



# **Evolution of the Resistance Profile of Klebsiella Species to Antibiotics Over Ten Years at the Yaoundé University Teaching Hospital, Cameroon**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study aimed to study the resistance profile of *Klebsiella* species to antibiotics, highlighting their resistance phenotypes at the Yaoundé University Teaching Hospital (YUTH).

**Study Design:** We carried out a retrospective and prospective study.

**Place and Duration of Study:** Bacteriology unit, Yaoundé University Teaching Hospital, from January 2010 to December 2020.

**Methodology:** Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusions method on Muller Hinton agar according to the AST committee of the French Society of Microbiology (CASFM, 2020). The data was digitized and analyzed using descriptive statistics to understand the antibiotic resistance patterns of *Klebsiella* species.

**Results:** During this study, we identified 589 strains of *Klebsiella* species. *Klebsiella pneumoniae pneumoniae* was the most frequent sub-specie identified (64.69%). Blood and urine specimens were the samples that were frequently encountered. Children under 10 and adults over 60 were the most infected groups of patients. The majority of the samples analysed came respectively from the Intensive Care Units (21.22%), Paediatrics (14.60%), and Medicine (12.56%). The *Klebsiella* species isolated showed a high resistance rate to beta-lactams (72%), phenolics (62.30%), and quinolones (60.41%). Over the years, we have observed a gradual decline in the resistance rate to aminoglycosides, going from 87.80% in 2010 to 11.11% in 2020 with amikacin, which remains the most active. Resistance to colistin, which is one of the last therapeutic resort antibiotics, showed very alarming resistance rates of 63.63% with low rates in 2016 (0%) and 25% in 2018. *Klebsiella* species retained good sensitivity to the piperacillin-tazobactam, as well as to imipenem and nitrofurantoin with low resistance rates for these last two antibiotics (8.33% and 15% respectively).

**Conclusion:** Based on our findings, which indicate a significant level of resistance among *Klebsiella* species to commonly prescribe antibiotics, it is crucial for both the health authorities in Cameroon and specifically at the YUTH to take prompt actions.

**Keywords:** Evolution of resistance; nosocomial infections; Yaoundé.

## 1. INTRODUCTION

Bacterial infections contracted both in hospitals and in the community are a serious public health problem (HiNordmann and Carrer, 2010). These infections are mostly due to Gram-negative bacilli which nowadays are increasingly incriminated in the phenomena of multi-drug resistance to antibiotics. The multi-resistance of *Klebsiella* species to antibiotics has experienced a worrying worldwide evolution in recent years (Grall et al., 2011). It has been shown that the presence of *E. coli* and *Klebsiella* species in vaginal cervical specimens from pregnant women increases the risk of preterm birth (Longla et al., 2013). *Klebsiella* species are implicated in many cases of hospitalization and many strains are resistant to antibiotics. Respiratory (pneumonia, lung abscess, pleurisy), and intestinal and urinary infections are other pathologies resulting from infection with *Klebsiella* species.

Since the 1980s, third-generation cephalosporins (3GC) have been among the drugs used for the treatment of severe infections due to Enterobacteriaceae. Rapidly, Extended-Spectrum  $\beta$ -lactamases (ESBL) enzymes with

plasmid transmission, responsible for resistance to penicillins, cephalosporins, and monobactams developed (Colodner, 2005). Carbapenems, thanks to their broader spectrum of activity, were until recently the only alternative to 3GC resistance. This resistance may be due to chromosomal mechanisms, the association of resistance mechanisms, or the production of enzymes hydrolyzing carbapenems. The last mechanism is the most worrying because the genes coding for these enzymes are usually located on mobile genetic elements (plasmids, transposons, integrons) allowing rapid dissemination (Gangoue-Pieboji et al., 2015). To overcome *Klebsiella* infections, several antibiotics are recommended with less and less success. This resurgence of failure to treat *Klebsiella* infections is mainly due to its particular ability to acquire mechanisms of resistance to usually active antibiotics. Better knowledge of the epidemiology of resistance will improve patient care. This can only be done by the monitoring of the evolution of resistance (Longla, 2013).

