

# *Klebsiella pneumoniae* isolated from street foods: characterization for extended spectrum $\beta$ -lactamases production and antibiotics resistance profile

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## ABSTRACT

In order to assess the risks associated to street foods consumption, this study aimed to characterize (phenotypic and genotypic) *Klebsiella pneumoniae* strains isolated from street foods firstly for extended spectrum  $\beta$ -lactamases production and secondly for antibiotic resistance. Three types of street foods (Russian salad, vegetable sauce and cooked rice) were investigated for this study. The sellers considered in this study are 'hawker seller', 'semi-fixed seller' and 'fixed seller'. A total of 216 foods samples are collected. After bacterial identification, their sensitivity to antibiotic was determined by the disk diffusion method. The phenotypic and genotypic characterization of *K. pneumoniae* strains that produce  $\beta$ -lactamase were made respectively by acidimetric and PCR method. This study revealed that about 20% of investigated street foods were contaminated by *K. pneumoniae*. Those *K. pneumoniae* strains producing penicillinase carried bla<sub>TEM</sub> (65%) and bla<sub>CTX-M</sub> (10%) genes while no strains carried bla<sub>SHV</sub>. And 20% of the strains tested carried together the bla<sub>TEM</sub> and bla<sub>CTX-M</sub>. Twenty seven percent of *K. pneumoniae* strains were resistant to imipenem. The presence of multi-drug resistant strains of *K. pneumoniae* in street foods shows that this bacteria was not neglected, and should deserve more attention of researchers worked in food safety.

**Keywords:** Street foods, food safety, *Klebsiella pneumoniae*, ESBL,  $\beta$ -lactamases.

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## INTRODUCTION

The African population has increased significantly during the past sixty years (from 5 in habitants/km<sup>2</sup> in 1950 to 36.7 inhabitants/km<sup>2</sup> in 2013). The expansion of African cities and changes in dietary patterns of populations have caused the development of defined street foods in recent years (FAO, 1989). The popular restoration was fairly low in the world (10%). Today, this population growth induces a growth of the socio-economic needs (Pothukuchi et al., 2008), particularly in the food sector (Signs et al., 2011).

Nowadays, local authorities, international organizations and consumers associations are increasingly aware of the socio-economic importance of street foods for sellers and health risks associated with them. The risk of food poisoning associated with foods sale in the vicinity of highways remains a very threat in many countries of the

world; microbiological contamination is one of the major problems. Lack of hygiene then food handling (Laurent, 2009), inadequate access to drinking water supply system and the elimination of wastes, as well as an unhealthy environment as the proximity of sewers and public dumps increase the risk to public health. In Cotonou and Abomey-Calavi, street foods consumption is in high expansion. According to the foregoing, street foods are today one of the main causes of foodborne resulting from the consumption of food contaminated with a harmful or pathogen microorganisms capable to produce toxins (Draper, 2008).

The food-borne infections are estimated to million annual cases in country concerned (Flint et al., 2005). There are more than 250 types of infections and

intoxications that can be caused by dozens of pathogens such as salmonella (Griffiths-Jones et al., 2006), *Staphylococcus aureus* (Attien et al., 2013), *Clostridium perfringens* and *Vibrio cholerae*, *Escherichia coli* and *Klebsiella pneumoniae* (Vincenot et al., 2008).

*K. pneumoniae* is an enteric bacterium which belongs to human commensal species (Kariuki et al., 2007). It focuses more and more the attention of researchers. However, some strains of *K. pneumoniae* can be pathogenic and cause gastroenteritis, urinary tract infections, meningitis, broncho-pulmonary infections and septicemia (Arafa et al., 2009). Pathogenicity of bacteria may be due either to the amount of bacteria ingested (Bollaerts et al., 2008), the susceptibility of the person (degree of immunity) (Flint et al., 2005) or is the resistance of strains to the antibiotics. Clinical *K. pneumoniae* strains have acquired of extended spectrum  $\beta$ -lactamases and have experienced ecological considerable success since the mid-1980. *K. pneumoniae* infections and extended-spectrum  $\beta$ -lactamases (ESBL) produced by them are an important cause of morbidity and mortality in hospital (Arafa et al., 2009). Thus, this study aims to assess the street food's degree of *K. pneumoniae* contamination, evaluated the capacity of *K. pneumoniae* strains isolated from their foods in Cotonou and Abomey-Calavi (Benin), to produce  $\beta$ -lactamase and establish the antibiotic profile of their strains.

## MATERIALS AND METHODS

### Street foods samples collection

Street foods samples were collected in four different areas (markets, student, residential and administrative) of Cotonou and Abomey-Calavi cities (Figure 1). 'Student area' very frequented by the students for their restoration, 'market area' where people goes to stock up on food crops, 'residential area' lived by rich people of Cotonou and Abomey and administrative area' where official services are implanted. These sites were randomly selected after a preliminary investigation. Three types of street foods such as Russian salad, vegetable sauce and cooked rice were collected. From each seller, the samples were taken twice a day. The first collection was made in early morning during that food is cooked freshly and the second in the afternoon when the sale is almost over. Samples were collected both in dry and rainy seasons for eight months (February to September 2014) from 54 sellers divided into 18 fixed sellers, 18 semi-fixed sellers and 18 hawker sellers. Per season, thirty-six samples of each food kind (salad, vegetable sauces and cooked rice) were collected in sterile Stomacher and then carried to laboratory in icebox at about 4°C for microbiological analysis. Thus, for the two seasons, 216 foods samples were analyzed.

### Assessment of samples

Once arrived at the laboratory, 10 g of each sample were aseptically mixed into Erlenmeyer containing 90 ml of Salt Tryptone (Bio-Rad, French) ( $10^{-1}$  dilution) and diluted serially up to a  $10^{-5}$  dilution. Then, 1 ml of each tube dilutions  $10^{-4}$  and  $10^{-5}$  were mixed with 15 ml of Plate Count Agar (~45°C) (Bio-Rad, France) and

poured in sterile Petri Dishes (Olutex Divine Concepts Ltd.). After complete solidification, a second stratum (~4 ml per box) of the agar was added before incubated at 30°C for 24 h. The colonies grown were counted (30 to 300 colonies per dish).

