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

M’langBritoh, A.1,2, Meité S.1,2, Boni, C.1,2, Zaba, F.1, Koffi, K. S.2, Guessennd, N.3, Kakou, N. S.3, Fayeg Kette, H.1,2, Dosso, M.1,2

1Unit of Bacteriology-Virology of the Central Laboratory of the Centre Hospitalier et Universitaire de Yopougon; 2Department of Bacteriology-Virology of the Faculty of Medicine of Abidjan; 3Institut 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.

Keywords: Klebsiella pneumoniae, capsular serotype - hypervirulence.

INTRODUCTION
Klebsiella pneumoniae is an opportunistic pathogen responsible for community infections and nosocomial infections such as pneumonia, septicemia, suppurrative 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 are related to the severity of infection. 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 hyperviscous 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 nonexistent. The aims of this study are to identify capsular serotypes and hypervirulent strains circulating at the Yopougon University Hospital in Abidjan.

MATERIALS AND METHODS

| SEROTYPE | PRIMER | SEQUENCE | PRODUCT SIZE | REFERENCES |
|----------|--------|----------|--------------|------------|
| K1       | MAGAF1 | GGTGCTCTTTACATCATTGC | 1283 | FANG ET AL. (2004) |
|          | MAGAR1 | GCAATG GCCATT TTGCGTAG |       |            |
| K2       | K2WZY-F1 | GAC CGGATATTCTC ATTTGACAG | 641 | TURTON ET AL. (2008) |
|          | K2WZY-R1 | CCTGAAGTAAAATCGTAGTAGAGC |       |            |
| K5       | K5WZF360 | TGGTAGTGATGCTGCCGA | 280 | TURTON ET AL. (2008) |
|          | K5WZXR639 | CCTGAACCCACCCCAATC |       |            |
| RMPA     | RMPAF  | ACTGGCTACCTCTGGCTTC | 516 | NADASY ET AL. (2007) |
|          | RMPAR  | CTTGCAATGAGCCCATCTTTCA |       |            |
| K. PNEUMONIAE 16S-23S ITS | PF | ATTTGAAAGGGTGTGCAACGAT | 130 | LIU ET AL. (2008) |
|          | PR1    | CTCACTCTGAAAGTTTTCTTCTGTTC |     |            |

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 conditions were: 95°C 15 min (1 cycle), (95°C 30 s, 58°C 90 s, 72°C 90s) (35 cycles), 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

| TABLE III: DISTRIBUTION OF ANTIBIOTICS RESISTANCE |
|--------------------------------------------------|
| Antibiotic            | Value (n) | %  |
| Amoxicilline+Acide    | 25/51     | 49 |
| clavulaniqueR         |           |    |
| CefoxitineR           | 15/51     | 29,5|
| CeftriaxoneR          | 25/51     | 49 |
| ImipênêmeR            | 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 idenfityed 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 = 64I)
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 hyperviscous 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.

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