To carry out this monitoring, many works both on a global and African scale have been carried out. In Cameroon, the work of Mbakop et al. (2012) at

the YUTH revealed that the rates of resistance to first-generation cephalosporins [1GC] (cefalotin), second-generation cephalosporins [2GC] (cefoxitin/ cefuroxime), third-generation cephalosporins [3GC] (ceftazidime), fourth-generation cephalosporins [4GC] (cefepime), monobactam (aztreonam) and carbapenem (imipenem) were respectively 84%, 52%, 45%, 50.3%, 38% and 12% (Mbakop et al., 2006). Betbeui *et al.*, (2015) found respective resistance rates of 85%, 55%, 54%, 56%, 51%, and 1% (Betbeui et al., 2015). Lyonga *et al.* (2019) found respective resistance rates of 86.5%, 83.8%, 78.4%, 78.4%, 76.7% and 2.7% (Mbamyah et al., 2021). Given the fact that the sensitivity or resistance of germs to antibiotics is a variable factor depending on time and space (Crude et al., 2001) we proposed to carry out a study over a broader period from January 2010 to December 2020, on the evolution of resistance of *Klebsiella* species to antibiotics at the YUTH.

## 2. MATERIALS AND METHODS

This study gathered retrospective data from January 2010 to June 2020 (10 years 6 month), and a prospective study from July to December 2020 (6 months) that took place in the bacteriology laboratory of YUTH. We recruited 589 patients with 534 strains from the retrospective study and 55 strains from the prospective study. The *Klebsiella* strains from clinical specimens of patients were isolated and identified using the API 20e biochemical gallery (Biomérieux SA, Lyon, France). The antibiotics used were those recommended by the AST committee of the French Society of Microbiology (CA-SFM-2020). The sensitivity of *klebsiella* isolates to antibiotics was determined by the disc diffusion method on Mueller Hinton agar. The antibiotics tested were chosen from the list of antibiotics to be tested for Enterobacteriaceae (Société, 2021). The interpretation criteria were those recommended by the CA-SFM and the interpretative reading of the AST made it possible to highlight the different phenotypes expressed by *klebsiella* species.

The antibiotics studied (Biorad, California, USA) for all the strains isolated were as follows: amoxicillin (25µg), amoxicillin + clavulanic acid 25/10µg), ticarcillin (75µg), ticarcillin + clavulanic acid (75/10µg), piperacillin (100µg), piperacillin-tazobactam (100/10µg), cefalotin (30µg), cefoxitin (30µg), cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), imipenem (10µg) and ertapenem (10µg). A

double synergy test was used to confirm the production of extended-spectrum β-lactamases. It was done by placing the cefotaxime, cefepime and aztreonam discs 15mm to the amoxicillin + clavulanic acid disc (Yimtchi et al., 2023). The data obtained were then digitised using the Census and Survey Processing System (CSPRO) 7.1 software. The Data was exported from the database into R software. Data was analyzed using descriptive statistics; this included the use of tables, graphs and percentages to explain the results.

## 3. RESULTS

### 3.1 Clinical Information

The majority of the *Klebsiella* species were isolated from patients aged under 10 years (24.31%) followed by patients aged 50-59 years (13.63%). The majority of these species came from the intensive care units (21.22%) followed by paediatrics (14.60%), and medicine (12.56%) units. The unit where the least *Klebsiella* species was recorded was the ENT service (0.34%). Concerning the source of isolation (type of specimen), most of the *Klebsiella* species were isolated from blood (31.75%) and Urine (25.30%) samples. The least represented samples were vaginal secretions and puncture fluids which represented respectively 2.72% and 3.23% (Table 1).

### 3.2 Distribution of *Klebsiella* species

Of the 589 *Klebsiella* species isolated, 64.69% (n= 381) were *Klebsiella pneumoniae pneumoniae*, 14.26% (n=84) *Klebsiella pneumoniae ozaenae*, 9.0% (n= 53) *Klebsiella oxytoca*, 8.83% (n= 52) *Klebsiella pneumoniae rhinoscleromatis*, and 0.68% (n= 4) *Klebsiella ornithinolytica*. A total of 15 (2.55%) of the isolates were not identified. This is illustrated in the Fig. 1.

### 3.3 Evolution of the Overall Resistance of *Klebsiella* species

#### 3.3.1 Evolution of the resistance rate of *Klebsiella* species by antibiotic family

Fig. 2 shows, the isolated *Klebsiella* species had a high resistance rate with an average of 72% over the years against Beta-lactams. Regarding the Quinolones, the rate of resistance fluctuated over the years. Although during the years 2014, 2016, and 2017, resistance rates remained

below 50%, however, resistance has increased permanently from 2018 to 2020. We recorded a decrease in the resistance rate against aminoglycoside over the years.

