



Resistance Profile of *Klebsiella pneumoniae* Strains Isolated at the Yaounde General Hospital and the Yaounde Gyneco-Obstetric and Pediatric Hospital

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Abstract

Introduction: Dissemination of resistant bacteria is responsible for a considerable increase in mortality, morbidity and cost of treatment. Our study aimed to determine the frequency of *Klebsiella pneumoniae* infections in two referral hospitals in Yaounde Cameroon, and to examine the antibiotic resistance profile.

Methods: A cross-sectional descriptive study was carried out for a five-month period. Samples were collected from in and out-patients at the Yaounde General Hospital and at the Yaounde Gyneco-Obstetric and Pediatric Hospital. The bacteria isolation was done using standard bacteriological procedures and the identification of *Klebsiella pneumoniae* species was done using API 20E system (Biomérieux). Antibiotic susceptibility testing was determined using the disc diffusion method on Mueller Hinton media and the interpretation of the antibiogram was performed as recommended by the *Comité de l'Antibiogramme de la Société Française de Microbiologie* (2019). The data collected were analyzed with Epi Info 7.0 software and Excel 2013.

Results: The frequency of *Klebsiella pneumoniae* infections was 2.48% (52/2096). The majority of *Klebsiella pneumoniae* strains were isolated from urinary tract infections 55.77% (29/52). Most isolates were recovered from in-patients 63.46% (33/52) received at the pediatrics unit 25.0% (13/52). Few isolates were resistant to imipenem and meropenem with a resistance rate of 3.85% (2/52) each, while a considerable number of isolates were highly resistant to antibiotics such as ticarcillin 96.15% (50/52), amoxicillin + clavulanic acid 94.23% (49/52) and piperacillin 86.54% (45/52). The majority of isolates 73.08% (38/52) were multidrug-resistant and one isolate was resistant to all tested antibiotics (superbug).

Conclusion: More than half of the isolates were multidrug-resistant and one isolate from an in-patient was found to be resistant to all tested antibiotics. These findings demonstrate the importance of establishing an effective surveillance system for antimicrobial resistance in Cameroon.

Keywords: Antimicrobial Resistance Profile; *Klebsiella pneumoniae*

Abbreviations: UTI: Urinary Tract Infection; BSI: Bloodstream Infection; PLA: Pyogenic Liver Abscess; ESBL: Extended Spectrum Beta-Lactamase; SMART: Study for Monitoring Antimicrobial Resistance Trends; EMB: Eosine Methylene Blue; CSF: Cerebrospinal Fluid; CLSI: Clinical Laboratory Standard Institute.

Introduction

Bacterial resistance to antimicrobial agents is a problem of growing importance in medical practice [1]. The spread of resistant bacteria is causing a considerable increase in mortality, morbidity and the cost of treatment [2]. *Klebsiella pneumoniae* is second to *Escherichia coli* as the most common gram-negative pathogen associated with a wide range of diverse infections, such as urinary tract infections (UTI), bronchopneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis and pyogenic liver abscess (PLA) [3,4]. Among the bacteria responsible for these infections, bacteria belonging to the family Enterobacteriaceae are the most prevalent because they produce beta-lactamases and have other resistance mechanisms against antibiotics [5]. Beta-lactamases, the main enzymes responsible for resistance in Enterobacteriaceae, were described for the first time in 1983 in Germany from *Klebsiella pneumoniae* [6], whose resistance to antibiotics has increased alarmingly in recent years [7]. Resistance of Gram-negative bacteria is a major public health problem [8] which is exacerbated by increasing global trends of *Klebsiella* species producing antibiotic resistance enzymes [9].

The global prevalence of Extended Spectrum Beta-Lactamase (ESBL) *Klebsiella pneumoniae* producers between 2005 and 2007 increased from 19.2 to 30.0 % according to the Study for Monitoring Antimicrobial Resistance Trends (SMART) program [10]. Though numerous studies to assess the infectious threat posed by multidrug-resistant bacteria have been carried out so far [11,12], it is still necessary to assess the epidemiological situation in real time in order to strengthen surveillance and the control of multidrug-resistant bacteria. In this study, we report the resistance profile of *Klebsiella pneumoniae* strains isolated at the Yaounde General Hospital and at the Yaounde Gyneco-Obstetric and Pediatric Hospital.

