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High Prevalence of Multidrug Resistant *Klebsiella* Species Isolated from the Yaounde University Teaching Hospital, Cameroon

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Abstract

Background and Purpose: *Klebsiella* species are amongst the most common causes of a variety of community-acquired and hospital-acquired infections (HAI), characterized by high morbidity and mortality rates. Most infections caused by *Klebsiella* species are usually treated using antibiotics. The aim of this study was to determine the antimicrobial resistance profile of *Klebsiella* species isolated from in-patients and out-patients at the Yaounde University Teaching Hospital. The data generated will go a long way to improve on the choice of an adequate empiric antibiotic treatment for infections caused by *Klebsiella* species. **Methodology:** A cross-sectional descriptive study was carried out over a period of 6 months, spanning from February 2019 to July 2019 with a sample size of 37 isolates, obtained from 6 different clinical specimens. Identification of isolates was done using API 20E identification system (Biomerieux SA, Lyon, France). Susceptibility to antibiotics was tested as described by Kirby-Bauer in 1956. Inhibition diameters were interpreted according to recommendations from the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019). **Results and Conclusion:** Among the 37 *Klebsiella* isolates identified, *Klebsiella pneumoniae* was the most prevalent species isolated with a percentage of 54.1%, followed by *Klebsiella rhinoscleromatis* 18.9%, *Klebsiella ozaenae* 16.2% and *Klebsiella oxytoca*, 10.8%. The resistance pattern of *Klebsiella* to amoxicillin, amoxicillin/clavulanate, tircacil-

lin, tircacillin + clavulanic acid, piperacillin, piperacillin + tazobactam, cefalotin, cefuroxim, ceftazidime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, aztreonam, amikacin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole, nalidixic acid, pipemidic acid, norfloxacin, ciprofloxacin, levofloxacin, ofloxacin, and moxifoxacin was as follows; 100%, 86.5%, 97.3%, 83.6%, 86.5%, 16.2%, 86.5%, 83.8%, 78.4%, 32.4%, 78.4%, 76.7%, 2.7%, 2.7%, 76.7%, 13.5%, 75.7%, 73.0%, 91.9%, 51.4%, 48.6%, 64.9%, 48.6%, 48.6%, 73.0% and 62.2% respectively. Multidrug resistance was observed in 94.6% of the *Klebsiella* isolates. **Conclusion:** This study shows that the level of multidrug resistance is high. The isolates expressed good sensitivity to carbapenems, piperacillin + tazobactam, amikacin and high resistance to all other antimicrobials tested. Therefore, antimicrobial susceptibility testing prior to prescriptions should be encouraged and sensitization of the population about consequences of inappropriate antibiotic treatment and auto medication should be enforced as a means to curb antimicrobial resistance.

Keywords

Klebsiella Species, Antimicrobial Resistance, Multidrug Resistance

1. Introduction

Klebsiella species are found in nature in water, soil and animals and they can colonize medical devices and the healthcare environment [1] [2]. *Klebsiella* species are among the most common causes of a variety of community-acquired and hospital-acquired infections (HAIs). These diseases cause an increase in morbidity and mortality [3].

They are considered opportunistic pathogens colonizing mucosal surfaces without causing pathology; however, from mucosae *Klebsiella* may disseminate to other tissues causing life-threatening infections including bronchopneumonia, urinary tract infections (UTIs), bloodstream infections and sepsis [4].

Most infections caused by *Klebsiella* species are usually treated using antibiotics. The high rate at which antibiotics are used all around the world in human therapy and in veterinary medicine has given rise to antimicrobial resistance [5]. Antimicrobial resistance is associated with increased patient morbidity and mortality and contributes to escalating health care cost and prolonged stay in hospital [6] [7].

Today, antimicrobial resistance is a global public health problem [8] [9]. The aim of this study was to determine the antimicrobial resistance profile of *Klebsiella* species isolated from the Yaounde University Teaching Hospital. This data will be useful in choosing an adequate antibiotic treatment for infections caused by *Klebsiella* species.