### 3.3.2 Evolution between 2010 and 2020 of the antibiotic resistance rate of *Klebsiella* species

After analyses of the cumulative resistance rate per year, It was noted that the resistance rate of *Klebsiella* species, against antibiotics increased progressively from 2010 (68.13%) until 2012

(75.06%). In addition, 2014 recorded the lowest resistance rate, which stood at 49%. Finally, there was an increasing trend in the resistance rate from 2016 (59.80%) to 2020 (71.11%) (Fig. 3).

### 3.4 Resistance Profile of *Klebsiella* Species to Families of Antibiotics

Fig. 4 allows us to assess in aggregate the resistance over the entire study period of *Klebsiella* species to the 6 families of antibiotics. It was seen that the family to which *Klebsiella*

**Table 1. Clinical information of the isolated *Klebsiella* species from the period of January 2010 to December 2020, at the YUTH**

| Variables                | Frequency  | Percentage (%) |
|--------------------------|------------|----------------|
| <b>Age group (years)</b> |            |                |
| 0-9                      | 132        | 22.41          |
| 10-19                    | 28         | 4.75           |
| 20-29                    | 54         | 9.17           |
| 30-39                    | 55         | 9.34           |
| 40-49                    | 53         | 9.00           |
| 50-59                    | 74         | 12.56          |
| 60-69                    | 71         | 12.05          |
| 70-79                    | 51         | 8.65           |
| ≥80                      | 25         | 4.24           |
| No information           | 46         | 7.81           |
| <b>Total</b>             | <b>589</b> | <b>100.0</b>   |
| <b>Department/ Units</b> |            |                |
| Outpatient               | 45         | 7.64           |
| Paediatrics              | 86         | 14.60          |
| Gynaecology              | 25         | 4.24           |
| Emergency                | 51         | 8.66           |
| Medicine                 | 74         | 12.56          |
| Surgery                  | 42         | 7.13           |
| Neonatology              | 35         | 5.94           |
| ENT                      | 2          | 0.34           |
| General intensive care   | 125        | 21.22          |
| Surgical reanimation     | 24         | 4.07           |
| No info                  | 77         | 13.07          |
| Other                    | 3          | 0.51           |
| <b>Total</b>             | <b>589</b> | <b>100.00</b>  |
| <b>Samples</b>           |            |                |
| Blood                    | 187        | 31.75          |
| Urine                    | 149        | 25.30          |
| Pus                      | 80         | 13.58          |
| Vaginal secretions       | 54         | 9.17           |
| Puncture fluid           | 50         | 8.49           |
| Venous catheter tip      | 19         | 3.23           |
| Urinary catheter tip     | 16         | 2.72           |
| No information           | 16         | 2.72           |
| Other                    | 18         | 3.06           |
| <b>Total</b>             | <b>589</b> | <b>100.00</b>  |

species were the most resistant was that of beta-lactams with a resistance rate of 95.59%. It was followed by antibiotics from the amphenicol family, with an overall resistance rate of 62.30%. A low resistance rate was recorded against polymixin (46.89%) and aminoglycoside (53.00%) families.

### 3.5 Evolution between 2010 and 2020 of the Resistance Rate of *Klebsiella pneumoniae pneumoniae*

*Klebsiella pneumoniae pneumoniae* displayed very high resistance to beta-lactams throughout the study period reaching 100%. However, we observed a gradual decrease in resistance from 2017 to 2019 then a rise to 100% in 2020. The resistance of *Klebsiella pneumoniae pneumoniae* against tested quinolones has been fluctuating through the years with the highest resistance reaching 82.35% in 2012. Resistance to aminoglycosides declined gradually over the years (Fig. 5).

## 4. DISCUSSION

From the study that took place in the bacteriology laboratory of YUTH. We recruited 589 patients with 534 strains from the retrospective study and 55 strains from the prospective study.

### 4.1 Clinical Information

The majority of Most (24.31%) of the patients were children under 10 years old, followed by patients in the age group of 50-59 years who represented 13.63% of the sample. These results are similar to that of Nirwati *et al.*, in Indonesia who had isolated more *Klebsiella* species strains in patients in the 60-year-old age group (38.10%). Given the fact that patients under the age of 5 and those aged 60 most often have a fragile immune system, this would explain why these age groups are the most represented in our study.