### Phenotypic characterization of *K. pneumoniae* strains

The selective medium used was Eosin Blue of Methylene (EMB) agar (Bio-Rad, French), 0.1 ml of suspension of each tube of previously dilutions ( $10^{-4}$  and  $10^{-5}$ ) has put aseptically into sterile Petri dishes after the melted and cooled (45°C) the EMB agar. Then, the suspension was spread on agar surface before incubated at 37°C for 24 h. *K. pneumoniae* strains are a mucoid colonies. It has 4 mm of diameter, curved, shiny, opaque and often confluent. The *K. pneumoniae* strains were confirmed using various biochemical tests such as H<sub>2</sub>S and gas production, lactose, and glucose fermentation, mobility, Vogues Proskauer and methyl red, catalase/oxidase/indole/urea production and mannitol/citrate utilization.

### Antibiotic susceptibility profile of isolated strains

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion method on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted as recommended by the AntibioGram Committee of the French Society of Microbiology (CASFM, 2012). Ten (10) antibiotics (BioMérieux, France) were used in this study such as Amoxicillin (AMX 30  $\mu$ g), Amoxicillin + clavulanic acid (AMC 20/10  $\mu$ g), Cefotaxime (CTX 30  $\mu$ g), Ceftriaxone (CRO 30  $\mu$ g), Imipenem (IPM 30  $\mu$ g), Tobramycin (TM 10  $\mu$ g), Nalidixique acid (NA 5  $\mu$ g), Ciprofloxacin (CIP 5  $\mu$ g), Gentamicin (GEN 15  $\mu$ g), Ofloxacin (OFX 5  $\mu$ g) Cotrimoxazol (COT 23.75  $\mu$ g) and Pefloxacin (PFX 5  $\mu$ g).

### Phenotypic detection of the penicillinase

The production of penicillinase by the isolated *K. pneumoniae* strains was performed by tube acidimetric method (Koneman, 2006). Six hundred milligrams of benzyl penicillin was diluted into 400  $\mu$ l of distilled water before adding 300  $\mu$ l of aqueous phenol red solution (1%, w/v) and the solution was completed with 300  $\mu$ l of NaOH (1 N). The pH of this solution was then adjusted to 8 using NaOH (1 N). The final reaction volume was 1 ml. Two young isolated *K. pneumoniae* colonies were suspended in 500  $\mu$ l of distilled water and were mixed to 150  $\mu$ l of benzylpenicillin solution. The *K. pneumoniae* ATCC 35657 strains was used as a control positif. An appearance yellow or orange color within one hour at 37°C indicates penicillinase activity.

### Phenotypic detection of producing Extended Spectrum Beta-Lactamase (ESBL)

The screening of *K. pneumoniae* strains producing ESBL on the isolated *K. pneumoniae* strains was performed by double disk synergy test (Jarlier et al., 1988; Thomson and Sanders, 1992). Indeed, the tested strains ( $10^6$  bacteria/ml) were flooded onto Mueller-Hinton according to the recommendations of the French Society of Microbiology (CASFM, 2012). The antibiotic discs used to perform this test are amoxicillin + clavulanic acid and the third generation Cephalosporins namely Cefotaxime (30  $\mu$ g) and Ceftriaxone (30  $\mu$ g). The amoxicillin + clavulanic acid disc was placed at the center of the inoculated Mueller-Hinton agar petri dish whereas the cefotaxime and ceftriaxone discs were placed at both sides (about 15 to 20 mm) of the amoxicillin + clavulanic acid disc. After incubation at 37°C for 18 h, the enhancement of the zones of

