The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 3592-3597

Hypervirulent *Klebsiella pneumoniae* at Benha University Hospitals

Sherin M. Emam, Sawsan Abdelrahman, Amany Abdelaziz Hasan*, Marwa S. EL- Melouk
Department of Microbiology and Immunology, Faculty of Medicine, Benha University, Egypt
***Corresponding author:** Amany Abdelaziz Hasan, **Mobile:** (+20)01274424188, **E-Mail:** dr.amany18.abdelaziz@gmail.com

ABSTRACT

Background: Hypervirulent *Klebsiella pneumoniae* (hvKP) is a virulent subtype of K. Pneumoniae. It commonly causes a serious community acquired infections, but it can also cause hospital acquired (nosocomial) infections. Emergence of antimicrobial resistance in hvKP ia a cause of concern.

Objective: Phenotypic and molecular characterization and differentiation of classical and hypervirulent *klebsiella pneumoniae* isolated from Benha University Hospital. **Subjects and methods:** Klebsiella pneumoniae was isolated by cultivation on Mac-Conkey agar media. Identified phenotypically and subjected to antibiotic sensitivity test. Conventional PCR was performed for detection of RmpA and RmpA2 virulence genes.

Results: Eight strains out of 70 (11.4%) *K.Pneumoniae* were identified as probable hvKP by detection of RmpA2 that was detected in all hvKP while rmpA was detected in 2 out of 8 hvKP isolates. Both cKP and hvKP isolates exhibited high resistance rates for most of the tested antibiotics.

Conclusion: Particularly in the presence of antibiotic resistance, HvKP pose a new hazard. Both Rmp A and Rmp A2 virulence genes were strongly associated with hvKP.

Keywords: Hypervirulent, *Klebsiella pneumoniae*, Hypermucoviscosity, String test, Rmp A, Rmp A2.

INTRODUCTION

Hypervirulent *Klebsiella pneumoniae* (hvKP) is a capsulated gram negative bacilli, non motile and non spore forming. It is a virulent subtype of *Klebsiella pneumoniae*, identified for the first time in Taiwan in the mid-1980s [(1)].

hvKP along with classical (cKP) are two groups of *Klebsiella pneumoniae*. Both are worldwide pathogens, but in the last three decades, the incidence of hvKP has increased all over the world [(2)].

In Egypt, hvKP represent a significant percentage among *Klebsiella pneumoniae* isolates causing serious community and hospital acquired infections [(3)].

Classical Klebsiella infections are usually non-virulent, it is frequently causing pneumoniae, urinary tract, wound and blood infections [(4)]. HvKP causes many more severe infections including pyogenic liver abcess, meningitis, urinary tract infection, necrotizing fasciitis and endophthalmitis in immunocompromised and also young healthy individuals [(4)].

To differentiate hvkp from cKP strains, one can use both genotypic and phenotypic traits. They were recognised based on distinctive phenotypic characteristics and clinical symptoms. Nevertheless, as genomic analysis has advanced, clinical research has relied more and more on genotypic markers to identify important virulence genes, which are more reliable for identifying hypervirulence [(5)].

Phenotypic characters in hyper virulent *Klebsiella pneumoniae* refers to hypermucoviscosity, it is a typical feature of hvKP strain but it is not specific [(6)].

Hvkp have a large 200 to 220 kb virulent plasmid. This virulent plasmid have many virulence factors encoded genes, as the regulator of mucoid phenotype (Rmp A and RmpA2) gene which is a plasmid-mediated regulator of capsular polysaccharide synthesis, siderophores (aerobactin (*iucA*) and salmochelin (iroB)), which are responsible for regulation of iron acquisition by bacteria enhancing their growth, replication and virulence and the putative metabolite transporter (peg34) [(6, 7)].

A virulence factor that is situated on a plasmid that controls the hvKP high-mucus phenotype is called rmpA (regulator of mucoid phenotype A). RmpA and an isoform rmpA2 have been shown to regulate capsular polysaccharide (CPS) biosynthesis. The rmpA2 gene shares 80 % identity with DNA sequence of rmpA [(8)].