The majority of the patients (73.85%) were hospitalized, while 16.89% were community patients. According to the study by Ebongue *et al.*, on the evolution of antibiotic resistance of Enterobacteriaceae isolated at the Douala General Hospital, the majority of strains came from hospitalized patients, accounting for 58.39%. This difference could be explained by the fact that we were particularly interested in *Klebsiella* species and not in all

Enterobacteriaceae; moreover, this bacterium is most often indexed as being a germ with nosocomial transmission, which could explain the tendency to encounter it in hospitalized patients.

During the study, the majority of samples analysed came from the general Intensive Care Unit (21.22%), paediatrics (14.60%), and medicine (12.56%). These results are similar to those of Müller-Schulte *et al.*, in Ivory Coast, who obtained most of their samples from the paediatric department (29%), internal medicine (24%), and intensive care (14 %). As well as those obtained during a study carried out in Cameroon (Betbeui *et al.*, 2015) revealing a predominance in the units of Paediatrics (48.4%) and medical reanimation (21.3%). This may be due on the one hand to an increased and abusive use of antibiotics or an insufficient application of hygiene rules in these departments and, on the other hand, to a high concentration of patients and caregivers in these intensive care settings that can lead to transmission of the germ between patients (Mirabaud, 1996).

The strains of *Klebsiella* species were mainly isolated from blood (31.75%) followed by urine (23.50%) and suppuration (13.58%). Eloïse Müller-Schulte also found in their study that the majority of samples were blood (39%) and urine (39%) (Müller-Schulte *et al.*, 2014). However, these results are different from those obtained by Ebongue *et al.*, where the most frequent of the samples in their study was urine (68.7%) followed by suppuration (13.5%). Though these results differ, it is clear that in the majority of cases, urine samples appear most often. This can be explained by the fact that *Klebsiella* species is found to be a bacterium of the intestinal commensal flora and can easily be found in the urinary tract following poor hygiene, particularly in women given their genital anatomy. The *Klebsiella* species were isolated from patients aged under 10 years (24.31%) followed by patients aged 50-59 years (13.63%). The majority of these species came from the intensive care units (21.22%) followed by paediatrics (14.60%), and medicine (12.56%) units. The unit where the least *Klebsiella* species was recorded was the ENT service (0.34%). Concerning the source of isolation (type of specimen), most of the *Klebsiella* species were isolated from blood (31.75%) and Urine (25.30%) samples. The least represented samples were vaginal secretions and puncture fluids which represented respectively 2.72% and 3.23% (Table 1).

