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Research Article

FIRST DETECTION OF CLASS I INTEGRONS ISOLATED FROM CLINICAL STRAINS OF *KLEBSIELLA PNEUMONIAE* PRODUCING BROAD-SPECTRUM BETA-LACTAMASES IN ABIDJAN (COTE D'IVOIRE)

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ABSTRACT

One of the important causes of multidrug resistance in Gram-negative bacteria is the production of extended-spectrum beta-lactamases. Antibiotic resistance genes are usually found on mobile structures: integrons. The aim of this study was to determine the prevalence of class 1, 2 and 3 integrons in strains of extended spectrum beta-lactaminase producing *Klebsiella pneumoniae*. Bacterial sensitivity was determined by the agar diffusion method. The double synergy test was applied for screening extended-spectrum beta-lactamase-producing isolates. To identify the integrating strains integrons, int1, int2 and int3 specific primers were used. 91 multidrug-resistant *Klebsiella pneumoniea* strains producing broad-spectrum beta-lactamases were collected. Antibiotic resistance rates were high for most antibiotics tested. However imipenene and cefoxitin were the most active molecules with respectively 1% and 31.8% as the resistance rate. Only class 1 integrons with a prevalence of 47.5% were identified. This observed prevalence confirms that cassettes of integrons carrying antimicrobial genes are strongly implicated in the dissemination of antibiotic resistance in Abidjan.

INTRODUCTION

Klebsiella pneumoniae, commensal pathogen and ubiquitous species, is an enteric bacterium that is implicated in a variety of nosocomial or community-based bacterial infections worldwide[1].

The irrational use of antibiotics, particularly beta-lactams, in the treatment of bacterial infections has promoted the emergence of multidrug-resistant strains that sometimes produce broad-spectrum beta-lactamases (ESBL)[2].

Originally, antimicrobial resistance was long considered to be chromosomal. But since 1980, new genetic elements that may acquire or lose genes for antibiotic resistance have been described by Stokes and Hall and referred to as integrons[5]. They are unable to self-replicate and are usually carried by plasmids and transposons. Several publications therefore argue that large-sized conjugative plasmids are responsible for the rapid spread of antimicrobial resistance in ecosystems.

In addition, integrons play a major role in the emergence and spread of antibiotic resistance by capturing resistance genes and transferring them from one DNA molecule to another or from one bacterium to another[6]. The functional platform of integrons includes an insertion site capable of inserting cassettes carrying genes. At this site, specific recombinations result in a reorganization of gene expression[7]. Once mobilized, genes can be hosted by many mobile elements other than integrons: insertion sequences (IS) such as Ecp1, IS26 and IS903[8,9]. Thus, these moving elements are therefore a major problem in the management of resistance to anti-infectives.

Frequently isolated in enterobacteria, integrons belong to a diverse family of mobile elements, but three classes in particular, classes 1, 2 and 3, are of clinical importance[10]. Integrons have been the subject of several publications[11,12,13,14].

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Moreover, in a study called dysenteriae and Shigella flexneri isolated in Senegal, Gassama *et al*.[15] had to detect class 1 and 2 integrons respectively in 26 and 16 strains. This team also observed that 14 strains harbored both class 1 and 2 integrons. In Côte d'Ivoire, this study appears to be the first of its kind. The objective of the present study is to determine the prevalence of integrons in order to obtain epidemiological data for better surveillance of antimicrobial resistance and to evaluate their role in the spread of antibiotic resistance in *Klebsiella pneumoniae* in Abidjan.

MATERIAL AND METHODS

Collection of strains

Strains of *Klebsiella pneumoniae* were collected at the Institut Pasteur biobank of Côte d'Ivoire (IPCI). These strains have been isolated from various biological products for diagnostic purposes and identified by conventional biochemical and microbiological tests at the Department of Bacteriology and Virology at the Institute. The study focused on strains preserved in brain heart broth supplemented with glycerol from January 2011 to June 2016.

Bacterial sensitivity

Antibiogramme

The susceptibility of the strains to antibiotics was determined by the method of diffusion of disks in agar medium (Müeller-Hinton) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (EUCAST-CASFM, 2016). The antibiotic discs tested are amikacin (30 µg), gentamicin (30 µg), imipenem (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), cefalotine (30 µg) cefoxitin (30 µg), tobramicine (10 µg) and amoxicillin-clavulanic acid (20/10 µg).