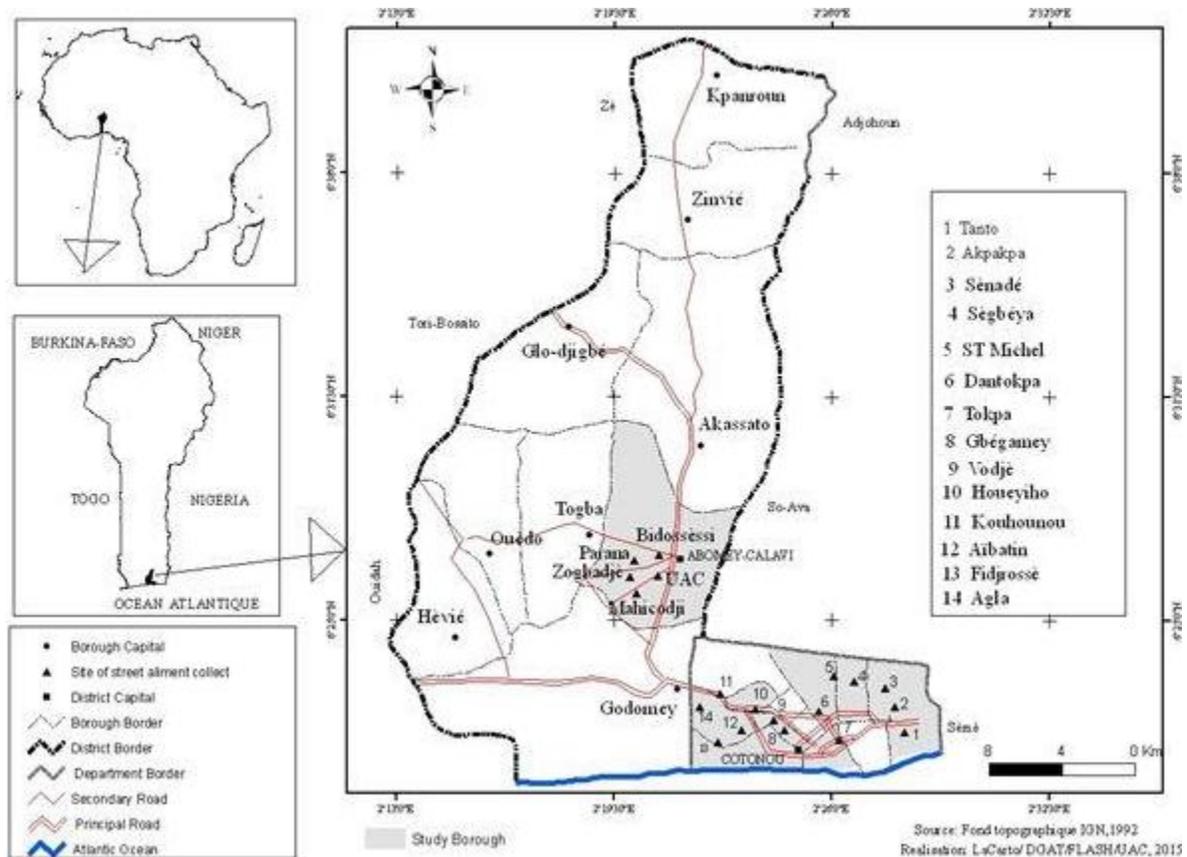


Figure 1. Map showing the collection sites of different street foods.

inhibition of any of the cephalosporin disc towards the clavulanic acid disc confirms the strains as an ESBL producer (Allouch et al., 1995).

#### Genotypic detection of Extended Spectrum $\beta$ -Lactamase (ESBL)

Polymerase Chain Reactions (PCR) was performed on total DNA of all confirmed ESBL producer *K. pneumoniae* to detect genes encoding ESBL production (TEM, SHV and CTX-M). The DNA template was extracted by suspending a loop of *K. pneumoniae* colonies in 500  $\mu$ l of sterile pure water and boiling for 10 min at 95°C. The suspension was then centrifuged for 5 min at 12,000 rpm, and 10  $\mu$ l of the supernatant were used as target DNA. DNA extracts were stored at -20°C until used.

The primers for bla<sub>TEM</sub>, bla<sub>SHV</sub> and bla<sub>CTX-M</sub> were used for multidrug resistance gene investigation by PCR amplification in 30  $\mu$ l containing for each: 5  $\mu$ l of DNA, 0.5  $\mu$ M of each primer (F and R), 1.5 mM MgCl<sub>2</sub>, 250  $\mu$ M dNTPs, 1X PCR buffer (Invitrogen) and 1U Taq DNA polymerase (Invitrogen). The PCR program used for amplification consisted i- for bla<sub>TEM</sub> (initial denaturation 94°C for 5 min followed by 30 cycles 94°C for 30 s, 52°C for 30 s, 72°C for 1 min and a final elongation step 10 min at 72°C), ii- for bla<sub>SHV</sub> (initial denaturation was performed at 96°C for 5 min, 30 cycles of 96°C for 15 s, 50°C for 15 s, 72°C for 1 min and a final elongation step 10 min at 72°C) and iii- for bla<sub>CTX-M</sub> (initial denaturation was performed at 95°C for 5 min, 35 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 2 min and a final elongation step 10 min at 72°C). The primers sequences and the expected fragments are presented in Table 1.

PCR products (10  $\mu$ l), after electrophoresis at 150 V for 30 min on a 1.5% agarose gel containing ethidium bromide, were visualized with an UV trans-illumination. A 100 bp ladder standard was used as molecular weight marker.

#### Statistical analysis

The software Microsoft Office Excel 2010 was used for processing of the data. The software Epi Info 6 version 6.04cfr January 1999 has helped to make the test of Chi-square. The test is considered statistically significant if  $p < 0.05$ .

## RESULTS

### Street foods sellers' environment

The sellers often settle street foods on the sidewalk and near stagnant water. In addition, some sellers reject wastewater and garbage in the streets close to their sale sites. After preparation, the foods are summarily covered and often arranged on tables, near busy streets and are not more heated before be served most of the time. The majority of sellers serve foods with their hands. Also, at the same time as they serve the meals they handle coins or tickets that are for the dirtiest and carriers of germs. It

**Table 1.** Primers used to search genes in this study.

|                               | Targets genes        | Primers        | Primers sequences (5'→ 3')                                  | Amplicon size (bp) | References                     |
|-------------------------------|----------------------|----------------|---|--------------------|--------------------------------|
| <i>E. coli</i> multiresistant | bla <sub>TEM</sub>   | OT-A<br>OT-B   | 5'-ATTGGGTGCACGAGTGGGTTAC-3'<br>5'-ATAATTGTTGCCGGAAGCTAG-3' | 465                | Gangoué- Piéboji et al. (2005) |
| <i>E. coli</i> multiresistant | bla <sub>SHV</sub>   | SHV-A<br>SHV-B | 5'-CACTCAAGGATGTATTGTG-3'<br>5'-TTAGCGTTGCCAGTGCTCG-3'      | 620                | Piéboji et al. (2007)          |
| <i>E. coli</i> multiresistant | Bla <sub>CTX-M</sub> | CTX-F<br>CTX-R | 5'-CGCTTTGCGATGTGCAGATT-3'<br>5'-ACCGCGATATCGTTGGTAAT-3'    | 450                | Gassama-Sow et al. (2004)      |

should be noted that sellers and some fixed semi sellers have not enough water for dishes. In addition, the packagings used are inadequate (plastic bags, paper bags of cement, etc.).

According to information collected from sellers, the foods preparation starts very early in morning (between 5 am and 6 am) in homes and commercial premises (fixed). Of the three types of sellers, hawker sellers are the first to begin their selling (from 9 am to 12 am), then semi-fixed sellers (from 10 am to 4 pm) and finally fixed sellers (from 12 am to 12 pm).

### Street foods contamination by mesophilic microorganisms

The rate of street foods contamination by mesophilic microorganisms revealed that, salad samples are more contaminated by mesophilic microorganisms ( $4.76 \times 10^6$  CFU/g) followed by rice ( $1.53 \times 10^6$  CFU/g) and vegetable sauce ( $0.27 \times 10^6$  CFU/g).

