviscosity phenotype	EF221827	531
K2_rev	TGGTAGCCATATCCCTTTGG			
kfu_for	GGCCTTTGTCCAGAGCTACG	Iron transport and phosphotransferase function	AB115591	638
kfu_rev	GGGTCTGGCGCAGAGTATGC			
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 hypermucoviscosity phenotype	AY762939	1,283
magA_rev	GCAATGGCCATTTGCGTTAG			

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).

Results

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)
magA (n=113 ; 54,6%)	1 (100%)	13 (59,1%)	10 (62,5%)	8 (47,1%)	4 (80.0%)	77 (52,7%)	14 (66,7%)
iutA (n=80 ; 38,6%)	0	9 (40,9%)	11 (68,8%)	5 (29,4%)	1 (20.0%)	54 (37.0%)	6 (28,6%)
allS (n=100 ; 48,3%)	0	11 (50.0%)	7 (43,8%)	9 (52,9%)	3 (60.0%)	70 (47,9%)	7 (33,3%)
kfu (n=102 ; 49,3%)	0	11 (50.0%)	8 (50.0%)	9 (52,9%)	2 (40.0%)	72 (49,3%)	10 (47,6%)
K2 (n=105 ; 50,7%)	0	14 (63,6%)	10 (62,5%)	7 (41,2%)	2 (40.0%)	72 (49,3%)	9 (42,9%)
rmpA (n=49 ; 23,7%)	0	8 (36,4%)	4 (25.0%)	7 (41,2%)	1 (20.0%)	29 (19,9%)	3 (14,3%)
entB (n=189 ; 91,3%)	1 (100%)	16 (72,7%)	15 (93,8%)	17 (100%)	5 (100%)	135 (92,5%)	19 (90,5%)
mrkD (n=198 ; 95,7%)	1 (100%)	22 (100%)	16 (100%)	16 (94,1%)	5 (100%)	138 (94,5%)	20 (95,2%)
ybtS (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).

entB (n=189 ; 91,3%)	Pos	Neg	B/A	B/U
Eye E (n=1)	1 (0,5)	0 (-)		
Blood B (n=22)	16 (8,5)	6 (33,3)	0,0267	0,012
Pneumonia P (n=16)	15 (7,9)	1 (5,6)		
Abdomen A (n=17)	17 (9,0)	0 (-)		
Cutaneous C (n=5)	5 (2,6)	0 (-)		
Urinary U (n=146)	135 (71,4)	11 (61,1)		
Multiple (n=21)	19 (10,1)	2 (11,1)		

iutA (n=80 ; 38,6%)	Pos	Neg	P/A	P/U	M/P
Eye E (n=1)	0 (-)	1 (0,8)			
Blood B (n=22)	9 (11,3)	13 (10,23)			
Pneumonia P (n=16)	11 (13,8)	5 (3,9)	0,0381	0,017	0,022
Abdomen A (n=17)	5 (6,3)	12 (9,4)			
Cutaneous C (n=5)	1 (1,3)	4 (3,1)			
Urinary U (n=146)	54 (67,5)	92 (72,4)			
Multiple (n=21)	6 (7,5)	15 (11,8)			

rmpA (n=49 ; 23,7%)	Pos	Neg	B/U	A/U
Eye E (n=1)	0 (-)	1 (0,6)		
Blood B (n=22)	8 (16,3)	14 (8,9)		
Pneumonia P (n=16)	4 (8,2)	12 (7,6)		
Abdomen A (n=17)	7 (14,3)	10 (6,3)	0,0987	0,0619
Cutaneous C (n=5)	1 (2,0)	4 (2,5)		
Urinary U (n=146)	29 (59,2)	117 (74,1)		
Multiple (n=21)	3 (6,1)	18 (11,4)		

Table 4. Individual distribution and association of virulence genes according to the nature of the clinical isolation specimen.

	Cure (n=184)	death (n=23)	p-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
iutA (n=80 ; 38,6%)	73 (39,7%)	7 (30,4%)	0,5
allS (n=100 ; 48,3%)	88 (47,8%)	12 (52,2%)	0,83
kfu (n=103 ; 49,8%)	89 (48,4%)	13 (56,5%)	0,51
k2 (n=106 ; 51,2%)	91 (49,5%)	14 (60,9%)	0,38
rmpA (n=49 ; 23,7%)	42 (22,8%)	7 (30,4%)	0,44
entB (n=189 ; 91,3%)	169 (91,8%)	20 (87.0%)	0,43
mrkD (n=199 ; 96,1%)	175 (95,1%)	23 (100%)	0,6
ybtS (n=65 ; 31,4%)	55 (29,9%)	10 (43,5%)	0,23

Table 5. Distribution of virulence genes according to lethality.