Confirmation of Extended Spectrum Beta-Lactamase Production

The double synergistic method was used for the detection of *Klesbiella pneumoniae* ESBL according to Jarlier *et al.*[16]. This consisted of placing the 3rd generation cephalosporin disks (cefotaxime, ceftriaxone and ceftazidime) and aztreonam at 30 mm around a central disk of amoxicillin clavulanic acid according to the recommendations of the French Committee of Antibiogram of the French Society. of Microbiology, EUCAST-CASFM[17]. The presence of ESBL is materialized by a distortion of the zone of inhibition and those with regard to the disk containing clavulanic acid, thus describing a "champagne cork" image. Only isolates of *Klebsiella pneumoniae* ESBL with resistance to aminoglycosides and fluoroquinolones were included in the study. Reference strain *E. coli* ATCC 25922 was used during antibiograms for the purpose of carrying out the positive control.

Genotyping strains of Klebsiella pneumoniae ESBL

Extraction of plasmid DNA from *Klebsiella pneumoniae* isolates and that of DH5a reference strain having plasmid pTRC99A were extracted by the alkaline lysis method with phenolyzation. The polymerase chain reaction (PCR) allowed us to detect the integrons of the different classes (int1, int2 and int3). Specific primer pairs were used to amplify the fragments of the genes encoding the enzymes (Table 1). The amplification was carried out in a volume of 50 µl with the thermal cycler (Perkin® Elmer Gen Amp Lapplied Biosystems 9700). The amplification conditions are summarized in Table 2. The reaction medium is composed of 5 µl of plasmid DNA, 0.3 U Taq polymerase (Promega), 10 µM mixture of dNTPs, 10 µM MgCl 2, 10 µM of each primer, and a 5 × PCR buffer. The PCR products were subjected to 1.5% agarose gel electrophoresis at 135 volts.

Table 1 List of primers used for detection

Genes		Sequences 5'-3'	References	Taille de l'amplicon (bp)
*intI*1	Amorce F	CCTCCCGCACGATGATC	Goldstein *et al*., 2001	**280**
	Amorce R	TCCACGCATCGTCAGGC		
*intI*2	Amorce F	TTATTGCTGGGATTAGGC	Goldstein *et al*., 2001	**233**
	Amorce R	ACGGCTACCCTCTGTTATC		
*intI*3	Amorce F	AGTGGGTGGCGAATGAGTG	Goldstein *et al*., 2001	**600**
	Amorce R	TGTTCTTGTATCGGCAGGTG		

Table 2 Conditions of gene amplification

Amplification steps	Condition / term
Initial denaturation	95 °C / 5 min
Cyclical denaturation	94 °C / 1 min
hybridization	60 °C / 1 min
Cyclic elongation	72 °C / 1 min
Final elongation	72 °C / 10 min
Number of cycles	35

RESULTS

Collection of bacterial strains

A total of 91 strains of *Klebsiella pneumoniae* ESBL were collected at the Institut Pasteur biobank of Côte d'Ivoire. The distribution of its strains by biological products reveals that *Klebsiella pneumoniae* was elevated in the urine (36.2%), followed by pus (24.2%) and blood (20.8%). The most offending hospital services were respectively the outpatient department and the pediatric ward, with respective rates of 35.2% and 14.3%.

Susceptibility of Klebsiella pneumoniae strains

The resistance of *Klebsiella pneumoniae* strains to aminoglycosides revealed high levels of resistance to tobramicin and gentamicin with respectively 84.6% and 74.7%. Only amikacin was the most active molecule with 4.4% resistance. For Floroquinolones, the ciprofloxacin resistance rate was 67%, 50.5% for nalidixic acid and 73.6% for norfloxacin. As for beta-lactams, the strains tested were sensitive to imipenem (99%) with the exception of one strain. Resistance was 31.8% for cefoxitin, 92.3% for ceftazidine and cefotaxime, 93.4% for ceftriaxone, 100% for cefalotine, and aztreonam, level a. was 90.1% and finally 54.9% for amoxicillin + clavulanic acid.

