International Journal of Advanced Scientific Research
ISSN: 2456-0421
Impact Factor: RJIF 5.32
www.allscientificjournal.com
Volume 2; Issue 6; November 2017; Page No. 09-12

Klebsiella pneumoniae multi-resistant to antibiotics, involved in bacterial infections in patients hospitalized in Abidjan (Côte d'Ivoire)

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Abstract
The aim of this study was to determine the resistance of *Klebsiella pneumoniae* to beta-lactams, fluoroquinolones and aminoglycosides in hospitalized patients. From January 2011 to June 2016, 350 *Klebsiella pneumoniae* were isolated from various biological products and identified by standard bacteriological tests. The antibacterial sensitivity was determined by the disk diffusion method. The production of expanded-spectrum beta-lactamase was determined by the double-synergistic method. Of the 350 strains, 91 were producing broad-spectrum beta-lactamase. Resistance levels were 90.1%, 54.9% and greater than 90%, respectively, for aztreonam, amoxicillin + clavulanic acid and for third generation cephalosporins. Imipenene and cefoxitin were the most active molecules with respectively 1% and 31.8% of the resistant strains. Concerning the classes of antibiotics quinolone and aminoside, the isolates had a resistance rate higher than 50% except amikacin (4.4%). The emergence of multiresistant *Klebsiella pneumoniae* is a real public health problem in Cote d'Ivoire.

Keywords: *klebsiella pneumoniae*, multiresistance, extended-spectrum beta-lactamases, bacterial infection

1. Introduction
The emergence and spread of multiresistant *Enterobacteriaceae* is increasingly common in the public health sector. This situation is favored and sustained by the mechanisms of resistance to antibiotics through the production of broad spectrum beta-lactamases (ESBL) in these microorganisms [1]. One of the species commonly implicated in hospital infections is *Klebsiella pneumoniae*. The main reservoirs of this species are hospital services, especially Intensive Care Units [2], but also in the environment [3]. Moreover, it is a ubiquitous microorganism. It has been associated with the expansion of nosocomial infections leading to prolonged hospitalization, increased morbidity and mortality, and consequently high costs of health care [4, 5]. Several publications have shown the involvement of this bacterium in antibiotic resistance. In Senegal, for example, Camara *et al.*, Have confirmed a high prevalence of *Klebsiella pneumoniae* ESBL with high resistance to fluoroquinolones [6]. In Côte d'Ivoire, the resistance of bacteria to antibiotics has become a real concern for nursing staff and patients, as evidenced by several publications [7, 8, 9, 10]. However very little data are available on the co-resistance to antibiotics of *Klebsiella pneumoniae* BLSE, hence our interest. The objective of this study was to assess the rate of resistance to beta-lactams, fluoroquinolones and aminoglycosides in *Klebsiella pneumoniae* ESBL in patients hospitalized from January 2011 to June 2016.

2. Material and methods
2.1 Bacterial Strains
It is a retrospective study from January 2011 to June 2016. The strains were isolated from various biological products from patients hospitalized in several health centers in Abidjan. Conventional bacteriological tests made it possible to isolate and identify the isolates of *Klebsielle pneumoniae* at the Pasteur Institute of Côte d'Ivoire (IPCI).

2.2 Antibiogram
Sensitivity to antibiotics was determined by the diffusion method of discs on agar medium (Müeller-Hinton) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [11]. The antibiotics commonly used in the treatment of ESBL-producing Gram-negative bacillus infections were selected. For example, imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), Cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), cefalotine (30 µg) cefoxitin (30 µg) tobramicin Amoxicillin-clavulanic acid (20/10 µg).

2.3 Detection of ESBL production
The double synergy method was used for the detection of *Klesbiella pneumoniae* ESBL according to Jarlier *et al.*, [12]. This consisted in placing the cephalosporin discs of 3rd generation (cefotaxime, ceftriaxone and ceftazidime) and

aztreonam at 30 mm around a central disc of amoxicillin clavulanic acid according to the recommendations of the French Committee of Antibiogramme of the French Society Of Microbiology [11]. The reference strain *E. coli* ATCC 25922 was included during the antibiograms in order to carry out the positive control.

2.4 Statistical analysis

During data processing, the duplicates were eliminated, so a patient matched à strain. The calculated resistance rates are the cumulation of the intermediate strains and the levels of the resistant strains. The data was analyzed by Graph Pad 5 software.