### Street foods contamination by *K. pneumoniae* strains

Globally, twenty percentages of foods samples

(216) were contaminated by *K. pneumoniae*. Forty-four strains of *K. pneumoniae* were isolated from these samples. In dry season, 26% of sampled foods are contaminated by *K. pneumoniae*. Indeed, 28 *K. pneumoniae* strains were isolated from samples (108) taken in dry season. The rate of contamination of street foods by *K. pneumoniae* in rainy season is 15%. The difference in proportion is statistically significant ( $p < 0.0001$ ).

The contamination of street foods by *K. pneumoniae* is not depended of the time of samples collection. Figure 2 shows that more than half (61%) samples collected in morning were contaminated with *K. pneumoniae* in dry season while only 39% of the samples of the evening were contaminated. Fifty percentages of foods sampled at morning and evening were contaminated by *K. pneumoniae* in rainy season. The proportion difference is statistically significant ( $p < 0.05$ ).

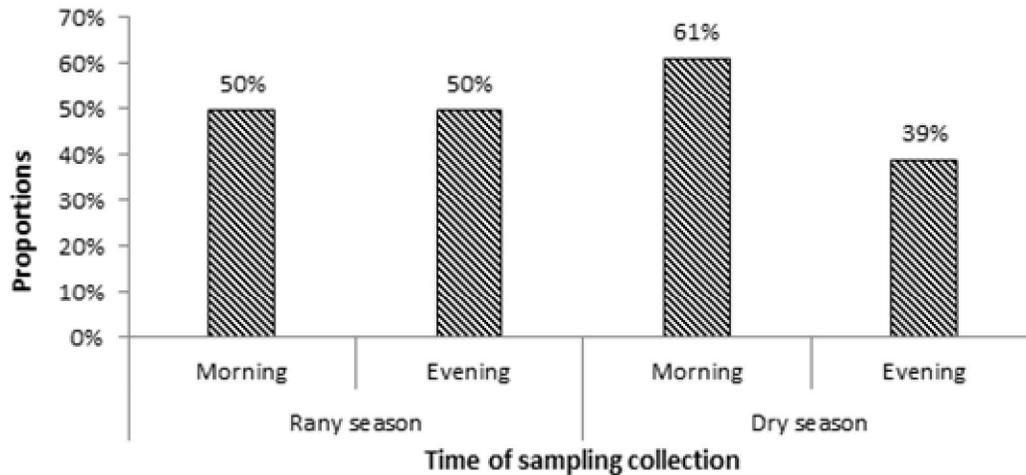
The contamination of street foods samples is variable depending on the type of sellers. Figure 3A shows that samples from transportable sellers were more contaminated (41%) followed by those of sellers (34%), and finally those of fixed sellers (25%). Street foods samples collected from fixed sellers are less contaminated than those gathered in the semi-fixed and the hawker ( $p < 0.001$ ).

In the dry season, samples from transportable sellers were more contaminated (39%) followed by those of sellers (36%), and finally those of fixed sellers (25%). Street foods samples collected at fixed sellers are less contaminated than those gathered in the semi-fixed and the hawker (Figure 3B) ( $p < 0.05$ ).

Figure 3C shows that the samples taken from semi-fixed sellers of the rainy season were most contaminated (44%) followed by those hawker sellers (31%), and finally those of fixed sellers (25%). Street foods samples collected at fixed sellers are less contaminated than those gathered in the semi-fixed and hawker ( $p < 0.05$ ).

The *K. pneumoniae* strains were isolated in the three types of sampled street foods (lettuce, rice, vegetable sauce) in different proportions (Figure 4). In rainy season, salad was most contaminated (54%) than rice (25%) and vegetable sauce (19%). In the same way, in dry season salad was the most contaminated (54%) followed by rice (39%) and vegetable sauce (7%). The difference of contamination according to types of food is significant ( $p < 0.001$ ). The degree of contamination of the street foods by *K. pneumoniae* strains depending to samples sites.

The samples collected in residential areas were the most contaminated (41%) followed by those collected in markets (23%), administrative areas



**Figure 2.** Contamination of street foods by *K. pneumoniae* according to the collection periods.

(18%) and student areas (18%) (Figure 5A). The difference in contamination proportion is statistically significant ( $p < 0.0001$ ). Figure 5B shows that the samples collected in residential areas during the dry season were the most contaminated (43%) followed to those collected in administrative areas (21%), student areas (21%) and finally to those collected in the markets (14%). Thus, it is found that the samples collected in the markets were less contaminated than those collected in the other areas ( $p < 0.0001$ ). Figure 5C shows that the samples collected in residential areas (38%) and markets (38%) were the most contaminated with those collected in student circles (13%) and administrative areas (13%) during the rainy season ( $p < 0.0001$ ).

### Susceptibility of *K. pneumoniae* strains to antibiotics

The susceptibility of *K. pneumoniae* strains (44) isolated from different street foods was variable depending to the 12 tested antibiotics (Figure 6). *K. pneumoniae* strains expressed strong resistance to antibiotics of cephalosporin's family such as CTX (77%), AMC (82%) and CRO (95%). Strong resistance was also observed with the fluoroquinolones antibiotics as OFX (88%) and CIP (91%). The most active antibiotic was IPM (27%) ( $p < 0.05$ ).

### Phenotypic and genomic detection of the penicillinase and ESBLs

Among the 44 isolated *K. pneumoniae* strains, 43.18% were penicillinase-producing. None of *K. pneumoniae* strains were expanded spectrum  $\beta$ -lactamase (ESBLs) producers.