Methods

Specimen

A total of 2096 specimens were consecutively collected for bacteriological analysis within a period of five months. Specimens were collected from in and out-patients at the Yaounde General Hospital and the Yaounde Gyneco-

Obstetric and Pediatric Hospital. *Klebsiella pneumoniae* was isolated from specimen collected from consenting patients. The specimens analyzed included: urine, blood, pus, cerebrospinal fluid (CSF), venous catheter and urinary catheter tips, higher-vaginal smears.

Transport and Storage of Samples

The isolates were transported from the bacteriology laboratories of the Yaounde General Hospital and the Yaounde Gyneco-Obstetric and Pediatric Hospital to the Center for Study and Control of Communicable Diseases, Faculty of Medicine and Biomedical Sciences, the University of Yaounde I, for bacteriological analysis. The isolates were transported in a cooler bag following triple packaging. These samples were sub-cultured on Eosine Methylene Blue (EMB) media to ensure their viability before being stored in brain heart infusion broth in the freezer at -20°C.

Isolation and Identification

Clinical specimens were cultured on Eosin Methylene Blue agar and suspected colonies were subcultured on nutrient agar to obtain pure cultures and biochemical tests were carried out using the Api 20E identification system in accordance with the manufacturer's manual (Biomérieux SA, Lyon, France).

Antimicrobial Susceptibility Testing

This was done using the Kirby Bauer Disc diffusion method with reference to the *Comité de l'Antibiogramme de la Société Française de Microbiologie* (2019) performance guideline for antimicrobial susceptibility testing [13]. *E. coli* ATCC 25922 control strain was used to perform quality control of antibiotic disc. Twenty one antibiotics were tested, namely: amoxicillin (AMX, 25 µg), amoxicillin + clavulanic acid (AMC, 20/10 µg), piperacillin (PIP, 30 µg), piperacillin + tazobactam (PPZ, 30/6 µg), ticarcillin (TIC, 75 µg), ticarcillin + clavulanic acid (TCC, 75/10 µg), cefalotin (CTT, 30 µg), cefuroxime (CFM, 30 µg), ceftazidime (CAZ, 10 µg), cefotaxime (CTX, 5 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), aztreonam (ATM, 30 µg), gentamicin (CN, 10 µg), amikacin (AK, 30 µg), tobramycin (TM, 10 µg), norfloxacin (NOR, 10 µg); nadilixic acid (NA, 30 µg) and sulfamethoxazole + trimethoprim (STX, 1.25/23.75 µg).

Detection of Multi-Drug Resistant (MDR) Isolates

Isolates of *Klebsiella pneumoniae*, which showed non-susceptibility to at least one agent in more than three

antimicrobial classes, were considered MDR [14].

Detection of Extended-Spectrum β -Lactamase (ESBL) Producing Isolates

Screening consisted in identifying species resistant or of intermediate susceptibility to either or both ceftazidime (10 μ g) and cefotaxime (5 μ g). Confirmation was based on the combination disc test [15].

Ethical Considerations

This study received approval from the ethics committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. All the patients signed the consent form before specimen collection. All the samples were assigned codes after collection.

Results

From the 2096 specimens collected for bacteriological analyses, 416 came from the Yaounde General Hospital and 1680 came from the Yaounde Gyneco- Obstetric and Pediatric Hospital. Among these specimens, 52 revealed a positive culture for *Klebsiella pneumoniae*, representing a frequency of 2.5%. The majority of isolates, 63.4% (32/52) were from

the Yaounde Gyneco-Obstetric and Pediatric Hospital and 34.6% (18/52) were from the Yaounde General Hospital. *Klebsiella pneumoniae* infections were predominant in children 0-4 years of age who had an infection rate of 32.7% (17/52) followed by people over the age of 55 years who had an infection rate of 13.5% (07/52). The female population was the most infected by *Klebsiella pneumoniae* infections as they had an infection rate of 61.5% (32/52). From the 52 positive cultures for *Klebsiella pneumoniae*, 63.5 % (33/52) isolates were from in-patients and 36.5 % (19/52) from out-patients.

The frequency of *Klebsiella pneumoniae* in the different units in descending order was: pediatric unit 25.0 % (13/52), medicine and gynecology units, 13.5 % (7/52) each, surgery, 9.6 % (5/52), emergency and intensive care unit, 7.7 % (4/52) each, urology, 5.8 % (3/52), neonatal and oncology units 3.8 % (2/52) each. The frequency of *Klebsiella pneumoniae* in the different clinical specimens in descending order was: urine 55.8 % (29/52), higher vaginal smear 17.3 % (9/52), blood 11.5 % (6/52), pus, cerebrospinal fluid (CSF), veinous catheter and urinary catheter tips 3.8 % (2/52) each and seminal fluid 1.9 (1/52). These results are better illustrated in Figure 1 below.