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-acquired infection (n=160)	Healthcare-associated infection (n=47)	p-value
Number of gene per bacteria (mean; SEM)	4,71 (0,12)	5,23 (0,20)	0,0362
Gene name (n)			
magA (n=113 ; 54,6%)	84 (52,5%)	29 (61,7%)	0,32
iutA (n=80 ; 38,6%)	62 (38,8%)	18 (38,3%)	1
allS (n=100 ; 48,3%)	80 (50.0%)	20 (42,6%)	0,41
kfu (n=103 ; 49,8%)	77 (48,1%)	24 (51,1%)	0,74
k2 (n=106 ; 51,2%)	76 (47,5%)	29 (61,7%)	0.0986
rmpA (n=49 ; 23,7%)	33 (20,6%)	16 (34.0%)	0.0778
entB (n=189 ; 91,3%)	145 (90,6%)	44 (93,6%)	0,77
mrkD (n=199 ; 96,1%)	152 (95.0%)	47 (100%)	0,2
ybtS (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 *k2*, *rmpA* and *ybtS* genes tended to be associated with nosocomial origin (p<0.1).

CoAmoxiClav	R (n=59)	S (n=148)	pvalue
Number of gene per bacteria (mean; SEM)	4,69 (0,20)	4,88 (0,12)	0,41
Gene name (n)			
magA (n=113 ; 54,6%)	37 (62,7%)	76 (51,4%)	0,17
iutA (n=80 ; 38,6%)	14 (23,7%)	66 (44,6%)	0.0069
allS (n=100 ; 48,3%)	26 (44,1%)	74 (50%)	0,53
kfu (n=102 ; 49,3%)	23 (39.0%)	80 (54,1%)	0.0644
k2 (n=105 ; 50,7%)	28 (47,5%)	78 (52,7%)	0,54
rmpA (n=49 ; 23,7%)	13 (22%)	36 (24,3%)	0,86
entB (n=189 ; 91,3%)	57 (96,6%)	132 (89,1%)	0,11
mrkD (n=198 ; 95,7%)	58 (98,3%)	141 (95,3%)	0,44
ybtS (n=65 ; 31,4%)	21 (35,6%)	44 (29,7%)	0,41

Cotrimoxazole	R (n=37)	S (n=169)	pvalue
Number of gene per bacteria (mean; SEM)	4,51 (0,29)	4,89 (0,11)	0,16
Gene name (n)			
magA (n=113 ; 54,6%)	25 (67,6%)	88 (52,1%)	0,1
iutA (n=80 ; 38,6%)	7 (18,9%)	73 (43,2%)	0.0084
allS (n=100 ; 48,3%)	16 (43,2%)	84 (49,7%)	0,59
kfu (n=102 ; 49,3%)	13 (35,1%)	90 (53,3%)	0.0685
k2 (n=105 ; 50,7%)	18 (48,6%)	88 (52,1%)	0,72
rmpA (n=49 ; 23,7%)	5 (13,5%)	44 (26%)	0,14
entB (n=189 ; 91,3%)	35 (94,6%)	154 (91,1%)	0,74
mrkD (n=198 ; 95,7%)	37 (100%)	162 (95,9%)	0,36
ybtS (n=65 ; 31,4%)	11 (29,7%)	54 (32%)	0,85

Cephalosporin	R (n=34)	S (n=175)	pvalue
Number of gene per bacteria (mean; SEM)	4,82 (0,30)	4,83 (0,11)	0,97
Gene name (n)			
magA (n=113 ; 54,6%)	21 (61,8)	92 (52,6)	0,35
iutA (n=80 ; 38,6%)	8 (23,5)	72 (41,1)	0,0565
allS (n=100 ; 48,3%)	15 (44,1)	85 (48,6)	0,71
kfu (n=102 ; 49,3%)	13 (38,2)	89 (50,9)	0,19
k2 (n=105 ; 50,7%)	19 (55,9)	86 (49,1)	0,57
rmpA (n=49 ; 23,7%)	6 (17,6)	43 (24,6)	0,51
entB (n=189 ; 91,3%)	33 (97,1)	156 (89,1)	0,21
mrkD (n=198 ; 95,7%)	32 (94,1)	166 (94,9)	0,7
ybtS (n=65 ; 31,4%)	12 (35,3)	53 (30,3)	0,55

Table 7. Presentation of antibiotic resistance based on the presence of virulence genes. *iutA* was significantly associated with resistance to Amoxicillin-Clavulanic Acid, and Cotrimoxazole (p<0.05). *iutA* tended to be associated with cephalosporin susceptibility (p<0.1). *Kfu* tended to be associated with resistance to Amoxicillin-Clavulanic Acid and cotrimoxazole (p<0.1).

Discussion

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.

Reference

1. Compain F. *et al.* J. Clin. Microbiol. 2014.
2. Podschun R. *et al.* Clin. Microb. Rev. 1998.
3. Russo T.A *et al.* Clin. Microb. Rev. 2019.
4. Chang S.C. *et al.* Diagn. Microbiol. Infect. Dis 2010.
5. Brisse S. et al. PLOS ONE 2009
6. Bachman MA, Infect. Immun. 2011.
7. Ma LC. et al. J. Infect. Dis. 2007.
8. Hsieh PF, et al. J. Infect. Dis. 2008
9. Jagnow J. et al. Microbiology 2003

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