Detection of different classes of integron

The results of the PCR made it possible to detect out of the 91 analyzed strains, 43 which possess the integrons. In this study, a prevalence of 47.25% was observed. Of the three classes of integrons desired to detect, only the class 1 integrons (280 bp)

were observed. Of the 91 strains, 43 had class 1 or 47.25%. Figure 1 shows the electrophoretic profile of the class 1 integrons with respective positive bands of 280 base pairs.

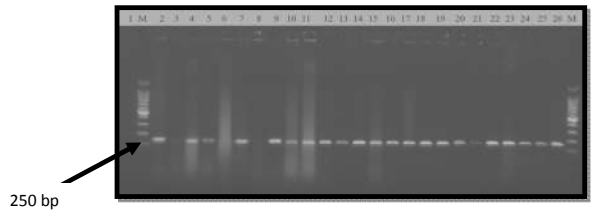

Fig 1 1.5% agarose gel electrophoresis showing simplex PCR for the detection of class 1 integrons.

Lane M: molecular weight marker (Invitrogen, 1Kb DNA Ladder). Lane 1: Negative Control Lane 2: Int1 Positive Control (280 bp) Lane 3, 6 and 8: Negative samples for int1, Lane 4, 5, 7, 9 to 26: Positive samples for int1

DISCUSSION

The spread of multidrug resistance in different ecosystems has become a major problem in the treatment of infections caused by pathogenic bacteria[17] including *Klebsiella pneumoniae*.

It has been reported according to Bao *et al*.[18] and Rangaiahagari *et al*.[19] that *E. coli* and *Klebsiella sp* are the most isolated species in bacteriology. In this report, 36.2% of *Klebsiella pneumoniae* was isolated from the urine. This observed prevalence of isolation was reported respectively by Hashemi *et al*.[20] in Iran with 69.3% and Raji *et al*.[21] with 64.7% in Nigeria. However, high levels of blood cultures positive for *Klebsiella pneumoniae* ESBL have been described by some authors[22,23]. This may be due to the fact that *Klebsiella pneumoniae* septicemia often starts with an infection of the urinary tract.

Antimicrobial resistance rates were high for most antibiotics tested. These results corroborate those of Murshed *et al*.[24] and Rezaee *et al*.[25] who reported high rates of antibiotic resistance above 60%. However, imipenene and cefoxitin were the most active molecules with 1% and 31.8% respectively of the resistant strains. The increase in antibiotic resistance may be due to the irrational use of antimicrobial agents in the treatment of bacterial infections[26]. Surveys have suggested that, regardless of the pattern of antibiotic consumption, resistance genes could be transferred between bacterial populations. Indeed, the acquisition of resistance genes by horizontal transfer is currently thought to play a major role in the development of multiresistance strains[27].

Regarding the integrons, our results indicated the presence of class 1 integrons (47.25%) only. The prevalence for this class of integron thus observed was relatively high in other surveys. For example, Jones *et al*.,[28] reported that 47% of mutipresistant isolates had class 1 and 2 integrons, whereas no class 3 was detected. Also, research by Farshad *et al*.[29] found frequencies of 25.6% for the class 1 integron and 41.10% for the class 2 integer, while Ranjbaran *et al*[30] found one study, reported the presence of class 1 and 2 integrons with respective rates of 86% and 8%. This study found a significant correlation between the presence of class 1 integrons and various resistance genes. These results demonstrate the strong association between the presence of class I integrons and the acquisition of antimicrobial resistance. Another explanation related to multidrug resistance could be a process of co-selection of various genes on cassettes of integrons selected by antibiotherapy and horizontal diffusion[31].

Thus, the presence of integrons suggests that these isolates could be a dangerous reservoir for the transmission of antibiotic resistance genes in microorganisms. Surveillance of epidemiology, as well as more in-depth study of the characteristics of these integrons are warranted to provide updated information on the current situation of resistance gene dissemination in *Klebsiella pneumoniae*.

CONCLUSION

This study detected integrons in strains of *Klebsielle pneumoniae* producing broad-spectrum beta-lactamases. The prevalence of these mobile genetic elements shows a considerable rate (47.25%) which raises concerns in the management of bacterial resistance to antimicrobials. The discovery of integrons thus serves as a basis for further study on the characterization of integron cassettes and antimicrobial resistance diffusion mechanisms.

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