3. Results

Of a total of 350 strains of Klebsiella pneumoniae isolated from January 2012 to June 2016, 91 were ESBL-producing with co-resistance to aminoglycosides and fluoroquinolones. The prevalence observed for these ESBL isolates with antibiotics was 26%. The distribution of ESBL-producing strains according to biological products is shown in Figure 1. Of these biologicals, *Klebsiella pneumoniae* was elevated in urine (36.2%), followed by blood (20.8%) and pus 24.2%).

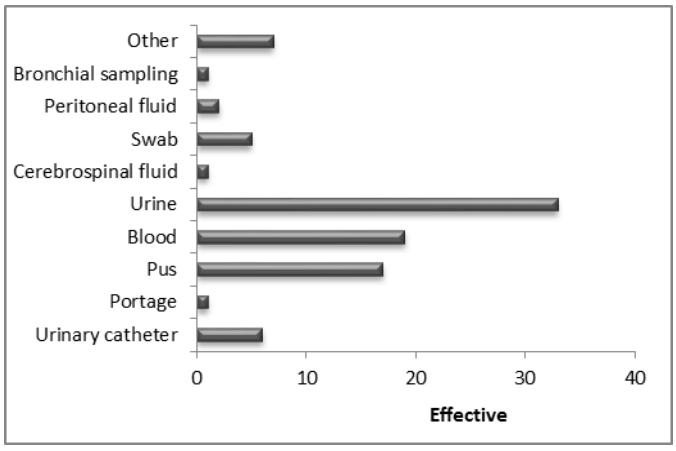

Fig 1: Distribution of strains of *Klebsiella pneumoniae* in the various pathological products.

The sensitivity of *Klebsiella pneumoniae* to the antibiotics posed is as follows.

For beta-lactams, the strains tested were all susceptible to imipenem (99%) with the exception of one strain. The resistance was 31.8% for cefoxitin, 92.3% for ceftazidin and cefotaxime, 93.4% for ceftriaxone, 100% for cefalotine, as for aztreonam, the level was 90.1% and finally 54.9% for amoxicillin + clavulanic acid.

For Floroquinolones, the rate of resistance to quinolones was 67% for ciprofloxacin, 50.5% for nalidixic acid and 73.6% for norfloxacin.

For aminoglycosides, the resistance of strains to aminoglycosides revealed that only amikacin was the most active molecule with only 4.4% resistance. As for tobramicin and gentamicin, all strains were resistant to 84.6% and 74.7%, respectively.

Fig. 2 shows the sensitivity to antibiotics of the 91 clinical strains of Klebsiella. Pneumoniae to the antibiotics tested.

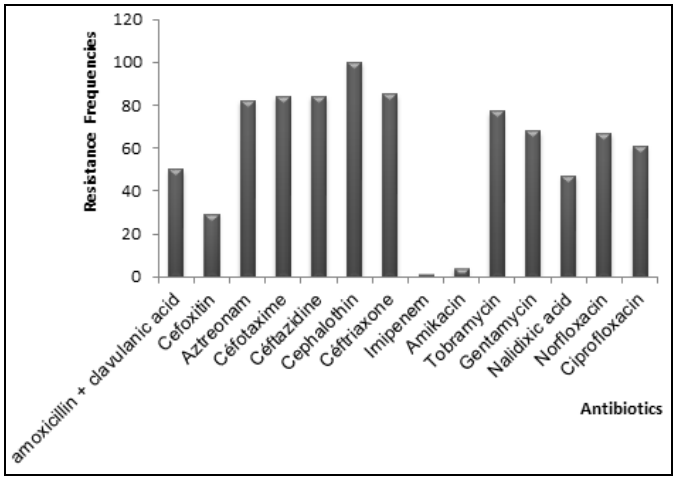

Fig 2: Antibiotic susceptibility of the 91 clinical strains of *Klebsiella*. *pneumoniae* to the 14 antibiotics tested.