2. Methodology

A cross sectional descriptive study was carried out for 6 months, spanning from

February to July 2019. Isolates were collected from 6 different clinical specimens: blood, pus, wound, urinary catheter, venous catheter and urine. The isolates were from in-patients and out-patients consulted at the Yaounde University Teaching Hospital. These specimens were cultured on eosin methylene blue agar (EMB) and incubated at 37˚C for 18 - 24 hours. Based on colony morphology, suspected *Klebsiella* spp. colonies were identified using API 20E identification kits (Biomerieux SA, Lyon, France). *Klebsiella* species were identified from 37 specimens. Antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method was done following recommendations from the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2019) [10] and inhibition diameters were reported.

The 26 antibiotics tested were from the following classes: quinolones, penicillins, cephalosporins, monobactams, carbapenems, aminoglycosides and sulphonamides. Seven quinolones were tested namely: nalidixic acid (30 µg), pipemidic acid (20 µg), norfloxacin (5 µg), ciprofloxacin (10 µg), levofloxacin (5 µg) ofloxacin (5 µg) and moxifoxacin (5 µg). The penicillins tested were: amoxicillin (25 µg), amoxicillin/clavulanate (20/10µg), tircacillin (75 µg), tircacillin + clavulanic acid (75/10µg), piperacillin (30 µg), and piperacillin + tazobactam (30/6µg). Cephalosporins tested included: cefalotin (30 µg), cefuroxime (30 µg), ceftriaxone (5 µg), ceftazidime (10 µg), cefotaxime (5 µg), and cefepime (30 µg). Among the carbapems imipenem (10 µg) and meropenem (10 µg) were tested. The following aminoglycosides were tested: amikacin (30 µg), gentamicin (10), and tobramycin (10 µg). One sulphonamide: trimethoprim/sulfamethoxazole (1.25/23.75µg) was tested and aztreonam (30 µg) was the only monobactam tested. Quality control of antibiotic discs was done using *Escherichia coli* ATCC 25922. Data analysis was carried out using Microsoft Excel 2016 and SPSS version 25.

3. Results

Thirty seven *Klebsiella* isolates were identified using the API 20E identification system. Among the species isolated *Klebsiella pneumoniae* was the most prevalent with 54.1%, followed by *Klebsiella rhinoscleromatis* 18.9%, *Klebsiella ozaenae* 16.2% and *Klebsiella oxytoca*, 10.8% as shown on **Figure 1** below.

Klebsiella species were most isolated from pus 24.3%, urinary catheter and urine with a frequency of 21.6% each and isolated least from venous catheter 5.4% as shown on **Table 1**.

The antimicrobial resistance profile of the *Klebsiella* species to all the antibiotics tested according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2019) [10] standards are represented on **Figure 2**.

The natural resistance of *Klebsiella* species to ampicillin was confirmed with a 100% resistance. The isolates were most resistant to tircacillin (97.3%) and sulfamethoxazole + trimethoprim (91.9%). The isolates showed the least resistance to imipenem and meropenem with 2.7% each as shown on **Figure 2**.

Multidrug resistance was observed in 94.6% of the *Klebsiella* isolates as shown on **Figure 3**. No wild type was found.