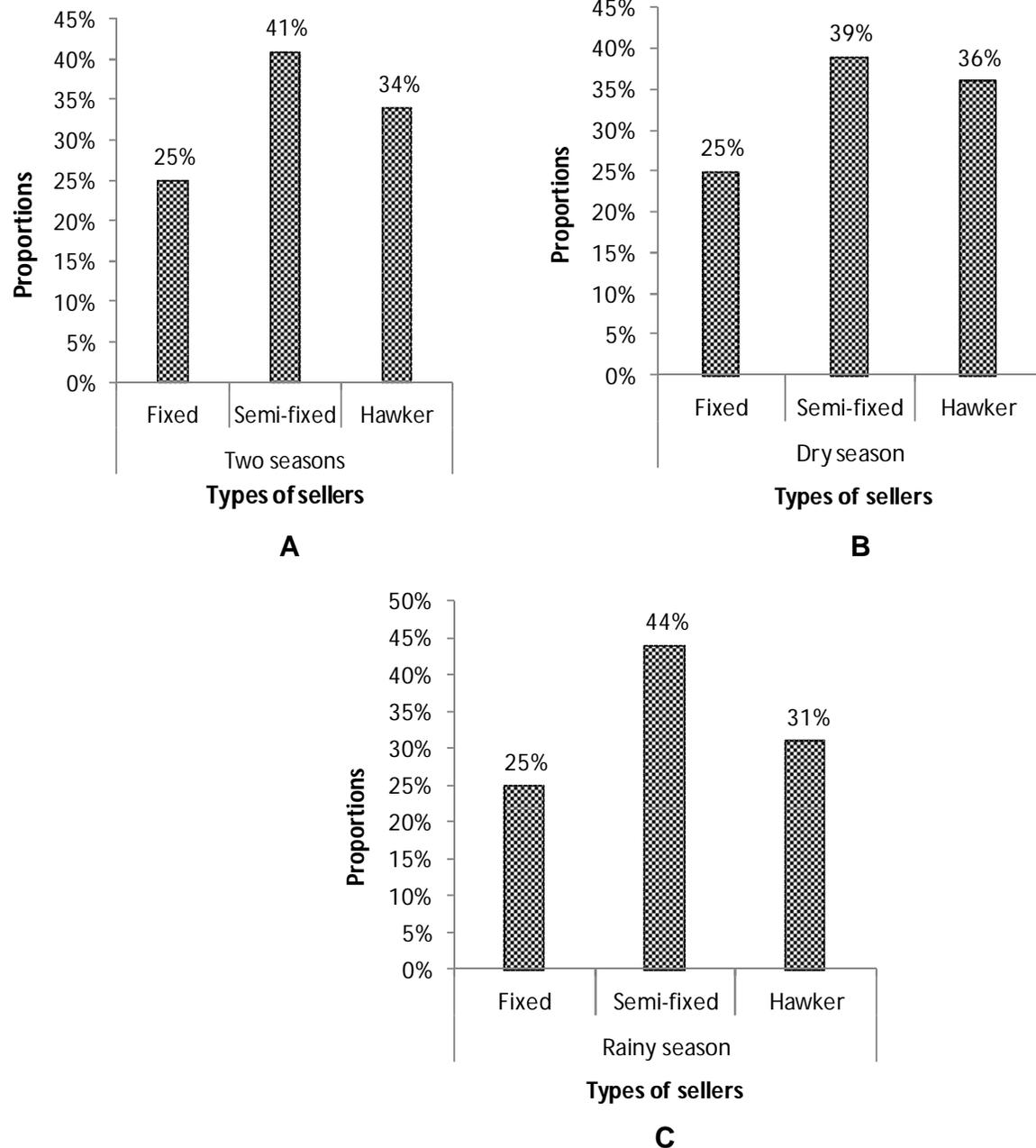
The total DNA of those strains, were used to seek the

presence or no of the  $bla_{TEM}$ ,  $bla_{SHV}$  and  $bla_{CTX-M}$  genes. This investigation displays that 65% of the tested strains carried the  $bla_{TEM}$  genes, 10% carried the  $bla_{CTX-M}$  and 20% of the strains tested carried the  $bla_{TEM}$  and  $bla_{CTX-M}$  (Table 2) and (Figure 7A, B and C). No strains carried the  $bla_{SHV}$  gene. The distribution of *K. pneumoniae* strains has considerably varied according to the season ( $p < 0.0005$ ).

### DISCUSSION

Street foods contamination comes from various sources. In this study, it was found that foods from street sellers often settled on the public sidewalks near stagnant water and garbage with foods arranged on tables, on the floor. These foods are partially covered and are not heated before served. Also, it was noticed that street foods sellers serve foods with their hands. These same hands are used to take or to give the coins or tickets that are dirty and carriers of germs. This observation has noticed by Mensah et al. (2002) to Ghana. The water often used by street foods sellers and some semi-fixed sellers is extremely dirty (Sina et al., 2011). These different practices may be induced the contamination of street foods. The lack of hygiene in the commercialization process of street foods leads to the microbial foodborne disease that can reach one or more people at a time.