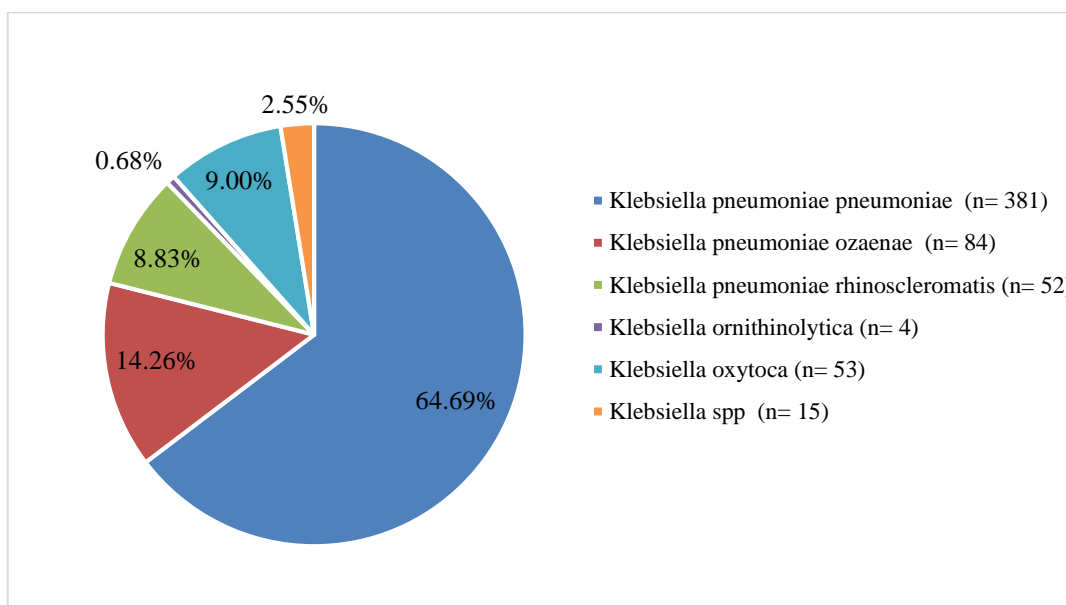


Fig. 1. Distribution of *Klebsiella* species identified

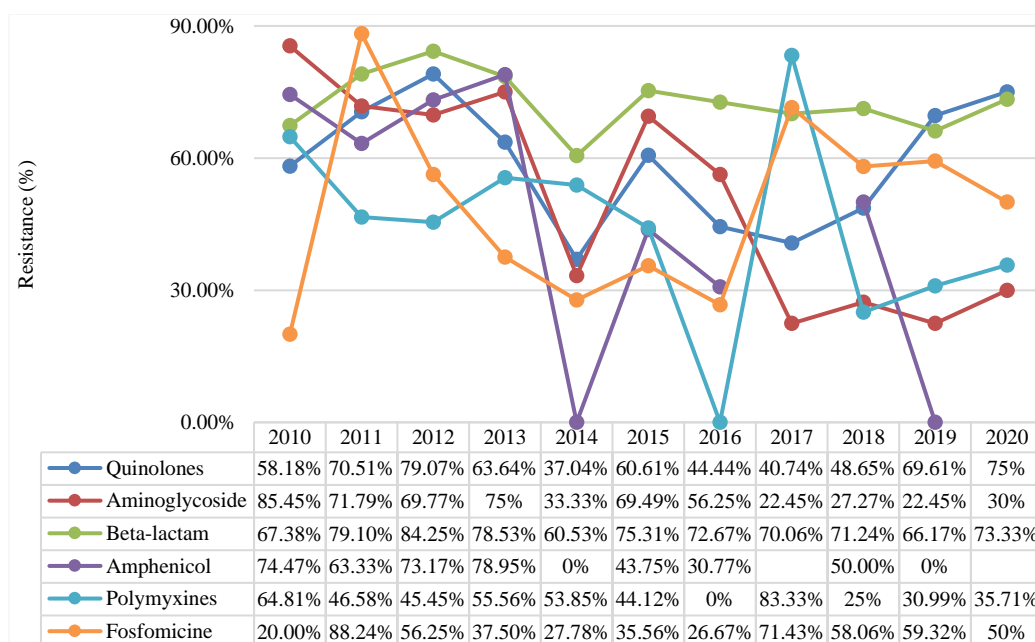


Fig. 2. Evolution of the overall resistance rate of all *Klebsiella* species by antibiotic family

#### 4.2 Evolution of the Overall Resistance of *Klebsiella* Species

We noted that the resistance of *Klebsiella* species to antibiotics was on the increase from 2010 until 2012 when it reached 75.06%. However, 2014 recorded the lowest resistance rate, at 49%. Finally, we noted that since 2016, this resistance rate has been on an upward trend, reaching 71.11% in 2020. This study

allows us to assess the resistance in an aggregated way over the entire period of study of *Klebsiella* species with 6 families of antibiotics. As observed, the family for which *Klebsiella* species are by far the most resistant are the beta-lactams with a resistance rate of 95.59%. It is followed by antibiotics from the amphenicol family, the overall resistance rate of which is 62.30%, and quinolones (60.41%).

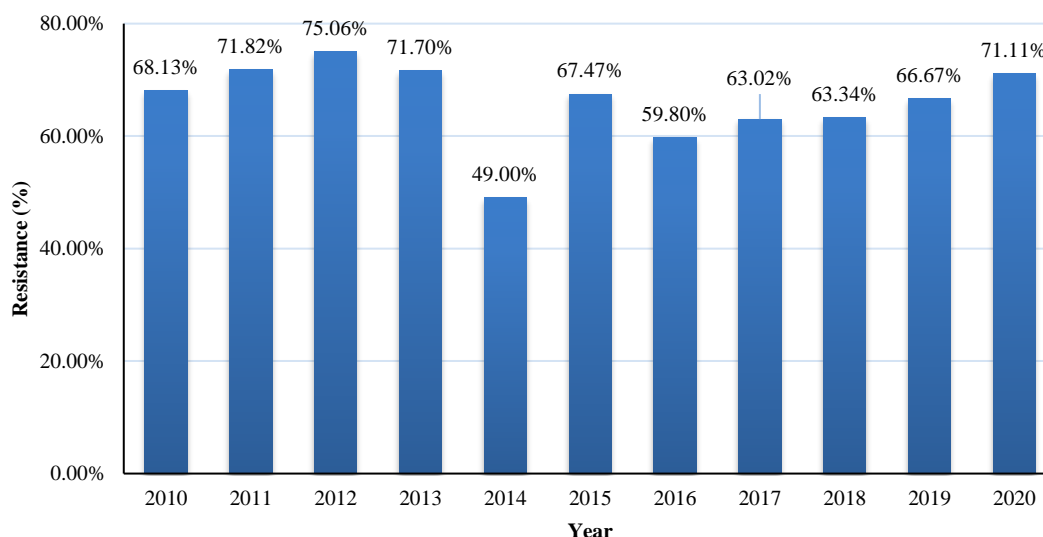


Fig. 3. Evolution between 2010 and 2020 of the antibiotic resistance rate of *Klebsiella* species.