Klebsiella pneumoniae infections is affected by the kind and number of virulence factors, additionally, the infectivity of *Klebsiella pneumoniae* is aggravated by its ability of obtaining multiple drug resistance [(3)].

According to antimicrobial susceptibility, classical *Klebsiella pneumoniae* is more resistant to antimicrobials than Hvkp. However in recent years, reports have indicated the existence of and rising prevalence of MDR-hvKP isolates [(9)].

This study was conducted to differentiate between classical and hypervirulent *Klebsiella pneumoniae* and detect rmpA and rmpA2 virulence genes by conventional PCR.

SUBJECTS AND METHODS

A cross sectional study was conducted at Microbiology and Immunology Department, Faculty of Medicine, Benha University during the period from July 2021 to July 2022.

One hundred and fourty two non-duplicate clinical samples (sputum, bronchoalveolar lavage, urine, sputum and blood) were obtained from patients admitted to the adult Intensive Care Unit (ICU) and Chest Department of Benha University Hospital. All the patients underwent a full personal and clinical history taking, they were 53 females and 89 males, their ages ranged from 18-60 years old.

Isolation and identification of *K. pneumoniae*:

All samples were inoculated on Mac conkey agar medium at 37oC for 24 h. For blood samples, 5ml blood were collected under complete aseptic condition in heparin containing blood collection tube, added to 50 ml broth, incubated in a blood culture bottle at 37oC for up to 7 days, inspected daily for any turbidity and subcultured on Macconkey agar. *K. Pneumoniae* were identified by colony morphology, gram stain and biochemical tests [(10)].

Phenotypic identification of hypermucoviscous *Klebsiella pneumoniae*:

String test was carried out by stretching *K. pneumoniae* colonies using the bacteriological loop. The test was considered positive and the strain was identified as hypermucoviscous (hmvKP) if a viscous string with >5mm in length was formed [(11)].

Antibiotic susceptibility testing:

All isolates of *K. pneumoniae* were subjected to disc diffusion antibiotic susceptibility testing in accordance with CLSI recommendations 2021 using the following antibiotic discs (Oxoid, UK), Amikacin (AK, 30µg), piperacillin /tozabactam (TPZ, 30µg), aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5µg), imipenem (IPM, 10µg), sulphamethoxazole/trimethoprim (SXT, 25µg), cefepime (FEP, 30µg) and cefotoxime (CTX, 30µg).

Rmp A and Rmp A2 genes detection:

Multiplex- PCR was carried out for detection of RmpA and RmpA2 using the specific primers (Table 1).

Table (1): Sequence of primers used in the study.

Primer name	Sequence	Product size	Reference
RmpA	F: ACT GGG CTA CCT CTG CTT CA R: CTT GCA TGA GCC ATC TTT CA	535	**Siu *et al.*** [(12)]
RmpA2	F: CTT TAT GTG CAA TAA GGA TGT T R: CCT CCT GGA GAG TAA GCA TT	451	**Lee *et al.*** [(13)]

A) Genomic DNA extraction: The whole bacterial genomic DNA was extracted using Qiagen QIAamp DNA Mini purification kits **(Qiagen, Germany)** according to the manufacture instructions.

B) DNA amplification: in Eppendorf tube an amplification mixture of a total volume 50 µl was prepared as follow: 25 µl (2x Taq Red PCR Master mix, 5 µl Template DNA, 2.5 µl of each primer and 10 µl RNase free water. The HotStar Taq DNA polymerase was first activated during the initial heat stage of the PCR protocol, which was carried out at 95 °C for 15 minutes. Then 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min. Finally, a 10-minute final extension at 72 °C [(14)].