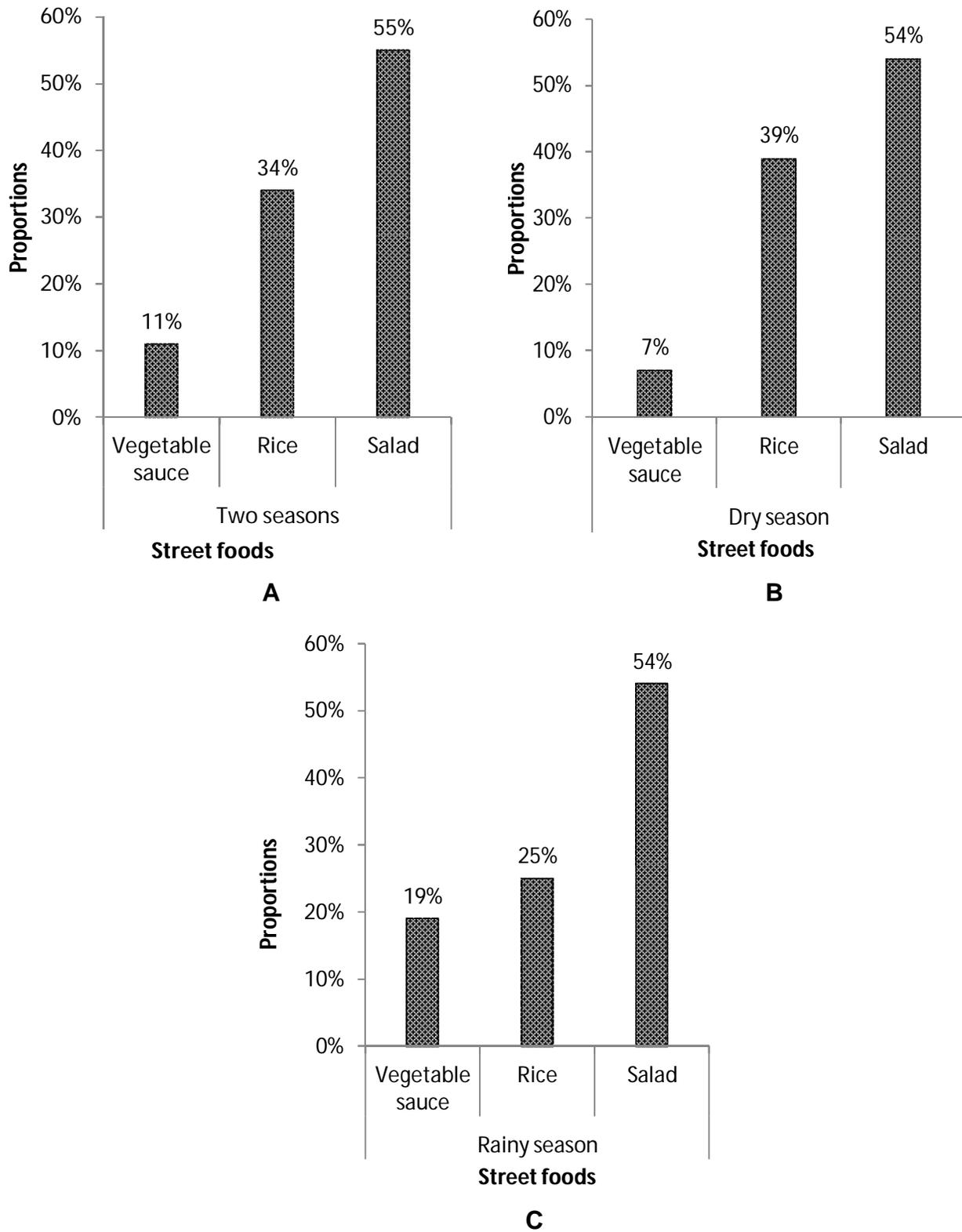
Globally, twenty percentages of investigated street foods are contaminated by *K. pneumoniae*. The contamination level depends to the season (dry or rainy). Indeed, 26% of street foods sampled in the dry season contained, *K. pneumoniae* strains while 15% of street foods sampled in rainy season are contaminated by *K. pneumoniae*. The difference of contamination rate is statistically significant ( $p < 0.0001$ ). Similarly, 66% of samples collected in the morning were contaminated by



**Figure 3.** *K. pneumoniae* contamination rates of sampled street foods according to the season and the type of sellers.

*K. pneumoniae* (Figure 2) to 34% in the evening. Also, the lowest contamination rate (Figure 3) of samples collected from fixed sellers compared to other sellers types ( $p < 0.001$ ) can say that fixed sellers put more care in the sale of foods than other types of sellers. The *K. pneumoniae* contamination rate varied according to different street foods (salad, rice and vegetable sauce) (Figure 4). In both dry and rainy seasons, the salad was the most contaminated followed to rice and vegetable sauce ( $p < 0.001$ ). This strong contamination could be

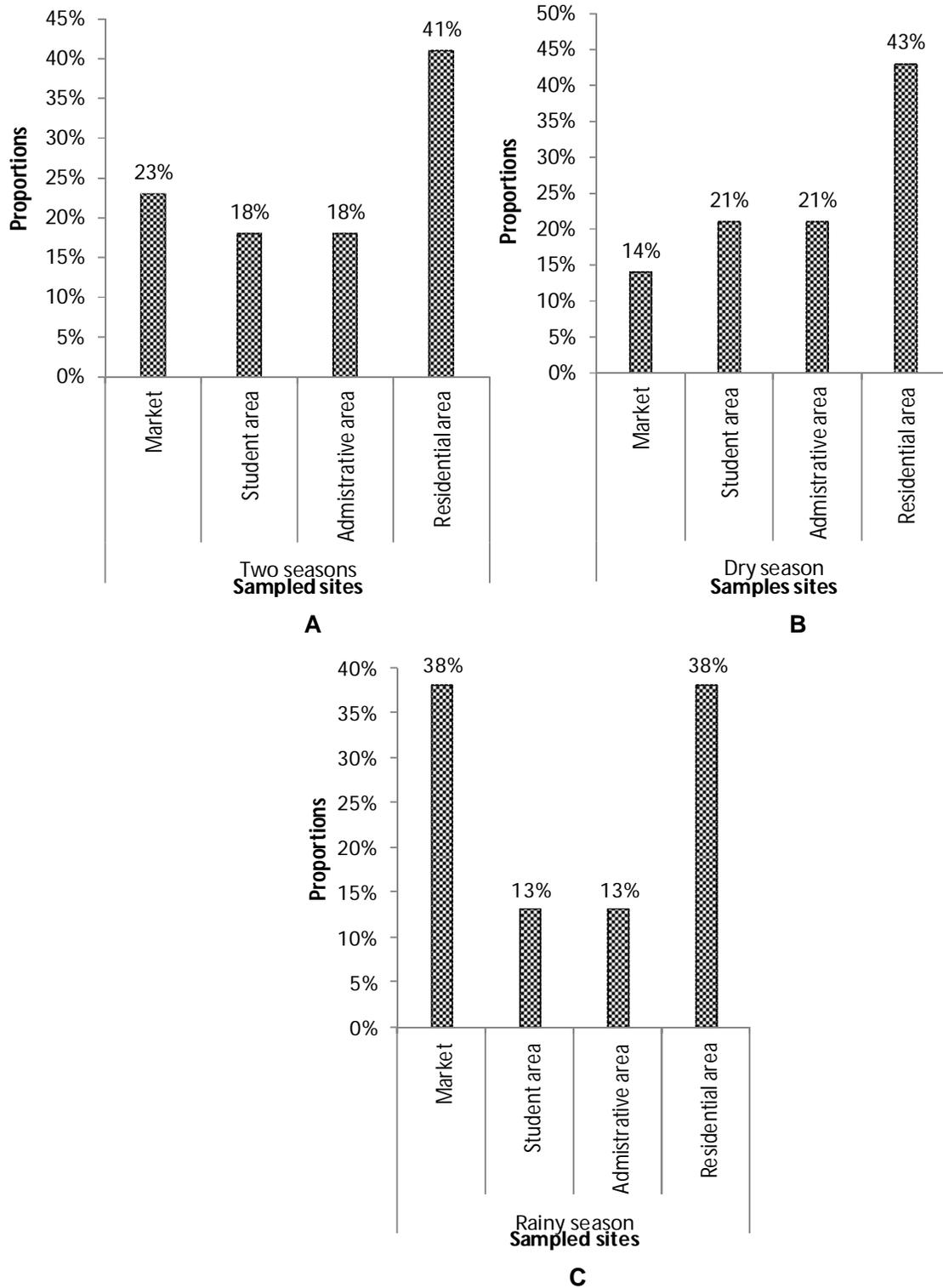
explained by the fact that vegetable sauce is a derived rawness of market gardening and does not require cooking or heating screening before being sold. Indeed, Moussé et al. (2015) had shown a strong contamination of market garden products and their watering waters by *E. coli* and other *Enterobacteriaceae* in Cotonou city. It should be noted that the growers of market garden products use the practices that induce permanent fecal contamination of watering waters. From this observation, we deduced that the strong salad contamination by