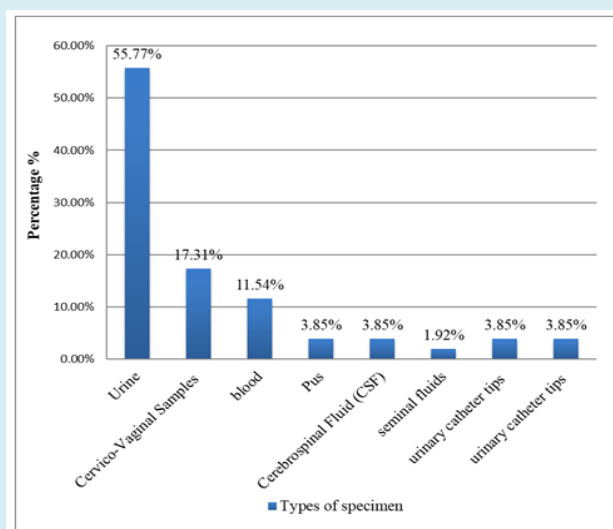
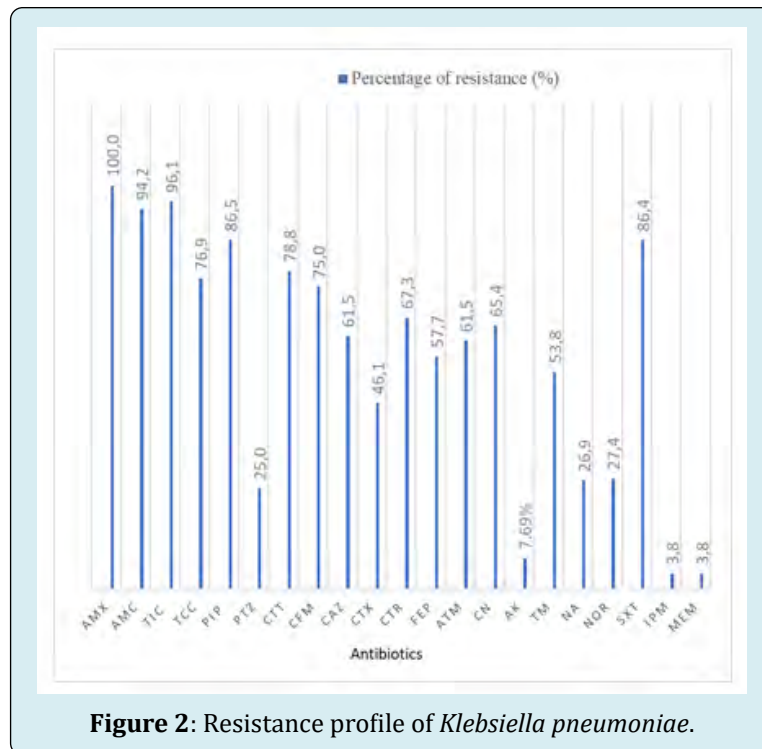


Figure 1: The prevalence of the *Klebsiella pneumoniae* isolates according to the different types of specimen.

Resistance to the penicillin (ticarcillin), amoxicillin + clavulanic acid, first generation cephalosporin (cephalothin), second generation cephalosporin (cefuroxime), third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone), fourth generation cephalosporin (cefepime), monobactam (aztreonam) and carbapenem (imipenem)

were 96.1% (50/52), 94.2% (49/52), 78.8 % (41/52), 75.0 % (39/52), 61.5 % (39/52), 46.1 % (24/52), 67.3 % (35/52), 57.7 % (30/52), 61.5 % (32/52) and 3.8 % (2/52) respectively. The resistance to quinolones (nalidixic acid) and fluoroquinolones (norfloxacin) were 26.9% (14/52) respectively. This profile is presented in Figure 2 below.



Antibiotics	Patients	
	In-Patients n= 33	Out-Patients n= 19
	Percentage of resistance (%)	Percentage of resistance (%)
Amoxicillin	100,0	100,0
Amoxicillin + clavulanic acid	97,1	87,50
Ticarcillin	100,00	87,5
Ticarcillin + clavulanic acid	82,3	62,5
Piperacillin	91,2	75,0
Piperacillin + tazobactam	32,3	6,2
Cefalotin	85,3	75,0
Cefuroxim	82,3	56,2
Ceftazidime	73,5	37,5
Cefotaxime	58,8	18,7
Ceftriaxone	76,5	43,7
Cefepime	64,7	43,7
Aztreonam	61,8	56,2
Gentamicin	70,6	50,0
Amikacin	8,8	6,2
Tobramycine	59,4	37,5
Nadilixic Acid	24,2	25,0
Norfloxacin	28,1	13,3
Trimethoprim + Sulfamethoxazole	93,9	68,7
Imipenem	5,9	0,0
Meropenem	5,9	0,0
Mean	62,0	45,3

Table 1: Resistance profile of *Klebsiella pneumoniae* from in-patients and out-patients.