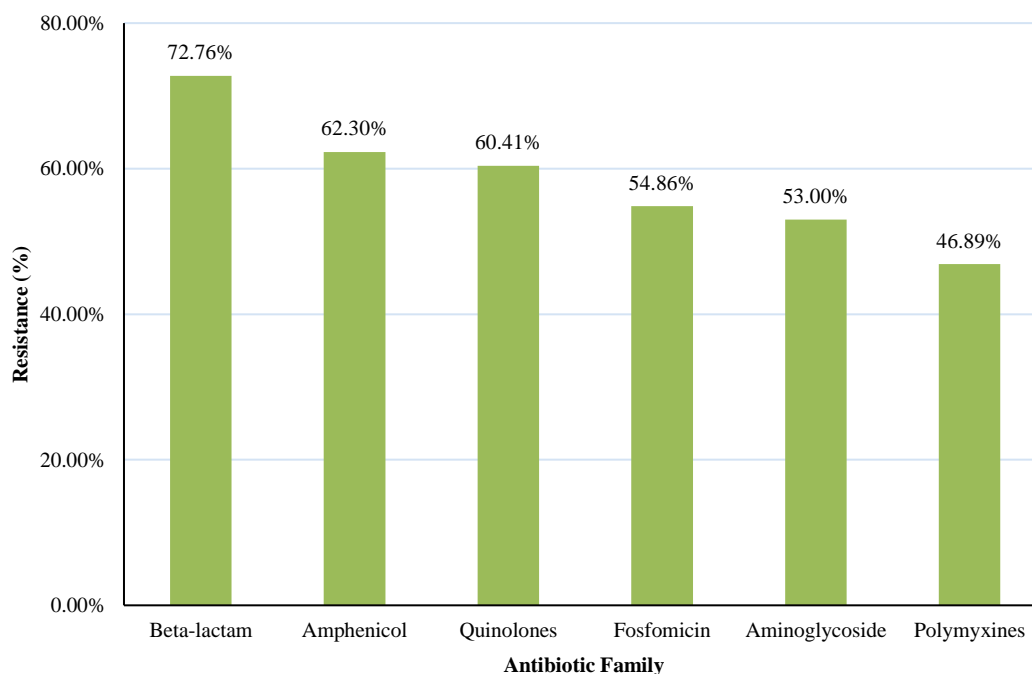
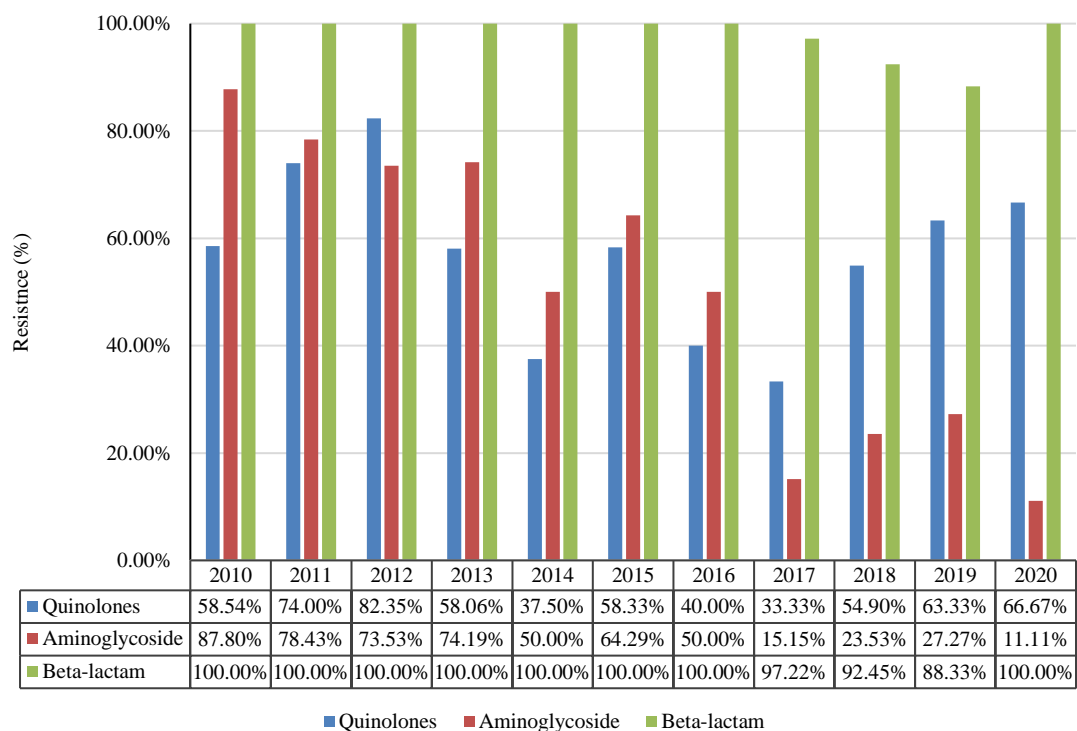


