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Multidrug Resistance Pattern of Klebsiella Isolates from Clinical Samples in a Tertiary Health Facility in Southeast Nigeria

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Abstract

The development of multi-drug resistant (MDR) Klebsiella is a global public health issue with mortality rates among patients infected with MDR Klebsiella as high as 64.0%. This study aimed to investigate the prevalence of infection and multidrug resistance pattern of Klebsiella isolates in Federal Medical Centre Umuahia (FMCU). We examined the MDR pattern of Klebsiella isolates, and explored the association between Klebsiella spp and clinical specimen in FMCU. Eighty-four (84) isolates were obtained from 2,510 clinical specimens between January 2022 and June 2022 in FMCU and identified using standard microbiological procedure and biochemical panel tests. Isolates were subjected to antimicrobial activities with penicillin, second and third-generation cephalosporin, floroquinolones, aminoglycosides, and carbapenem using the modified Kirby Bauer disc diffusion methods. Resistivity was determined using the Clinical and Laboratory Standard Institute (CLSI) criteria. Isolates resistant to three or more antimicrobial class were considered MDR. Prevalence and associations were determined using descriptive statistics and $\chi 2$ respectively and level of significance (α level) was set at 0.05. Fifty-four (54; 64.3%) isolates were identified as K. pneumoniae and 30 (35.7%) identified as K. oxytoca. Isolates from females were 48 (52.0%) and males 36 (43.0%). Overall prevalence of MDR- Klebsiella was 55.0%. K. oxytoca were more resistant to the antibiotics used compared to K. pneumonia. The distribution of isolates by specimen were urine (30; 35.7%), wound (25; 29.8%), sputum (16; 19.0%), urogenital (7; 8.3%), blood (3; 3.6%), and aspirate (3; 3.6%). There was a statistically significant association between Klebsiella spp and clinical specimen ($\chi 2= 18.89$; $p < .05$). Our findings indicated a high prevalence of MDR Klebsiella. This emerging challenge of MDR Klebsiella pose a significant threat to public health in Federal Medical Centre Umuahia and the communities in southeast Nigeria in general. These findings help as a justifiable reason for constant surveillance and necessary actions to address the challenges of MDR Klebsiella. This observation calls for strict policy on the regulation of antibiotic use and inappropriate prescription of antibiotics.

Introduction

There is an increase in trend in the development of multi-drug resistance (MDR) by Klebsiella globally which is a public health issue. The mortality rates among patients infected with MDR Klebsiella are as high as 64.0%. [1]. Klebsiella species have been implicated in most hospital-acquired human infections. However, Klebsiella pneumoniae is said to be the most important opportunistic pathogen of human infections followed by K. oxytoca, K. azaenae, and K. rhinoscleromatis [2]. Klebsiella pneumoniae has been identified as the cause of 3 - 30% of neonatal septicemias, 7 - 14% of nosocomial pneumonia, 2 - 4% of wound infections, and 4 - 15% of septicemias [2]. Other clinical infections associated with K. pneumoniae include endocarditis, urinary tract infections, osteomyelitis, cholecyctitis, diarrhoea, peritonitis, and meningitis [3].

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Antimicrobial agents generally act on microorganisms by either inhibiting the metabolic pathway that leads to DNA/RNA and protein synthesis or by interfering with the enzyme-substrate involved in the synthesis of the microbial cell wall [4]. However, microbes have developed different survival mechanisms through which they evade and overcome the effects of antimicrobial agents. The inhibition of cell wall synthesis occurs when antimicrobials bind the peptidoglycan layer in bacteria or ergosterol synthesis in fungi thereby blocking cell division and growth [4]. These microorganisms either undergo chromosomal mutations or they exchange their extrachromosomal DNA elements through transformation or conjugation as it is with K. pneumoniae. Chromosomal mutations or the extrachromosomal DNA elements can alter the composition of the cell membrane. This leads to reduced permeability, reduced uptake of antimicrobial agents into the cell, and lack of active target sites for antimicrobial agents to bind [5].

The emergence of extended-spectrum β-lactamase-(ESBL-) producing bacteria, especially in K. pneumoniae, has become a cause for concern for the management of bacterial infections [6]. This ESBL- producing bacteria are resistant to extended-spectrum beta-lactam antibiotics, aminoglycosides, and fluoroquinolones [6]. K. pneumoniae carbapenemase (KPC) fall in the Ambler class A plasmid-encoded enzymes, and can hydrolyze all beta-lactam antibiotics, including monobactams, extended-spectrum cephalosporins, and carbapenems [7]. The production of KPC was originally reported in North Carolina in 2001 where it was observed in a clinical isolate of Klebsiella pneumoniae [8]. The development of MDR Klebsiella infections has created a great challenge in the treatment process by reducing the efficacy of treatment. This leads to a prolonged and increased cost of treatment with deadly consequences and has become a major public health issue. Furthermore, the progress of disease control is greatly hindered by multi-drug resistant pathogens, with the possibility of spread through global tourism and trade. Studies on Klebsiella spp harbouring antibiotic resistance enzymes have been well documented [3, 4]. There have been several reports on the multi-drug resistance of K. pneumoniae and K. oxytoca worldwide [5, 6, 7]. A few of similar studies on MDR Enterobacteriaceae and Klebsiella have been conducted in the northeast and southwest Nigeria [9, 10, 11,12]. However, studies on MDR Klebsiella spp are scarce in southeast Nigeria. Thus, against this background, this study aimed to investigate the prevalence of infection and multidrug resistance pattern of Klebsiella in Federal Medical Centre Umuahia. We examined the resistance pattern of Klebsiella isolates, and explored the association between Klebsiella spp and clinical specimen in FMCU.