C) Detection of DNA amplification products: The amplified productes were visualized by UV trans-illuminator on (1.5 %) agarose gel using 50 bp ladder. RmpA and RmpA2 genes were detected at 535bp and 451bp respectively [(15)]. **(Fig. 1)**

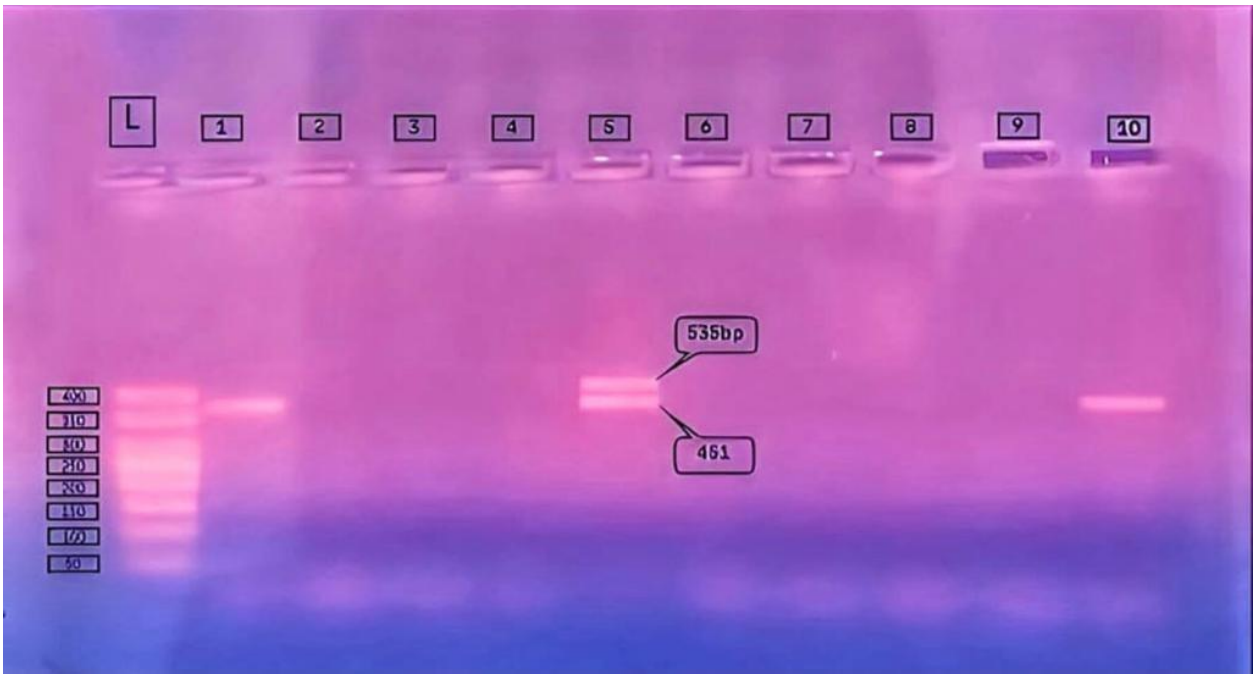

Figure (1). Agarose gel electrophoresis for the Multiplex PCR amplified products of *K. pneumoniae* rmpA and rmpA2 genes. L represents DNA molecular size marker (50 bp). Lanes (1,10) represent positive rmpA2 (451 bp) , while lane 5 represents positive rmpA2 (451 bp) and rmpA (535 bp). Lanes (2, 3, 4, 6, 7, 8, 9) represent negative samples.

Ethical approval:
This experiment was ethically approved by the Benha University's. After being fully informed, all participants provided written consent. The study was conducted out in line with the Helsinki Declaration.

Statistical analysis

The gathered data was updated, coded, tabulated, and uploaded to a computer using SPSS. Version 25.0 of IBM SPSS Statistics for Windows, IBM Corp., Armonk, New York. The type of data that was collected for each parameter was appropriately analysed using the data that were supplied. When using the Kolmogorov-Smirnov test to determine if a sample is normal, normality is presumed if the significance level is higher than 0.05. Numerical data was labelled as Mean±SD whereas non-numerical data was labelled as Frequency and Percentage. The Student T-Test and the Chi-Square test were applied to determine the statistical significance of the difference between the means of the two research groups. Findings were deemed significant if they had p values of 0.05 or above.

RESULTS

Patient characteristics

One hundred and fourty two clinical samples were obtained from patients attending the adult Intensive Care Unit (ICU) and Chest Department of Benha University Hospitals. These patients were (53 female and 89 male) aged from 18-60 years old (mean age= 34.5 SD ±9.6). Clinical samples were 67 respiratory secretions (including 25 endotracheal aspirate and 42 sputum), 35 urine, 23 pus and 17 blood samples. Different strains were isolated including 70 *Klebsiella pneumoniae*, 34 E. coli, 17 Staph. aureus, 11 Pseudomonas, 8 Enterobacter and 2 Candida species. Out of the isolated *K. pneumoniae* strains 8 strains were identified as HvKP.