Fig. 4. Resistance profile of *Klebsiella* species to families of antibiotics.

#### 4.2.1 Beta-lactams

Over the years, the resistance rate of *Klebsiella* species to Beta-lactams increased. *Klebsiella pneumoniae pneumoniae* displays very high resistance to beta-lactams throughout the study period (72%). Our results are similar to those of Vasaikar et al., (2017) in South Africa

which showed resistance rates of 60% with a maximum rate of 82.7% against antibiotics of the classes penicillins, cephalosporins, monobactam. This high resistance could reflect the fact that this family of antibiotics were the most used to deal with infections caused by *Klebsiella pneumoniae pneumoniae*.



**Fig. 5. Evolution between 2010 and 2020 of the resistance rate by families of antibiotics (quinolones, aminoglycosides, beta-lactams) of *Klebsiella pneumoniae pneumoniae***

#### 4.2.2 Quinolones

We recorded a high level of resistance to beta-lactams followed by antibiotics from the amphenicol family (62.30%) and quinolones (60.41%). This result, although different in terms of rates, has the same trend as that of Tlamçani *et al.*, in Egypt (Tlamçani., 2009) who found that 33% of *Klebsiella* species were resistant to quinolones and also in Cameroon by (Lyonga *et al.*, 2015) who found the resistance of *Klebsiella* species to quinolones (29.7%) in the city of Yaoundé. This class is the next after beta-lactams to be prescribed against Enterobacteria infections. In addition, certain antibiotics appear in empiric antibiotic protocols to deal with emergencies while awaiting the results of the AST. All this, coupled with the screaming phenomenon of self-medication in our context, could explain the increasing frequency of resistance due to the excessive consumption of these antibiotics.

#### 4.2.3 Aminoglycosides

*Klebsiella pneumoniae pneumoniae* displayed very high resistance to beta-lactams, while

resistance to aminoglycosides generally declined gradually. Over the years, we observed a gradual decline in the resistance rate, passing from values greater than or equal to 87.80% in 2010 to 11.11% in 2020 for the different species of *Klebsiella*. This downward trend in the resistance rate is in line with the results of the study carried out by (Soh *et al.*, 2021) who showed a resistance rate of 80% to 0% respectively for 2008 and 2019. The decrease may be because these antibiotics are generally associated with other classes of antibiotics in the various empiric antibiotic protocols and in addition, certain antibiotics of this class are usually prescribed in the light of an AST or are classified second line in the treatment protocols subject to an AST.

### 4.3 Resistance Profile of *Klebsiella* Species to Antibiotics

#### 4.3.1 Beta-lactams

According to the literature, antibiotic susceptibility results show that all strains of *Klebsiella* species studied had a natural resistance to amoxicillin and ticarcillin (Sirot *et al.*, 1987). The wild-type resistance phenotype was confirmed for all of our



strains (Société Française de Microbiologie, 2021). The piperacilin-tazobactam combination retained good activity on the strains. However, amoxicillin and ticarcillin combined with clavulanic acid had resistance rates greater than 80%.