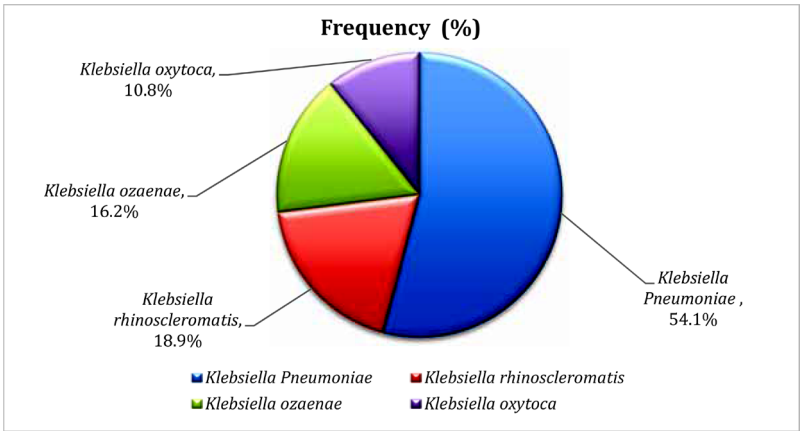
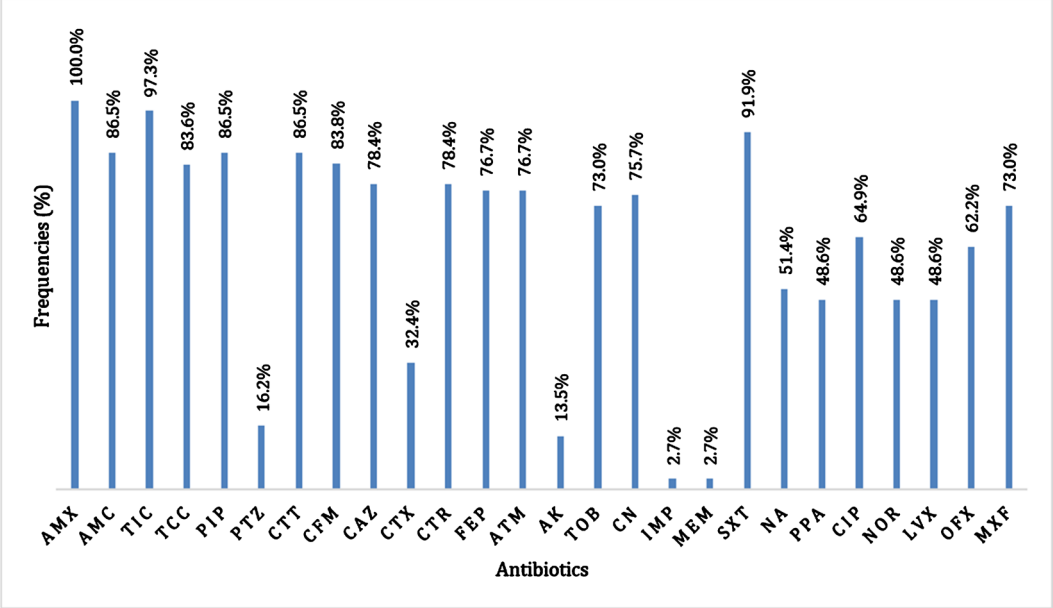


Figure 1. Distribution of *Klebsiella* species isolated.

Table 1. Distribution of clinical specimens.

Specimen	Number of subjects	percentage
Pus	9	24.3%
Urinary catheter	8	21.6%
Urine	8	21.6%
Blood culture	7	18.9%
Wound	3	8.1%
Venous catheter	2	5.4%
Total	**37**	**100**



AMX: Amoxicillin; AMC: Amoxicillin + clavulanic acid; TIC: Ticarcillin; TCC: Ticarcillin + clavulanic acid; PIP: Piperacillin; PTZ: Piperacellin + tazobactam; CTT: Cefalotin; CFM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; CTR: Ceftriaxone; FEP: Cefepime; IPM: Imipenem; MEM: Meropenem; AK: Amikacin; TOB: Tobramycin; CN: Gentamicin; SXT: Sulfamethoxazole + trimethoprim; ATM: Aztreonam; NA: Nalidixic acid; PPA: Pipemidic acid; NOR: Norfloxacin; CIP: Ciprofloxacin; LVX: Levofloxacin; OFX: Ofloxacin; MXF: Mixofloxacin.

Figure 2. Antimicrobial resistance profile of the *Klebsiella* spp.