The isolation of both cKP and hvKP was more frequently in males than females. A major risk factors for development of hvKP was DM as it was detected in 75% of hvKP isolates and only 25% of cKP with statistically significant difference ($P \leq 0.05$). While prolonged hospitalization was major risk factor for cKP (75.8%) with a statistically significant difference ($P \leq 0.05$). Classic *K. pneumoniae* were more frequently isolated from patients exposed to invasive procedures (88.7%) with a statistically significant difference ($P \leq 0.05$). Regarding history of antibiotic administration, cKP phenotypes were more frequently isolated from patients with a history of antibiotic administration while hvKP isolates were isolated more frequently from patients without antibiotic administration but with no statistical significant difference ($P > 0.05$) (Table 2).

Table (2): Comparison between cKP and hvKP phenotypes regarding demographic and clinical data among all studied patients.

	cKP N=62		hvKP N=8		*P*
	N	%	N	%	
Age (18-60) years (mean±SD)	62 mean (33.2)	88.56% SD±7.3	8 mean (35.1)	11.4% SD±8.1	0.376
Gender					
Male	39	62.9%	5	62.5%	0.982
Female	23	37.1%	3	37.5%	
Prolonged hospitalization	47	75.8%	2	25.0%	0.007
DM	16	25.8%	6	75.0%	0.010
Liver cirrhosis	15	24.2%	3	37.5%	0.415
Chronic renal failure	3	4.8%	1	12.5%	0.392
Pulmonary disease	15	24.2%	5	62.5%	0.038
Malignancy	2	3.2%	1	12.5%	0.309
Invasive procedures	55	88.7%	4	50%	0.005
Endotracheal intubation	18	29.0%	1	12.5%	0.322
Urinary catheter	26	41.9%	2	25.0%	0.357
Central venous catheter	11	17.7%	1	12.5%	0.711
Blood stream infections	11	17.7%	1	12.5%	0.711
Pneumonia	21	33.9%	2	25.0%	0.615
History of antibiotic administration	32	51.6%	3	37.5%	0.452
Surgical procedures	5	8.1%	2	25.0%	0.180

Antibiotic susceptibility testing

Both cKP and hvKP isolates exhibited high resistance rates for most of the tested antibiotics. Classic *K. pneumoniae* had higher resistance rates than hypervirulent against all tested antibiotics except for aztreonam (100% for hvKP and 96.8% for cKP) with a highly significant statistical difference for cefepime and sulfamethoxazole/trimethoprim (P<0.001), and a significant difference for amikacin, piperacillin/tozabactam, ciprofloxacin, imipenem (P ≤ 0.05 for each) (Table 3). For cKP, the highest resistance rate was for aztreonam (96.8%) followed by cefepime, ciprofloxacin and Piperacillin/tozabactam (95.2%, 90.3%, 83.9% respectively), then Sulfamethoxazole/trimethoprim and amikacin (75.8% and 62.9% respectively), While the least resistance rate was for imipenem (61.3%).

For hvKP, the highest resistance rate was for aztreonam (100%) followed by Piperacillin/tozabactam and ciprofloxacin 37.5% for both, then amikacin and cefepime 25.0% for both, while the least resistance rates were for Sulfamethoxazole/trimethoprim and imipenem with resistance rate 12.5% for both.

Table (3): Antimicrobial susceptibility patterns of ckp and hvKP isolates.

		HVKP				
		cKP N=62		hvKP N=8		*p*
		N	%	N	%	
Amikacin	**Sensitive**	19	30.6%	6	75.0%	0.046
	Intermediate	4	6.5%	0	0.0%	
	Resistant	39	62.9%	2	25.0%	
Piperacillin/tozabactam	**Sensitive**	3	4.8%	3	37.5%	0.004
	Intermediate	7	11.3%	2	25.0%	
	Resistant	52	83.9%	3	37.5%	
Cefepime	**Sensitive**	2	3.2%	4	50.0%	<0.001
	SDD	1	1.6%	2	25.0%	
	Resistant	59	95.2%	2	25.0%	
Ciprofloxacin	**Sensitive**	0	0.0%	0	0.0%	0.002
	Intermediate	6	9.7%	5	62.5%	
	Resistant	56	90.3%	3	37.5%	
Sulfamethoxazole/trimethoprim	**Sensitive**	9	14.5%	7	87.5%	<0.001
	Intermediate	6	9.7%	0	0.0%	
	Resistant	47	75.8%	1	12.5%	
Imipenem	**Sensitive**	19	30.6%	6	75.0%	0.022
	Intermediate	5	8.1%	1	12.5%	
	Resistant	38	61.3%	1	12.5%	
Aztreonam	**Sensitive**	1	1.6%	0	0.0%	0.876
	Intermediate	1	1.6%	0	0.0%	
	Resistant	60	96.8%	8	100.0%	

Twelve isolates were string test positive. eight of them were hvKP and 4 were cKP. All string negative test isolates were cKP. HvKP isolates were confirmed by detection of either rmpA or rmpA2 virulence genes by PCR. 8 isolates were rmpA2 gene positive and 2 were rmpA gene positive.

Table (4): Relation between string test, RmpA and RmpA2

		RmpA			RmpA2		
		Negative	**Positive**	**Total**	**Negative**	**Positive**	**Total**
String test	**Negative**	58	0	58	58	0	58
	Positive	10	2	12	4	8	12
	Total	68	2	70	62	8	70
K		0.249			0.768		
Sensitivity (%)		16.7			66.7		
Specificity (%)		100			100		
PPV (%)		100			100		
NPV (%)		85.3			93.5		
Accuracy (%)		85.7			94.3		

DISCUSSION

In this study, 17.1 % (12/70) of *K. pneumoniae* isolates displayed hypermucoviscosity phenotype (hmvkp) as proved by positive string test while 82.9 % were string negative and were categorized as classic *K. pneumoniae*. But, after detection of virulence genes by PCR, it was proved that only 8 isolates out of 12 were hypervirulent. Such results of hypermucoviscosity came in consistence with the study done by **Li *et al.*** [16] which showed that 20.5% of *K. pneumoniae* isolates were hmvKP (hypermucoviscous) and 79.5% were cKP (classical).

The current percentage was higher than that reported by **El-Mahdy *et al.*** [3]**,** in Mansora, Egypt as 13.8% of *K. pneumoniae* isolates were hmvKP (hypermucoviscous) and 86.2% were cKP (classical).

Diabetes was strongly linked with hvKP infections among the examined underlying systemic disorders because more patients with hvKP isolates had diabetes than those with cKP isolates (75.0 vs. 25.8%). This finding came in parallel to that of **Li *et al.*** [16] and **Liu and Guo** [17] who found that diabetes mellitus was considered a predisposing factor for acquiring hvKP infections.

On the contrary, studies done by **Zhang *et al.*** [18]**, Li *et al.*** [16]**, El-Mahdy *et al.*** [3]**, Liu *et al.*** [19] and **Yao *et al.*** [20] demonstrated that the underlying systemic conditions were not significantly different between hvKP and cKP infections.

Multidrug resistance developed in hvKP despite the fact that hvKP isolates were thought to be less resistant than cKP, which is consistent with the findings of **Li *et al.*** [16] study. Both cKP and hvKP showed antibiotic resistance for the majority of the tested antibiotics when the disc diffusion technique for determining antibiotic susceptibility pattern was applied. Classic *K. pneumoniae* had higher resistance rates compared to hypervirulent *K. pneumoniae* except for Aztreonam. There is a highly significant statistical difference for cefepime and sulfamethoxazole/trimethoprim ($P<0.001$), and a significant difference for amikacin, piperacillin/tozabactam, ciprofloxacin, imipenem ($P \leq 0.05$ for each). These results agreed with the results of **Li *et al.*** [16]**, Liu and Guo** [17]**, Guo *et al.*** [21] **and Wu *et al.*** [22]**.**

In this study, the lowest resistance rates were for imipenem as all hvKP were imipenem susceptible except one intermediate 12.5% (1/8) and one resistant 12.5% (1/8), and 61.3% (38/62) of cKP were imipenem non-susceptible. This coincided with **Abd-Elmonsef *et al.*** [10] in Tanta, Egypt who found that lowest resistance rates were for carbapenems as non of hmvKP isolates were resistant to them and only 2.99% (2/67) of cKP were resistant to imipenem.

Out of 12 (12/70; 17.1%) string positive test isolates, 8 isolates were proved to be hvKP via detection of either rmpA or rmpA2 virulence genes by PCR and 4 isolates were cKP. All string negative test isolates were cKP. So, 8 (8/70; 11.4%) isolates only in all 70 *Klebsiella pneumoniae* isolates were hypervirulent. These results were slightly higher than that reported by **El-Mahdy *et al.*** [3]**,** in Mansora, Egypt as (13.8%) of *K. pneumoniae* isolates were hypermucoviscous by positive string test and (6.2%) were identified as hvKP by presence of virulence genes. So, catergorization of hypervirulent strains was based on presence of rmpA and/or rmpA2.

This study showed that out of 12 string positive isolates, 2 (16.7%) were rmpA gene positive and 10 (83.3%) were rmpA gene negative by PCR. No rmpA genes were detected among string negative isolates.

Out of 12 string positive isolates, 8 (66.7%) were rmpA2 gene positive and 4 (33.3 %) were rmpA2 gene negative by PCR. No rmpA2 genes were detected among string negative isolates.

According to the current results, both rmpA and rmpA2 genes were detected in hvKP isolates (25% (2/8) and 100% (8/8) respectively), but not detected in cKP isolates. These results were in coincided with a study done by **Lee *et al.*** [13] in Taiwan which found that rmpA or rmpA2 were detected in 91.4% of Hyper virulent (HV) isolates.

In **El-Mahdy *et al.*** [3] in Egypt, rmpA and rmpA2 were found in (2 and 3 respectively) of 4 hvKP isolates while not detected in cKP.

CONCLUSION

K. pneumoniae represents one of the most common causes of infections among Benha University Hospitals. The high prevelance of antibiotic resistance among classic *K. pneumoniae* isolates is a major challenge. In addition, the emergence of MDR hmvKP strains is more alarming particularly among hospital settings. Although the string test's accuracy in identifying metastatic Klebsiella is debatable, it is a quick and straightforward test that can be carried out in any laboratory to detect the presence of this bacteria. Genotypic detection by PCR provides helpful and confirmatory tool to diagnose hvKP.

Supporting and sponsoring financially: Nil.
Competing interests: Nil.

REFERENCES

1. **Liu C, Guo J (2018):** Characteristics of ventilator-associated pneumonia due to hypervirulent *Klebsiella pneumoniae* genotype in genetic background for the elderly in two tertiary hospitals in China. Antimicrob Resist Infect Control, 7: 95-99.
2. **Pajand O, Darabi N, Arab M *et al.* (2020):** The emergence of the hypervirulent *Klebsiella pneumoniae* (hvKp) strains among circulating clonal complex 147 (CC147) harbouring $bla_{NDM/OXA-48}$ carbapenemases in a tertiary care center of Iran. Ann Clin Microbiol

Antimicrob., 19(1):12. doi: 10.1186/s12941-020-00349-z.

3. **El-Mahdy R, El-Kannishy G, Salama H (2018):** Hypervirulent Klebsiella pneumoniae as a hospital-acquired pathogen in the intensive care unit in Mansoura, Egypt. Germs, 8(3):140-146.
4. **Russo T, Marr C (2019):** Hypervirulent Klebsiella pneumoniae. Clin Microbiol Rev., 32: 1-19.
5. **Parrott A, Shi J, Aaron J *et al.* (2020):** Detection of multiple hypervirulent *Klebsiella pneumoniae* strains in a New York City hospital through screening of virulence genes. Clin Microbiol Infect., 4: 583-589.
6. **Russo T, Johnson J (2018):** Diseases caused by gram-negative enteric bacilli. In: Jameson J, Fauci A, Kasper D *et al.* (ed), Harrison's principles of internal medicine, 20th ed. McGraw-Hill Education, New York, NY. Pp. 1146–1160. https://accessmedicine.mhmedical.com/content.aspx?bookid=2129&sectionid=192022222
7. **Chen L, Todd R, Kiehlbauch J *et al.* (2017):** Notes from the field: pan-resistant New Delhi metallo-beta-lactamase-producing *Klebsiella pneumoniae*—Washoe County, Nevada, 2016. MMWR Morb Mortal Wkly Rep., 66:33. doi: 10.15585/mmwr.mm6601a7.
8. **Yan Q, Zhou M, Zou M *et al.* (2016):** Hypervirulent *Klebsiella pneumoniae* induced ventilator-associated pneumonia in mechanically ventilated patients in China. Eur J Clin Microbiol Infect Dis., 35: 387–396.
9. **Lin Z, Zheng J, Bai B *et al.* (2020):** Types and Prevelance of *Klebsiella pneumoniae*. Front Microbiol., 11: 436. Doi: 10.3389/ fmicb.2020.00436.
10. **Abd-Elmonsef M, Khalil H, Selim A *et al.* (2016):** Detection of hypervirulent *Klebsiella pneumoniae* in Tanta University Hospital, Egypt. Br Microbiol Res J., 17:1-10.
11. **Zhan L, Wang S, Guo Y *et al.* (2017):** Outbreak by Hypermucoviscous *Klebsiella pneumoniae* ST11 Isolates with Carbapenem Resistance in a Tertiary Hospital in China. Front Cell Infect Microbiol., 7:182. doi: 10.3389/fcimb.2017.00182.
12. **Siu L, Fung C, Chang F *et al.* (2011):** Molecular Typing and Virulence Analysis of Serotype K1 *Klebsiella Pneumoniae* Strains Isolated From Liver Abscess Patients and Stool Samples From Noninfectious Subjects in Hong Kong, Singapore, and Taiwan. J Clin Microbiol., 49: 3761–3765.
13. **Lee C, Liu J, Su L *et al.* (2010):** Hypermucoviscosity associated with *Klebsiella pneumoniae* -mediated invasive syndrome: a prospective cross-sectional study in Taiwan. Int J Infect Dis., 14: 688–692.
14. **Compain F, Babosan A, Brisse S *et al.* (2014):** Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. J Clin Microbiol., 52(12):4377-80.
15. **Fattouh M, Nasr El-din A (2014):** Emergence of Carbapenem resistant *Acintobacter baumannii* in the intensive care unit in Sohag University Hospital, Egypt. Int J Curr Microbiol Appl. Sci., 3:732-744.
16. **Li J, Ren J, Wang W *et al.* (2018):** Risk factors and clinical outcomes of hypervirulent *Klebsiella pneumoniae* induced bloodstream infections. Eur J Clin Microbiol Infect Dis., 37: 679-89.
17. **Liu C, Guo J (2019):** Hypervirulent *Klebsiella pneumoniae* (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. Ann Clin Microbiol Antimicrob., 18(1):4. doi: 10.1186/s12941-018-0302-9.
18. **Zhang Y, Zhao C, Wang Q *et al.* (2016):** High prevalence of hypervirulent *Klebsiella pneumoniae* infection in china: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob. Agents Chemother., 60: 6115–6120.
19. **Liu Y, Li B, Zhang Y *et al.* (2014):** Clinical and molecular characteristics of emerging hypervirulent *Klebsiella pneumoniae* bloodstream infections in mainland China. Antimicrob Agents Chemother., 58: 5379–5385.
20. **Yao B, Xiao X, Wang F *et al.* (2015):** Clinical and molecular characteristics of multi-clone carbapenem-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in a tertiary hospital in Beijing, China. Int J Infect Dis., 37: 107–112.
21. **Guo Y, Wang S, Zhan L *et al.* (2017):** Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. Front Cell Infect Microbiol., 7: 24. Doi:10.3389/fcimb.2017.00024.
22. **Wu H, Li D, Zhou H *et al.* (2017):** Bacteremia and other body site infection caused by hypervirulent and classic *Klebsiella pneumoniae*. Microb Pathog., 104: 254–262.