Overall, we observed a slight increase in the level of antibiotic resistance compared to previous studies carried out in 2012, 2015, and 2019. Our results reveal that the resistance rates to 1GC (cefalotin), 2GC (cefoxitin), 3GC (ceftazidime), 4GC (cefepime), monobactam (aztreonam), and carbapenem (imipenem) were 71%, 71%, 75%, 100%, 75%, and 15% respectively in 2020. A study carried out by Lyonga et al., (2019) revealed resistance rates against 1GC (cefalotin), 2GC (cefoxitin/cefuroxime), 3GC (ceftazidime), 4GC (cefepime), monobactam (aztreonam) and carbapenem (imipenem) were respectively 86.5%, 83.8%, 78.4%, 78.4 %, 76.7% and 2.7% (Mbamyah et al., 2021). Again, Betbeui et al., (2015) found resistance rates of 85%, 55%, 54%, 56%, 51%, and 1% (Betbeui et al., 2015) respectively. Also, Mbakop et al. (2012) found resistance rates of 84%, 52%, 45%, 50.3%, 38%, and 12% (Mbakop et al., 2011) respectively. These results are similar to that carried out by Hamze et al., (2003) in Lebanon for four years which showed a resistance rate against 1CG (cefalotin) varying from 59 to 100%; 2GC (cefuroxime and cefoxitin) (49.3 to 100%) and (35 to 59.7%) respectively; 3GC (ceftazidime): 24.8 to 100%; 4GC (cefepime): 18 to 100%; aztreonam: 22.1 to 100% and imipenem: 0.6 to 0% (Hamze et al.,2003). This observed increase in the level of resistance to  $\beta$ -lactam antibiotics is probably the consequence of inappropriate prescribing and uncontrolled use of antibiotics from this family (Kang et al., 2022).

#### 4.3.2 Quinolones

In general, it was observed that the antibiotics used over the years have recorded resistance rates varying and greater than 33%. Contrary to our results, the multicentre study involving the centres of Cambodia, Madagascar, and Senegal (Huynh et al., 2023) showed respective resistance rates of 15.5%, 2.7%, and 3% for quinolones in general.

#### 4.3.3 Aminoglycosides

Of all the antibiotics used over the years, *Klebsiella pneumoniae pneumoniae* was most

sensitive to amikacin. Resistance to aminoglycosides has gradually dropped, with resistance rates dropping from 22% in 2008 to 0% in 2020. These results are by those of (Nirwati et al.,2019) who highlighted during their study that amikacin kept a good activity against *Klebsiella* species to the order of 95.80%. Thus, at the YUTH these antibiotics appear in the second line in the various protocols of the Reanimation and Paediatrics Services. That said, these drugs are generally less used or prescribed depending on the orientation of the AST.

## 5. CONCLUSION

*Klebsiella pneumoniae pneumoniae* was the most isolated species in the units of the YUTH. In addition, the most incriminated biological specimens were blood and urine, hence presenting a high risk of nosocomial infections within the various departments of the hospital. Also, children under 5 and adults over 60 were the most affected according to our study.

The different species of *Klebsiella* species identified during this study showed a high resistance rate against beta-lactams followed by amphenicols and quinolones. Concerning the most isolated species (*Klebsiella pneumoniae pneumoniae*), the piperacillin-tazobactam association retained good activity on the strains. In addition, amoxicillin and ticarcillin combined with clavulanic acid had resistance rates greater than 93%. Overall, cefoxitin was more active than third and fourth-generation cephalosporins. Among the carbapenems tested, imipenem retained good sensitivity.

Over the years, quinolones have had varying and higher resistance rates. Ciprofloxacin, on the other hand, was active against all strains for the years 2014 and 2018. However, over the years, we have observed a gradual decline in the resistance rate against aminoglycosides, with amikacin retaining good activity compared to other antibiotics of the same class. Finally, colistin showed alarming resistance rates.

Antibiotic resistance concerns everyone. Though the resistance rates against major drugs sometimes differ from one place to another, it is clear that no country is spared. The most resistant bacterial phenotypes are an integral

part of our practice in clinical bacteriology, hence there is a need to strengthen the epidemiological surveillance of bacteria, particularly *Klebsiella* species. This study drastically showed the urgency of decision-making by the Cameroonian health authorities in general and that of the YUTH, in particular, to fight antimicrobial resistance state the major findings of the study.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

The Study was approved and confirmed under the rules and regulations of research in the Department of microbiology and Immunology, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon. Therefore, at the beginning of the research point, ethical approval and authorization were issued while referring to the Yaoundé University Teaching Hospital. At the hospital, the regulations for research were approved by the ethical committee of the hospital and the microbiology laboratory as well.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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