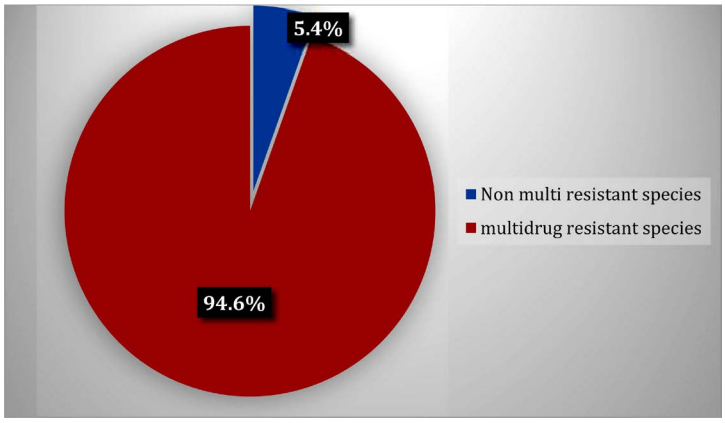


Figure 3. Multidrug resistant *Klebsiella* species.

3. Discussion

In this study the majority of isolates were *Klebsiella pneumoniae* 20/37 (54.1%). This trend is similar to previous work by Sunilkumar and collaborators in India (98%) [11] and Hansel and collaborators in the United Kingdom (76%) [12]. The frequency of *Klebsiella pneumoniae* confirms that it is one of the leading causes of infections [13].

The isolates were from 6 different types of clinical specimens: blood, pus, urinary catheter, urine, venous catheter, and wound. *Klebsiella* species were most isolated from pus (24.3%), a result similar to a study carried out in Algeria.

The isolates were resistant to all penicillins tested. Resistance to amoxicilin + clavulanic acid was high, 32/37 (86.5%), likewise to tircacillin 36/37 (97.3%), tircacillin + clavullanic acid 31/37 (83.6%) and piperacillin 32/37 (86.5%). However, the antimicrobial activity of piperacillin was improved with the combination of piperacillin with tazobactam resulting in a reduction of the rate of resistance of the isolates 6/37 (16.2%). All the isolates were resistant to amoxicilin 37/37 (100%), which was similar to that obtained by Arafat *et al.,* 2009 who in Algeria recorded 50/50 (100%) resistance to Amoxicillin [14]. *Klebsiella* spp. has a natural resistance to amoxicillin due to the presence of a Class A SHV-1 penicillinase [15].

This overall high rate of resistance of the isolates to the penicillins could be explained by the over use of these drugs in the treatment of common infections and ease of drug acquisition without prescription and even from road side vendors.

The resistance of the isolates to the cephalosporins was also very high with the following frequency recorded: cefalotine 32/37 (86.5%), cefuroxime 31/37 (83.8%), ceftazidime 29/37 (78.4%), ceftriazone 29/37 (78.4%) and cefepime 28/37 (76.7%). The least resistance of the isolates was to cefotaxime 12/37 (32.4%). This result is different from results reported by Betbeui and collaborators in 2015 from three referral hospitals in Yaounde. The study reported lower rates of resistance for the cephalosporins [16]. Thus there has been an increase over time in resistance to cephalosporins. The resistance of isolates to cephalosporins is as a result of the production of extended spectrum betalactamases that hydrolyze penicillins,

first, second, third, fourth generation cephalosporins and monobatams.

The resistance of the isolates to aztreonam was high 28/37 (76.7%). This result is similar to results had by Gangoue-Pieboji *et al.* 2006 who in Yaounde recorded 75% resistance to aztreonam [17]. This shows that resistance has remained high over time.

The resistance to carbapenems was low for both imipenem and meropenem 1/37 (2.7%). This result is similar to results by Gangoue-Pieboji *et al.,* who in Yaounde had 2% resistance to carbapenems [17]. The low resistance of the isolates to these antibiotics could be explained by the fact that these drugs are reserved as last line treatment, they are expensive and they can be administered only via the intravenous route [18].

The isolates expressed high resistance to the aminoglycosides such as tobramycin 27/37 (73.0%) and gentamicin 28/37 (75.7%) with least resistance recorded to amikacin 5/37 (13.5%). Again there is a general increase in resistance (except for resistance to amikacin) compared to results by Gangoue-Pieboji *et al.,* 2006, who reported a resistance of 35% [17].