**Figure 4.** Street foods contamination by *K. pneumoniae* according to the seasons and the types of street foods.

*K. pneumoniae* could be due to the precarious hygiene conditions of lettuce. In addition it is important to consider

the fact that vegetable farmers generally use the animals manures such as poultry manure as fertilizer which would

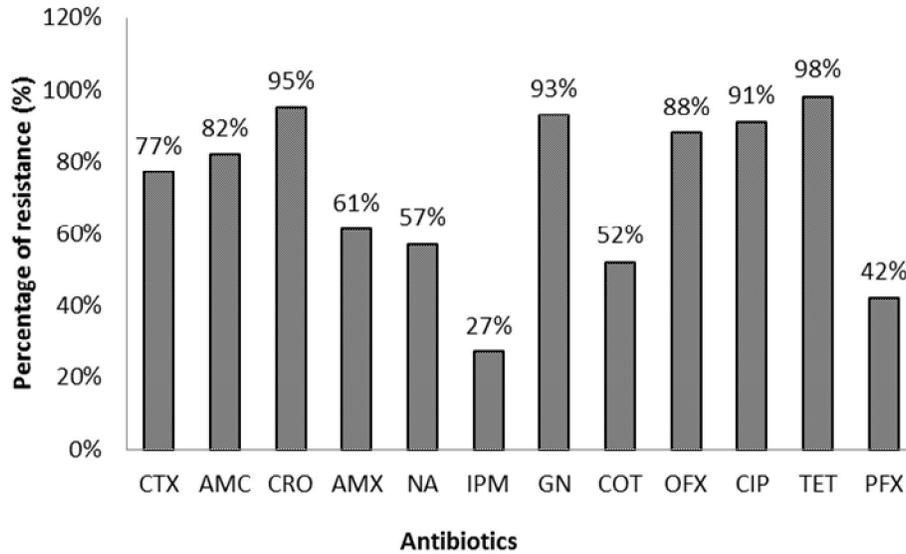


**Figure 5.** *K. pneumoniae* contamination rates of street foods collected according to seasons and sites.

a fecal permanent contamination of the irrigation water that comes from shallow wells (Moussé et al., 2015).

Fifty seven percentage of *K. pneumoniae* were produced penicillinase. It appears that the majority of the

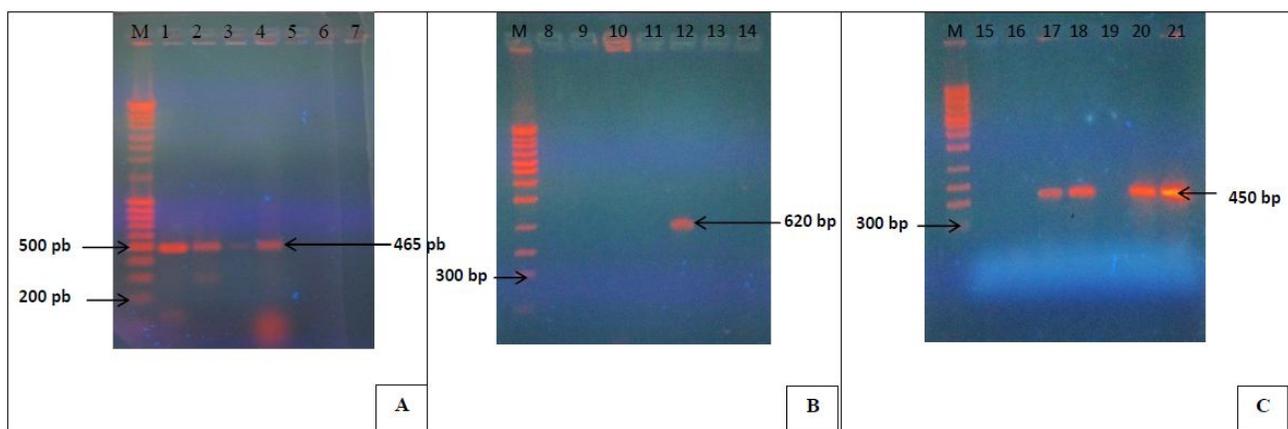
strains were resistant to penicillin. No isolated *K. pneumoniae* strains were produced expanded spectrum  $\beta$ -Lactamases. This result is different to those obtained by others authors. Lonchel et al. (2012) in Cameroon and



**Figure 6.** Resistance profile of *K. pneumoniae* strains isolated from street foods. AMX = Amoxicillin, AMC = amoxicillin/clavulanic acid, CTX = cefotaxime, NA= nalidixic acid, CRO = ceftriaxone, GN = gentamicin, IPM = imipenem, OFX = ofloxacin, CIP = ciprofloxacin, COT= cotrimozazol, TET = tetracycline and PFX = pefloxacin.