Out of the 52 isolates, the majority, 73.1% (38/52) were multidrug-resistant and one isolate was resistant to all studied antibiotics (super bug). The frequency of multidrug-resistant isolates was higher in in-patients, 68.4% (26/38) than in out-patients 31.6% (12/38). However, the difference between the frequency of multidrug-resistant strains and the category of the patients was not statistically significant (p -value = 0.37) Table 1.

Discussion

Our study consisted in highlighting the resistance profile of *Klebsiella pneumoniae*, isolated at the Yaounde General Hospital and the Yaounde Gyneco-Obstetric and Pediatric Hospital. A total of 2096 samples were collected for bacteriological analysis. We had 416 samples from the Yaounde General Hospital and 1680 samples from the Yaounde Gyneco-Obstetric and Pediatric Hospital. Out of these 2096 samples, 52 had a positive culture for *Klebsiella pneumoniae*, representing a prevalence of 2.5%. *Klebsiella pneumoniae* infections were common in people over 55 years of age, 13.5% (07/52) and even more common in children 0-4 years old, 32.7% (17/52). This can be explained in young children as a result of immaturity of their immune system and the elderly, due to weakened immune systems, hence these age groups contract infections more easily. These results are similar to those of Betbeui, *et al.* [12] who, worked in three reference hospitals in Cameroon on 99 isolates of *Klebsiella spp*, recovered 35.3% of *Klebsiella pneumoniae* strains in the pediatric unit. The isolates were less resistant to imipenem and meropenem, with a resistance rate of 3.8% (2/52) each and amikacin, 7.7% (4/52). The isolates were highly resistant to ticarcillin 96.1% (50/52), amoxicillin + clavulanic acid, 94.2% (49/52) and piperacillin, 86.5% (45/52). These results are similar to those reported by Betbeui, *et al.* [12] who reported a *Klebsiella* species sensitivity rate of 99.0 % to imipenem. Our study reveals that the resistance rate was very high against amoxicilin + clavulanic acid, 94.2% (49/52). These results corroborate those reported by Nzali, *et al.* [16] who found that all strains of *Klebsiella pneumoniae* were resistant to amoxicilin + clavulanic acid.

The majority of isolates, 73.1% (38/52) were multidrug resistant and it was reported more in isolates from in-patients, 68.4% (26/38) than among out-patients 31.6% (12/38) though the difference between the frequencies and the categories of the patients, was not statistically significant (p -value = 0.37). Although, it is known that bacterial isolates from hospitalized patients harbor more resistance determinants than those isolated from community patients due to selection pressure resulting from overuse of antibiotics within hospital settings, the present study does not reveal any link. The implications of these findings are such that the treatment of infections will be more difficult and resistance

mechanisms may spread to other bacteria species, causing susceptible isolates to become non-susceptible and hence therapeutic failure may occur.

Conclusion

The frequency of *Klebsiella pneumoniae* isolated from the different specimen was very low. Most *Klebsiella pneumoniae* were isolated from urinary tract infections. Most isolates were susceptible to carbapenem and highly resistant to most cephalosporins. The majority of isolates were multidrug resistant and no wild type was isolated. One isolate was resistant to all antibiotics tested. These findings demonstrate the importance of establishing an effective surveillance system for antimicrobial resistance in Cameroon.

Authors' Contributions

Emilia Enjema Lyonga conceived the study and designed it together with Aime Cesar Teukam Noukoupe and Hortense Kanga Gonsu. Emilia Enjema Lyonga, Aime Cesar Teukam Noukoupe, Modestine Djuissi, Florence Enyeji and Patience Mangum, conducted the laboratory aspect of the study with contributions from Martha Tongo Mesembe and George Mondinde Ikomey. The general supervision was carried out by Hortense Kanga Gonsu. Emilia Enjema Lyonga drafted the article with contributions from Aime Cesar Teukam Noukoupe and Martha Tongo Mesembe. All the authors reviewed the article. All the authors read and agreed to the final manuscript.

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