The isolates expressed high resistance to sulphamethoxazole + trimetoprim 34/37 (91.9%). This frequency is higher than results obtained by Arafa *et al.,* 2009 who in Algeria reported 73% of their isolates were resistant to sulphometoxazole + trimetoprim [14]. This antibiotic is often used for the treatment of UTI's but the high resistance rate recorded in this study, disqualifies it for such use.

Among the quinolones, the isolates were most resistant to moxifloxacin 27/37 (73.0%), ciprofloxacin 24/37 (64.9%), ofloxacin 23/37 (62.2%) and nalidixic acid 19/37 (51.4%). The isolates were least resistant to pipemidic acid, norfloxacin and levofloxacin 18/37 (48.6%) each. This result is different from results had by Chakraboty *et al.,* 2016 who recorded low levels of resistance to quinolones [19]. Resistance to ciprofloxacin is considered resistance to all quinolones because of acquisition of at least two mutations in either *gyr*A or *gyr*B and *par*C. These genes are capable of mediating low-level quinolone resistance that causes high-level resistance to arise in the presence of quinolones at therapeutic levels [20].

The emergence of multidrug resistant *Klebsiella* species has become a major public health concern worldwide and has been associated with outbreaks of infections in developing countries due to the indiscriminate use of antibiotics [21]. An isolate is said to be multidrug resistant if it is resistant to three or more antimicrobial classes. We found that 95% of the isolates were multidrug resistant species. Multidrug resistance in *Klebsiella* species varies in different parts of the world. In India, 54% was reported [22] and in Nigeria 75.8% [23]. The high level of multidrug resistance in this study could be due to an interplay of other resistance mechanisms co-expressed by the isolates such as extended spectrum beta-lactamases, quinolone resistance genes and *aac*(6')-*Ib-cr* enzymes which hydrolyze quinolones and aminoglycosides. Furthermore, prior antibiotic use in hospitals or through auto-medication, overuse of antibiotics in livestock and fish farming, poor infection control in health care facilitates and poor hygiene and

sanitation exercebates multidrug resistance.

This study however had some limitations. The sample size was small as isolates were collected from only one hospital, the Yaounde University Teaching Hospital. This study needs to be extended to other hospitals in Yaounde for a longer period to better understand resistance trends in Yaounde. For the purpose of infection control and clinical relevance of the results, it would be necessary for the resistance phenotypes to be characterized.

4. Conclusion

This study shows that the level of multidrug resistance is high. The isolates expressed good sensitivity to the carbapenems, piperacillin + tazobactam, amikacin and high resistance to all other antimicrobials tested. This indicates that with the exception of a few antibiotics, commonly used affordable antibiotics may not treat infections caused by *Klebsiella* spp. Therefore, antimicrobial susceptibility testing prior to prescriptions should be encouraged and sensitization of the population about the consequences of antibiotic abuse and auto medication should be enforced as a means to curb antimicrobial resistance. The surveillance of antimicrobial resistance should be put in place to monitor clinically relevant isolates.

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Authors' contributions

Emilia Enjema Lyonga Mbamyah conceived the study and designed it together with Florence Anjabie Enyeji, Judith Torimiro and Hortense Kamga Gonsu. Emilia Enjema Lyonga Mbamyah, Florence Anjabie Enyeji, Modestine Djuissi, Patience Mangum, and Dieudonné Sedena, Aime-Caesar Teukam, Agnes Bedie Eyoh, William Baiye conducted the laboratory aspect of the study with contributions from Martha Tongo Mesembe and George Mondinde Ikomey. The general supervision was carried out by Emilia Enjema Lyonga Mbamyah. Emilia Enjema Lyonga Mbamyah drafted the article with contributions from Florence Anjabie Enyeji[1] and Martha Tongo Mesembe. All the authors reviewed the article. All the authors read and agreed to the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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