**Table 2.** Distribution of the pencillinase genes carried by strains of *K. pneumoniae* according to seasons.

| Genes                                       | Street foods     |                | Total (%) |
|---|------------------|----------------|-----------|
|   | Rainy season (%) | Dry season (%) |           |
| Bla <sub>TEM</sub>                          | 40               | 25             | 65        |
| Bla <sub>SHV</sub>                          | 0                | 0              | 0         |
| Bla <sub>CTX-M</sub>                        | 5                | 5              | 10        |
| Bla <sub>TEM</sub> and bla <sub>SHV</sub>   | 5                | 0              | 5         |
| Bla <sub>TEM</sub> and bla <sub>CTX-M</sub> | 10               | 10             | 20        |
| Total                                       | 60               | 40             | 100       |



**Figure 7.** Detection of the presence of bla<sub>TEM</sub>, bla<sub>SHV</sub>, and bla<sub>CTX-M</sub> genes. (A) Columns 1, 2, 3, 4: bla<sub>TEM</sub> positive samples; Column 7: negative control; Columns 5, 6: negative samples bla<sub>TEM</sub>; Columns M: molecular weight marker. (B) Columns 12: bla<sub>SHV</sub> positive samples; Column 14: negative control; Columns 8, 9, 10, 11, 13: negative samples bla<sub>SHV</sub>; Columns M: molecular weight marker. (C) Columns 17, 18, 20, 21: bla<sub>CTX-M</sub> positive samples; Column 15: negative control; Columns 16, 18 negative samples bla<sub>CTX-M</sub>; Columns M: molecular weight marker.

Camara et al. (2014) in Senegal had shown respectively the rate of 16 and 31.7%. Note that the previously authors were worked on clinical strains. Thus, the difference can be explained by origin of the strains involved. Indeed, the strains of clinical origin have acquired resistance against antibiotics. This resistance is due to the improper and uncontrolled use of antibiotics in developing countries, which would result in the acquisition of the resistance factors to antibiotics by microorganisms (Kolar et al., 2001). On the other hand, our strains come from the street foods that have not yet faced the misuse of antibiotics.

The isolated *K. pneumoniae* strains were highly resistant to majority of tested antibiotics (until up 98% of resistance) (Figure 6). The most antibiotic active was obtained with Imipenem (27% of resistance) ( $p < 0.05$ ). Compared to the high resistance to antibiotics of the cephalosporin family, the rates obtained in our work are higher than those obtained by Abid et al. (2007) in Algeria on clinical strains (52%) for Amoxicillin + Clavulanic, well above the 3.7 and 8.8% acid found by Mathai et al. (2001) in America and also highly superior compared to the work of Arafa et al. (2009) where only 5% of the strains were resistant to Ceftriaxone. Also, regarding to the strong resistance of *K. pneumoniae* strains to antibiotics of fluoroquinolones family, our results are strongly higher than those obtained by Arafa et al. (2009) in Algeria that are 0% for ciprofloxacin (CIP). In this study, Imipenem are still active against strains of *K. pneumoniae* studied with a resistance of 27% rate. This rate is higher than that found by Abdallah et al. (2008) in Tunisia (19.6%) and in France by Amin et al. (2009), who have found a rate of 7.5% resistance to this antibiotic.

The *K. pneumoniae* strains isolated in the dry and rainy seasons have the bla<sub>TEM</sub>, bla<sub>SHV</sub> and bla<sub>CTX-M</sub> genes. Only bla<sub>SHV</sub> was not carried by the strains isolated in the dry season. In the clinic, the higher rate reached 95.45% for the presence of the bla<sub>TEM</sub>, bla<sub>SHV</sub> and bla<sub>CTX-M</sub> genes observed in the *K. pneumoniae* produced by ESBLs in some studies (Gazin et al., 2012; Gharout-Sait et al., 2012; Messai et al., 2008; Ramdani-Bougouessa et al., 2011). This difference in proportion may be attributed to the strains in this study come mainly from foods samples. The prevalence of *K pneumoniae* strains producers the  $\beta$ -lactamases SHV compared to those of TEM type was also noted recently in European and American studies and more recently in Far East countries (Chanawong et al., 2001). It is confirmed more and more that the SHV genes are ubiquities at *K. pneumoniae* (Babini and Livermore, 2000). However, previous investigators have shown that amino acid substitution at this position is important in the development of the ESBL phenotype in TEM  $\beta$ -lactamases (Petit et al., 1995). In this study, 20% of *K. pneumoniae* strains carried simultaneously bla<sub>TEM</sub> and bla<sub>CTX-M</sub>. CTX-M-type  $\beta$ -lactamases are the ESBLs most commonly produced by isolates in Algerian hospitals (Messai et al., 2008; Ramdani-Bougouessa et al., 2006), as in isolates from our study. However, their

co-production with other  $\beta$ -lactamases is frequent, such as SHV and/or TEM, conferring resistance phenotypes which are additive, with the respective consequences at clinical and therapeutic levels (Paterson and Bonomo, 2005; Ramdani-Bougouessa et al., 2006).

Food safety can help to prevent and improve the population's health, the work performance and thus contribute to reduce the poverty by increasing incomes. The cleaning-up remains the best solution against infectious diseases especially in developing countries. In Benin, the population eats a lot of street foods which is an accessible source of food. In addition, street foods are cheap, varied and available everywhere. This sector is beyond the control of the health authorities and thus is potential sources of poisoning.

This study allowed characterizing *K. pneumoniae* strains isolated from three types of street foods (salad, rice, vegetable sauce). The contamination levels are varied according to the foods types, sales locations and seller's types. These contamination rates are factors that will contribute to maintain the poverty. It is necessary to develop the quality standards for street foods in Benin for a sustainable development. With high resistance rates, prescribing of antimicrobial treatment to the patient should be avoided before identifying the pathogenic microorganisms.

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