Materials and Methods

This cross-sectional hospital-based study was conducted in the Department of Medical Microbiology in the Federal Medical Centre (F.M.C) Umuahia. FMC Umuahia is a 405-bed tertiary hospital centrally located and draws clientele and patients mainly from the southeast and south-south part of the country. The hospital serves as a referral hospital to the surrounding towns in Abia state, Ebonyi state, and some towns in Imo state. The Microbiology laboratory received an average of 15 clinical samples for microscopy, culture, and sensitivity (M/C/S) per day from inpatients and outpatients of the hospital.

The Study

In this study, we examined eighty-four (84) isolates of Klebsiella spp from 2,480 clinical specimens. The samples comprised all samples except stool samples submitted for microscopy, culture, and sensitivity from different wards and outpatients between January 2022 and June 2022. Isolates from the same patient following a repeat culture were excluded from the study. Klebsiella isolates were collected consecutively until the sample size of 84 isolates was attained. The sample size was determined using the G* Power software [13]. Every form of identifier that could lead to patients' identity were removed. Age, gender, and source of specimen were extracted from the test request forms. The antimicrobial resistibility reports of Klebsiella isolates were stratified by the source of isolates (specimen) (wound, urine, sputum, urogenital, blood, and aspirate) according to the methods of Sanchez et al. [3]. The isolates were also stratified by specie type.

Isolation and Identification: Isolation of Klebsiella was done by inoculating clinical specimens on the appropriate bacteriological culture media (MacConkey, Blood agar, and Cysteine Lysine Electrolyte Deficiency agar) using standard conventional procedures. Identification of Klebsiellae was done using morphological characteristics on MacConkey agar media (Large mucoid lactose fermenting colonies and Gram-negative rods on staining). All isolates that exhibited colonies suspected to be Klebsiella species were tested biochemically according to the methods of Cruickshank [14]. The biochemical test employed included Indole test, Vogues Proskauer, Citrate utilization, urease production, H2S production on Triple Sugar Iron (TSI) agar, and fermentation of sugars (sucrose, glucose, mannitol, lactose, adonitol, inositol, adonitol, L-sorbose, D- melezitose). Other tests included catalase, oxidase, growth on potassium cyanide, and motility test.

Antibiotic Susceptibility Test

The antibiotics used for sensitivity determination were

broad-spectrum penicillin, second and third-generation cephalosporin, fluoroquinolones, aminoglycosides, and carbapenems. They included ampicillin (AMP 10µg), amoxicillin+clavulanic acid (AMC 20 µg+10 µg), cefoperazone (CPZ 30 µg), cefuroxime (CRX 30 µg), ceftriaxone (CRO 30 µg), ceftazidime (CAZ 30 µg), ciprofloxacin (CIP 5 µg), ofloxacin (OFL 5 µg), gentamycin (CN 10 µg), and imipenem (IPM 10µg) (Antec Products).

Antimicrobial activities were determined using the modified Kirby Bauer disc diffusion methods according to Clinical and Laboratory Standard Institute (CLSI) [15]. Suspensions of the test organisms' equivalent to 0.5 McFarland standards were inoculated on the surface of Mueller - Hinton Agar (MHA) (Oxoid, England) plates. The plates were allowed to stand for 10 minutes at room temperature after which the different antibiotics discs were placed on the surface of the inoculated plates and then incubated at 37¬¬0C for 18-20hrs. Sensitivity or resistance to antimicrobial agents was determined based on the zone diameters of each of the antibiotics according to the CLSI criteria. Klebsiella isolates were considered multidrug-resistant (MDR) if isolates were resistant to three or more antimicrobial agents from the different classes/groups of antimicrobial agents [15].

No institutional review board was needed for this study because no personal identifying information collected.

Statistical analysis

The distribution of data and prevalence of multi drug resistant Klebsiella spp were determined using descriptive statistics, while resistance was determined using proportions. Results were expressed in numbers and percentages. Chi-square ($\chi 2$) test was used to determine associations between Klebsiella spp and clinical specimen (source of isolate). Data was fed into Microsoft Excel, cleaned, coded, and then transported into Statistical Package for Social Science (SPSS) statistic software version 23 for statistical analysis. Level of significance was set at 0.05. