ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JANUARY 2018 ISBN 1595-689X VOL19 No.1
AJCEM/1810 http://www.ajol.info/journals/ajcem
COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i1.10
AFR. J. CLN. EXPER. MICROBIOL. 19 (1):70- 75

FIRST MOLECULAR INVESTIGATION OF CAPSULAR SEROTYPING AND HYPERVIRULENT (HVLP) OF *K. PNEUMONIAE* IN UNIVERSITY HOSPITAL CENTER OF YOPOUGON COTE D'IVOIRE

M'lan-Britoh, A.[1,2], Meité S.[12], Boni, C. [12], Zaba, F.[1], Koffi, K. S.[2], Guessennd, N.[3], Kakou, N. S.[3,] Faye- Kette, H.[12], Dosso, M.[12]

[1]Unit of Bacteriology-Virology of the Central Laboratory of the Centre Hospitalier et Universitaire de Yopougon;
[2]Department of Bacteriology-Virology of the Faculty of Medicine of Abidjan; [3]Institut Pasteur of Ivory Coast

Correspondence: 21 B.P. 632 Abidjan 21 Dr M'lan-Britoh Alice (alicebritoh@yahoo.fr)

ABSTRACT
***Klebsiella pneumoniae* is a well known human pathogen. Although infectious in most nosocomial infections with a high level of resistance, capsular types and circulating hypervirulent strains in our context are not documented. The aims of this study are to identify capsular serotypes and hypervirulent strains circulating at the Yopougon University Hospital in Abidjan. 51 strains of *Klebsiella* were collected at Chu de Yopougon. The capsular serotypes were determined using PCR and the serotypes K1, K2 and K5 were searched. The hypervirulent strains were also investigated by PCR and by string test. The predominant serotypes were non-K1 / K2 (46/51, 90%). The serotypes found K5 and K2 in (4/51, 7.8%) and (1/51; 1.9%) respectively. The rmpA gene linked to hyperviscosity or hyperviscosity was not found although 25.5% (12/51) were positive for the stretch test. The capsular distribution of strains of *Klebsiella pneumoniae* seems different from Asian authors. The determination of non-K1non types K2 remains to be elucidated.**
Keyvords: *Klebsiella pneumoniae*, capsular serotype - hypervirulence

.

PREMIERE ETUDE D'INVESTIGATION MOLECULAIRE DE SEROTYPAGE CAPSULAIRE ET DE GENE D'HYPERVIRENCE DE *KLEBSIELLA PNEUMNIAE* AU LABORATOIRE DU CHU DE YOPOUGON EN COTE D'IVOIRE

M'lan-Britoh, A.[1,2], Meité S.[12], Boni, C. [12], Zaba, F.[1], Koffi, K. S.[2], Guessennd, N.[3], Kakou, N. S.[3,] Faye- Kette, H.[12], Dosso, M.[12]

**Unité d'Bacteriology-Virology du Laboratoire Central du Centre Hospitalier et Universitaire de Yopougon
d Bacteriology-Virology Ministère de la Faculté de médecine d'Abidjan
Institut Pasteur de Côte d'Ivoire**

Correspondance : 21 B.P. 632 Abidjan 21 Dr Mlan-Britoh Alicebritoh@yahoo.fr (Alice)

Résumé
Klebsiella pneumoniae est un pathogène nosocomial humain bien connu. Bien qu'incriminé dans la plus part des infections nosocomiales avec un niveau élevé de résistance, les types capsulaires et les souches hypervirulentes circulantes dans notre contexte ne sont pas documentés. L'objectif de cette étude est d'identifier les sérotypes capsulaires et les souches hypervirulentes circulant au CHU de Yopougon Abidjan., 51 souches de *Klebsiella* ont été collectés au Chu de Yopougon. Les sérotypes capsulaires ont été déterminée à l'aide de la PCR et les sérotypes K1, K2 et K5 ont été recherchés. Les souches hypervirulentes ont été recherchées également par PCR et par le test d'étirement ou string test. Les sérotypes prédominants étaient les non K1/K2 (46/51; 90%). Les sérotypes retrouvés K5 et K2 dans respectivement (4/51; 7,8%) et (1/51 ; 1,9%). Le gène rmpA lié à l'hyperviscosité n'a pas été retrouvé bien que 25,5% (12/51) étaient positives au test d'étirement. La distribution capsulaire des souches de *Klebsiella pneumoniae* semble différente des auteurs asiatiques. D'ou l'intérêt de travaux plus approfondies afin de déterminer les types capsulaire des souches non K1 non K2.
Mots clefs : *Klebsiella pneumoniae* – serotype capsulaire – Hypervirulence

INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen responsible for community infections and nosocomial infections such as pneumonia, septicemia, suppurative and urinary infections, particularly in patients admitted to intensive care **(1)**.

The capsule is considered to be a major virulence factor for *Klebsiella*. It intervenes in the formation of biofilm and in the increase of the anti-opsonised effect allowing the bacterium to escape the immune response of the host (2,3,4**)**

Currently, 78 capsular antigens of *K. pneumoniae* are listed and the serotypes most frequently involved in human infection are serotypes K1, K2, K5, K54

Several reports have revealed that capsular types Some of these have reported a correlation between serotypes K1, K2 and abscesses of the liver, but serotypes are not the only determining factor in the occurrence of liver abscesses (**4**). Although the first case of a patient with liver abscess was described in China in the 1980s **(5)**, this strain of *K pneumoniae* responsible for liver abscess has been reported in Taiwan, Japan, Europe, North America, and Korea. **(6,7,8)**. This characterized emergent infection is often complicated by septic meningitis and purulent endophthalmitis. This new strain called "*Klebsiella pneumoniae hypervirulent* or HvKP" is a variant of the classical strain in terms of aspects of the colonies on the different agar plates. These strains are characterized by a hyperproduction of the capsule mediated by the rmpA / rmpA2 gene which gives these strains a hypervisic aspect. The association of hyperviscosity with the presence of the magA gene is also found, particularly in strains of serotypes K1, K2, K5, K20, K54 and K57 (9,10)
In Ivory Coast, *K pneumoniae* is involved in various infections (11) in human infection and in the colonization of area in hospitals (12)**. 2010**. Although the antibiotic resistance of *Klebsiella pneumoniae* has often been studied (13), data on circulating serotypes and hypervirulent strains are non-existent. The aims of this study are to identify capsular serotypes and hypervirulent strains circulating at the Yopougon University Hospital in Abidjan.

MATERIALS AND METHODS

are related to the severity of infection. This study was carried out at the Bacteriology-Virology Unit of the Central Laboratory of the Yopougon University Hospital for Classical Bacteriology and at the Molecular Biology Platform of the Pasteur Institute of Côte d'Ivoire for the molecular analysis of the strains of January To March 2016.

14. *Study population*

51 strains of which 47 were of human origin and four of environmental origin (1, 2, 34, and 36) were studied. 7 strains of *K oxytoca* , 17, 24, 33, 44, 48, 53, 55) were associated in molecular analyzes to investigate the specificity of *K pneumoniae* identification primer

15. *Bactériologie*

Isolates where biochemically identified by conventional bacteriology tests.Susceptibility testing for the strains was performed using the Kirby-Bauer disk diffusion test on Mueller-Hinton agar. Results were interpreted according to the recommendations and definitions from Comité Antibiogramme de la Société Française de Microbiologie (CASFM 2016) *E. coli* ATCC 25922 was used as the MIC reference strain.

A string test was performed to distinguish hvKP from cKP according to **Fang** study [8].

16. PCR assay

The" *K.pneumoniae* Pf/*K.pneumoniae* Pr1 "primer pair included in the multiplex PCR is for the identification of *K. Pneumoniae*. The following genes were targeted: *rmpA*, and the K1 K2 and K5 capsular serotypes. The primers targeting the capsular serotype specifying genes were previously described (Table 1).

TABLE 1: PRIMERS USED IN THE MULTIPLEX PCR

SEROTYPE	PRIMER	SEQUENCE	PRODUCT SIZE (PB)	REFERENCES
K1	MAGAF1	GGTGCTCTTTACATCATTGC	1283	FANG ET AL. (2004)
	MAGAR1	GCAATGGCCATTTGCGTTAG		
K2	K2WZY-F1	GACCCGATATTCATACTTGACAGAG	641	TURTON ET AL. (2008)
	K2WZY-R1	CCTGAAGTAAAATCGTAAATAGATGGC		
K5	K5WZXF360	TGGTAGTGATGCTCGCGA	280	TURTON ET AL. (2008)
	K5WZXR639	CCTGAACCCACCCCAATC		
RMPA	RMPAF	ACTGGGCTACCTCTGCTTCA	516	NADASY ET AL. (2007)
	RMPAR	CTTGCATGAGCCATCTTTCA		
K .PNEUMONIAE 16S-23S ITS	PF	ATTTGAAGAGGTTGCAAACGAT	130	LIU ET AL. (2008)
	PR1	TTCACTCTGAAGTTTTCTTGTGTTC		

Extraction of DNA was carried out by thermal shock by a freezing cycle (-20 ° C. for 1 hour and then heating on thermo block for 10 minutes at 95 ° C. The GoTaq G2 Flexi DNA polymerase kit (Promega Corporation, USA) was used for the PCR mixes containing 0.2µM of each primer, 7.5µM, MgCl, 0.5µM dNTPs, 3 unit Taq polymerase, 1X of buffer and 5µl of DNA template for a final volume

of 50µl. Amplification onditions were :95°C 15 min (1 cycle), (95°C 30 s ,58°C 90 s, 72°C 90s) (35cycles), 72°C 10 min (1 cycle). The revelation was made on a GelDoc Bioanalyzer (BioRad) after electrophoresis on 1.5% agarose gel.

RESULTS

Concerning the source of ours isolates, (92%) were isolated from clinical sources and (8 %), from hospital environment. 31% were isolated from biological products in the pediatric and 23% from intensive care unit. Of the 47 clinical isolates, 33(65%) were from urine and 7 (14%) from sputum (tableau II)

TABLE II: DISTRIBUTION OF BACTERIAL BY SPECIMEN AND WARDS

Wards	Value (n)	%
Surgery	3	6
Endocrinology	7	14
Médecine	3	6
Nephrology	4	8
Pediatric	16	31
Intensive care unit	12	23
Over	6	12
Specimen		
Aspirates	2	4
Catheter tip	2	4
hospital's environment	4	8
CSF	1	2
Sputum	7	14
Blood	2	4
Urine	33	65
Total	51	100

The bacterial isolates exhibited a high resistance to the antibiotics tested. In our study 14% of *strains* are resistant to at least three families of antibiotic at a time. The proportion of resistance to third generation céphalosporins, ciprofloxacine and gentamicin was 49%, 45% and 33%, respectively, (Table 5). The prevalence of ESBL producing strains was 30% in *K. pneumoniae*

TABLEAU III: DISTRIBUTION OF ANTIBIOTICS RESISTANCE

Antibiotic	Value (n)	%
Amoxicilline+Acide clavulaniqueR	25/51	49
CefoxitineR	15/51	29,5
CeftriaxoneR	25/51	49
ImipénèmeR	1/51	2
CiprofloxacineR	23/51	45
GentamycineR	17/51	33
AmikacineR	2/51	3,9
FosfomycineR	2/51	3,9

The molecular identification all (41) isolates gave positive results and identified as *K. pneumoniae*. Results of PCR amplification confirmed that all isolates were *K. pneumoniae*. Serotype of 9.8% where be identified by primers used whose 7,8% of K2 (Figure 1 and 2) and 2% of K5. Five of idenfied serotype came from urine 12% (4/33) and sputum 14,28% (1/7) .Of the 5 strains serotyped 2 had a positive String test (40%) however rmpA gene linked to hyperviscosity has not been found.

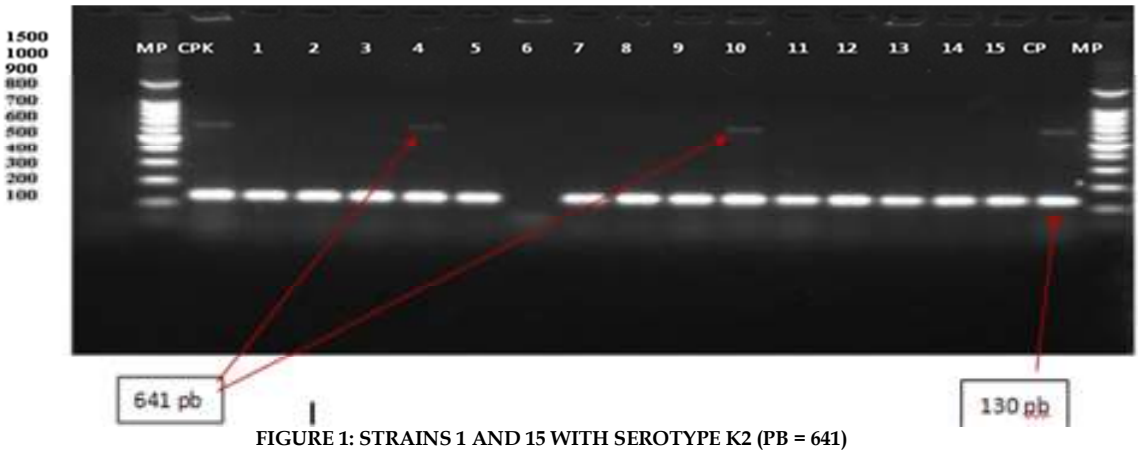

FIGURE 1: STRAINS 1 AND 15 WITH SEROTYPE K2 (PB = 641)

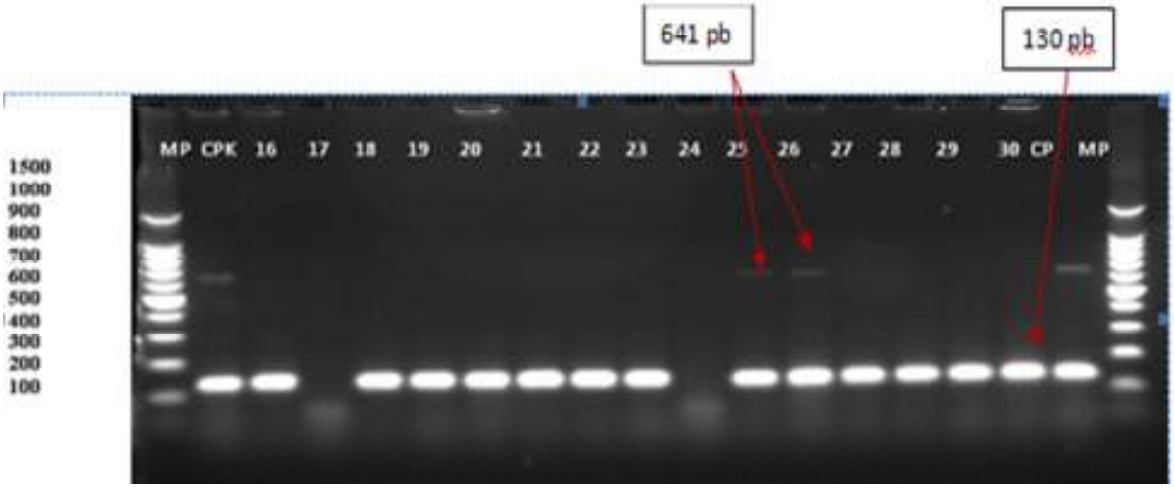

FIGURE 2: STRAINS 16 AND 30 WITH SEROTYPE K2 (PB = 641)

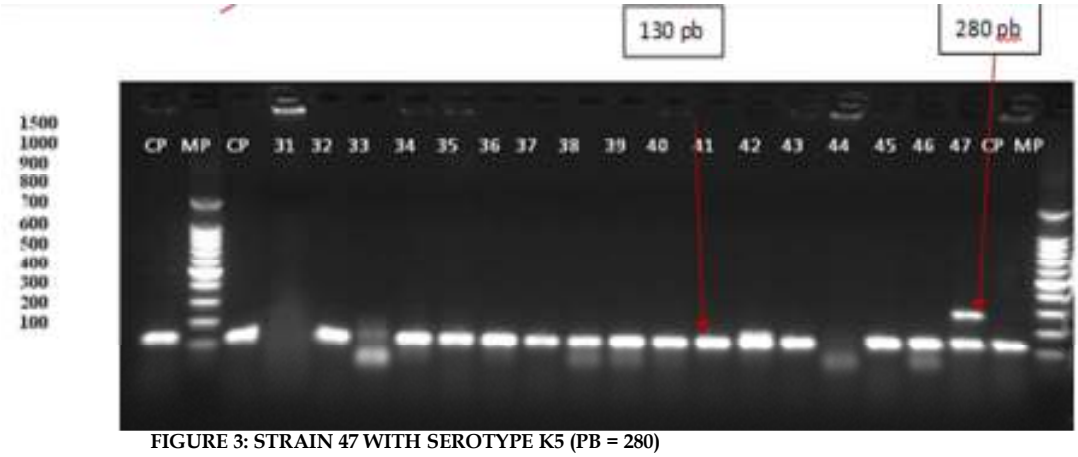

FIGURE 3: STRAIN 47 WITH SEROTYPE K5 (PB = 280)

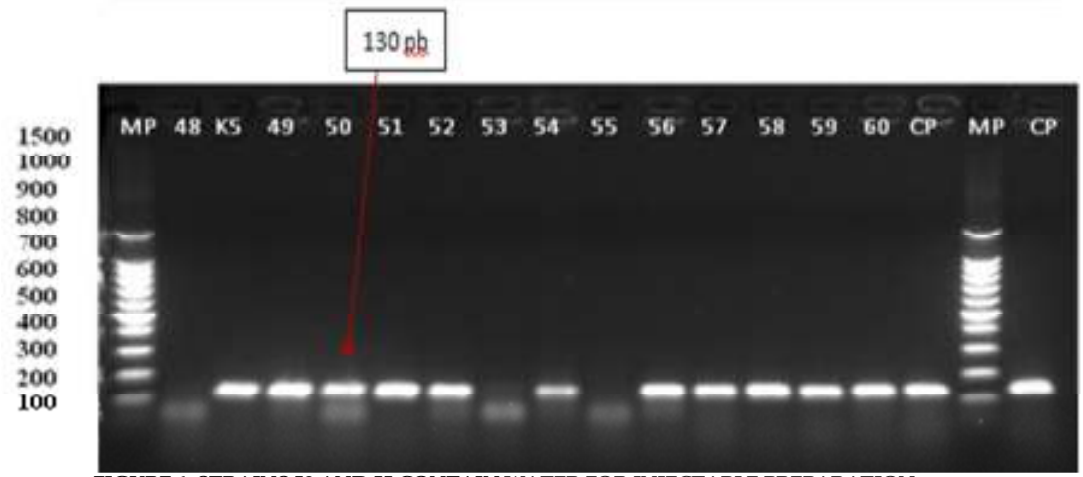

FIGURE 4: STRAINS 53 AND 55 CONTAIN WATER FOR INJECTABLE PREPARATION

MP = Molecular weight marker, wells CP, CPK and K5 contain ampicons of K pneumoniae already confirmed by PCR = positive controls and wells 6 and 31 contain water for injectable preparation = Negative controls

DISCUSSION

Most gave a band for the *K. pneumoniae* 16S–23S internal transcribed spacer region. Amplification of the 16S rRNA gene represents a highly accurate and versatile method for the identification of bacteria to the species level, even when the species in question is notoriously difficult to identify by biochemical methods (17). The K1 serotype was not found in our study. It is disagreed with results elucidated by other workers (15,16**)** who noticed that serotypes K1, K2 and Non-K1/K2 accounted for 14.3 % (7/49), 38.8 % (19/49) and 46.9 % (23/49) of all *K. pneumoniae* isolates, respectively. Our results were in agreement with those who reported that *K. pneumoniae* serotype K1 is dominant on the other serotypes and find K1 and serotype K2 was 52.3% and 22.7% (18 **19)** .

This could be related to the isolation site of our strains, more than 70% are non-invasive strains. Otherwise the capsular serotype K1 is recognized as the most virulent and the most encountered throughout the world especially in the countries of Asia where it is correlated to hepatic abscesses **(17).** In a study of strains of *K. pneumoniae* from 11 Asian countries serotype K1 are findings were **27,5**%, **12,6**% and **9,6**% in Taiwan, Korea and Vietnam respectively. Although cases have been reported in South Africa and Nigeria, no case of Liver Abscesses has been described in our context. This could explain the absence of serotype K1 in our series. But the absence of serotype K1 could also be related to the size of our sample. K. pneumoniae serotype K2, it was found in (4/51)7.8% of the strains.

Our results were in agreement with some workers **(**18,19,20**)** who reported that *K. pneumoniae* serotype K2 is dominant (64%) on the over serotypes of the three serotypes K1 K2 et K5 researched. Serotype K2 is one of the most common and most invasive capsular serotypes described throughout the world. A similarly wide range of capsular serotypes has been demonstrated in other studies. However, there are differences in the serotypes that appear most frequently in some countries. Non-K1/K2 strains constituted a very important proportion of the strains of our study with more than 90%. Our results were in agreement with **Adam et al. (2006)**(21) in Australia who noticed high prevalence of non K1/K2 strains in 96% of 293 strains. They are by far from **Lin et al. (2010)(22,)** who also found in their series a predominance of non-K1 / K2 serotypes (46.9%. However, they remain discordant with those of many Asian authors in whom serotypes K1 and K2 are predominant **(23).** In general, there is a variable global distribution of *Klebsiella* capsular serotypes

The rmpA gene is the regulator of capsular synthesis; many studies have suggested that this gene could be responsible for the hypervirulent phenotype of K. pneumoniae characterized by the hypervisic character of the strains and found the gene more often associated with serotypes K2 than K1 and non-K1 / K2 serotypes **(24)**. This gene was not found in our study

Conclusion

Capsular serotypes Non-K1/K2 were the most recovered hence the interest of more studies in order to identify them. Moreover, the determinants of hyper virulence were not found despite the presence of strains positive to the string test.

Acknowledgements

We are grateful to Molecular biology platform of institut Pasteur of Ivory Coast for collaboration

REFERENCES BIBLIOGRAPHIQUES

1. **Vardakas, K. Z., Matthaiou, D. K., Falagas, M. E., Antypa, E., Koteli, A., & Antoniadou, E. (2015). Characteristics, risk factors a nd outcomes of carbapenem-resistant *Klebsiella pneumoniae* infections in the intensive care unit. *Journal of Infection*, *70*(6), 592-599.**

2. **Cortés, G., Borrell, N., de Astorza, B., Gómez, C., Sauleda, J., & Albertí, S. (2002). Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. *Infection and immunity*, *70*(5), 2583-2590.**

3. **Lin, Y. T., Wang, Y. P., Wang, F. D., & Fung, C. P. (2015). Community-onset *Klebsiella pneumoniae pneumonia* in Taiwan: clinical features of the disease and associated microbiological characteristics of isolates from pneumonia and nasopharynx. *Frontiers in microbiology*, *6*, 122.**

4. **Fung, C. P., Chang, F. Y., Lee, S. C., Hu, B. S., Kuo, B. I., Liu, C. Y., & Siu, L. K. (2002). A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis?.*Gut*, *50*(3), 420-424.**

5. **Siu, L. K., Yeh, K. M., Lin, J. C., Fung, C. P., & Chang, F. Y. (2012). *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. *The Lancet infectious diseases*, *12*(11), 881-887**

6. **Ohmori, S., Shiraki, K., Ito, K., Inoue, H., Ito, T., Sakai, T., ... & Nakano, T. (2002). Septic endophthalmitis and meningitis associated with Klebsiella pneumoniae liver abscess. *Hepatology research*, *22*(4), 307-312.**

7. Rahimian, J., Wilson, T., Oram, V., & Holzman, R. S. (2004). Pyogenic liver abscess: recent trends in etiology and mortality. *Clinical infectious diseases*, *39*(11), 1654-1659.

8. Fang, C. T., Chuang, Y. P., Shun, C. T., Chang, S. C., & Wang, J. T. (2004). A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. *The Journal of experimental medicine*, *199*(5), 697-705.

9. Yeh, K. M., Lin, J. C., Yin, F. Y., Fung, C. P., Hung, H. C., Siu, L. K., & Chang, F. Y. (2010). Revisiting the importance of virulence determinant magA and its surrounding genes in Klebsiella pneumoniae causing pyogenic liver abscesses: exact role in serotype K1 capsule formation. *Journal of Infectious Diseases*,*201*(8), 1259-1267.

10. Shon, A. S., Bajwa, R. P., & Russo, T. A. (2013). Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed.*Virulence*, *4*(2), 107-118.

11. Meité S, C Boni-cisse, P Monemo, C J Babo, AP Mlan Tanoa, H Faye-Kette, M Dosso, A Amonkou.(2011) Profil de résistance des bactéries isolées des prélèvements bronchiques protégés chez des patients sous ventilation assistée en réanimation CHU de Yopougon. *Méd Afr Noire,* 2011, 58 (8/9) :416-422.

12. C Boni-Cissé, Meite S, N D Coulibaly, N Guessend AP Mlan, H Faye-Kette, M Dosso, KM Timite ,(2012) Prévalence des gènes qnr chez *Klebsiella pneumoniae* isolés dans le service de pédiatrie du CHU de Yopougon à Abidjan Côte d'Ivoire. *Rev int sc méd. 2012 ;14(2) :66-72.*

13. Méité S, Boni-cissé C. Monemo P, Mlan Tanoa AP, Faye-Ketté H, Dosso M.(2011) Surveillance microbiologique des surfaces au niveau d'un établissement hospitalier de niveau tertiaire : exemple du CHU de Yopougon, Abidjan, Côte d'Ivoire, *J. sci pharm Biol*, 2010, 11(1) :73-81.

14. Turton, J. F., Baklan, H., Siu, L. K., Kaufmann, M. E., & Pitt, T. L. (2008). Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella sp*. and comparison of isolates within these serotypes. *FEMS microbiology letters*, *284*(2), 247-252

15. Nadasy, K. A., Domiati-Saad, R., & Tribble, M. A. (2007). Invasive Klebsiella pneumoniae syndrome in North America. *Clinical Infectious Diseases*, *45*(3), e25-e28

16. Liu, Y., Liu, C., Zheng, W., Zhang, X., Yu, J., Gao, Q., & Huang, X. (2008). PCR detection of *Klebsiella pneumoniae* in infant formula based on 16S–23S internal transcribed spacer. *International journal of food microbiology*, *125*(3), 230-235

17. Turton, J. F., Perry, C., Elgohari, S., & Hampton, C. V. (2010). PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. *Journal of medical microbiology*, *59*(5), 541-547.

18. Victor, L. Y. (2007). Virulence characteristics of Klebsiella and clinical manifestations of *K. pneumoniae* bloodstream infections. *Emerging Infectious Disease*, *13*(7), 123-128.

19. Majid H. Al-Jailawi , Tamara H. Zedan , Kifah A. Jassim. (2014). Multiplex-PCR Assay for Identification of *Klebsiella pneumoniae* Int. J. Pharm. Sci. Rev. Res., 26(1),112-117

20. Chung, D. R., Park, M. H., Kim, S. H., Ko, K. S., Kang, C. I., Peck, K. R., & Song, J. H. (2012). Prevalence and molecular characterization of serotype K1 Klebsiella pneumoniae strains from various clinical specimen sources in 11 Asian countries. *Journal of Infection*, *64*(6), 622-625.

21. Chang, W. N., Huang, C. R., Lu, C. H., & Chien, C. C. (2012). Adult *Klebsiella pneumoniae* meningitis in Taiwan: an overview. *Acta Neurol Taiwan*, *21*(2), 87-96.

22. Lin, Y. T., Jeng, Y. Y., Chen, T. L., & Fung, C. P. (2010). Bacteremic community-acquired pneumonia due to *Klebsiella pneumoniae*: clinical and microbiological characteristics in Taiwan, 2001-2008. *BMC infectious diseases*,*10*(1), 1.

23. Chang, S. C., Fang, C. T., Hsueh, P. R., Chen, Y. C., & Luh, K. T. (2000). Klebsiella pneumoniae isolates causing liver abscess in Taiwan. *Diagnostic microbiology and infectious disease*, *37*(4), 279-284.

24. Majid H. Al-Jailawi , Tamara H. Zedan , Kifah A. Jassim. (2014). Multiplex-PCR Assay for Identification of *Klebsiella pneumoniae* Int. J. Pharm. Sci. Rev. Res., 26(1),112-117