4. Discussion

Enterobacteriaceae are a group of microorganisms frequently isolated from biological products following bacterial infections. *E. coli* and *Klebsiella sp* constitute the most encountered species according to Bao *et al.*, [13] and Rangaiahagari *et al.* [14]. In many studies, isolates were predominantly urine. In this study, 36.2% of *Klebsiella pneumoniae* was isolated from urine. This isolation predominance in this biological product was reported by Hashemi *et al.*, [15] in Iran with 69.3% and Raji *et al.*, [16] with 64.7% in Nigeria, respectively. However, high rates of *Klebsiella. pneumoniae* ESBL positive blood cultures have been described by some authors [17, 18]. This may be due to the fact that *Klebsiella. pneumoniae* septicemia often has a urinary tract infection (UTI). The study of the susceptibility of *Klebsiella pneumoniae* to antibiotics revealed a high level of resistance for the majority of antibiotics tested. This finding, also reported by various studies, is a consequence of the selection pressure due to the massive prescription and the often abusive use of broad-spectrum antibiotics in both hospital and community settings as well as transmission Crossing of acquired resistances to plasmid determinism [19, 20, 21]. Thus the isolates were highly resistant to third-generation cephalosporins (C3G). Indeed, more than 90 % resistance was observed, contrary to the results obtained by Hashemi which reveal a sensitivity of about 75% in 2013 [15]. Self-medication and the lack of guidelines for the management of bacterial infections would contribute to increasing levels of resistance to these antibiotics. The dissemination of bla genes (CTX-M, SHV and TEM) from one strain to another by horizontal transfer could also be evoked. Resistance to cefoxitin, which was 44.4% reported by Guessennd *et al.* [10], decreased in this study (31.8%). Certainly, a rational use of this molecule and a lack of production of cephalosporinase according to Bradford [22] would justify this rate obtained. Also a low prescription of cefoxitin or a membrane impermeability due to the alteration of porins [19] could also be one of the reasons. Imipenem and amikacin had good activity on enterobacterial strains. These results corroborate those reported by Moutachakkir [23] in a study on antibiotic resistance in uropathogenic *enterobacteria* in pediatric patients at the Marrakech University Hospital. On

the other hand, cases of resistance to Imipenem and amikacin have been described in a study by Hashemi *et al.*, Leading to increasingly limited therapeutic choices [15]. The latter found high resistance levels of 19% for imipenem and 30% for amikacin. This study revealed strong resistance to tobramicin and ciprofloxacin molecules. Resistance to aminoglycosides (aac gene) and quinolones (qnr gene) by horizontal transfer as well as the prescription of these molecules in the treatment of urinary tract infections would be the causes. The 91 strains of *Klebsiella pneumoniae* produced a broad spectrum beta-lactamase and were resistant to both the 3 families of antibiotics tested (beta-lactams, aminoglycosides and quinolones). These results reveal the presence of multi-resistant bacteria (BMR) in the city of Abidjan. This finding has been reported by Dadie *et al.*, [7] and Guessennd *et al.* [10]. Indeed, the existence of multi-resistant strains is the consequence of the selection pressure and the cross-transmission of acquired resistances to plasmid determinism [24].

The results of this study reflect the worrying increase in the frequency of antibiotic resistance in *Klebsiella pneumoniae*. In the face of these results, our hospitals and health centers should urgently adopt a number of measures to prevent the spread of this multiresistant bacterium. This is achieved through the strengthening of hospital hygiene measures, the rational prescription of Antibiotics guided by the results of the antibiogram, the screening and the technical and geographical isolation of the patients carrying a multiresistant bacterium.

5. Conclusion

This study revealed the presence of multi-resistance isolates with regard to the antibiotics tested. It also demonstrated the presence of broad spectrum beta-lactamase phenotype at the 91 strains of *Klebsiella pneumoniae*. The good sensitivity of the strains to the activity of amikacin and imipenem makes them, molecules of choice in combinations of antibiotics. The fight against bacterial resistance should integrate the rationalization of the prescription of antibiotics and strict compliance with hygiene measures in our locality. In view of these results, molecular characterization is necessary to study the different enzymes involved in resistance to these antibiotics.

6. Acknowledgements

We thank the staff of the Bio-Bank of the Institut Pasteur in Côte d'Ivoire for their support in collecting strains. This study received technical support from the Antibiotics, Natural Substances and Resistance Surveillance of Microorganisms to Anti-Infectives (ASSURMI) Unit.

7. Competing Interests

Authors have declared that no competing interests exist.

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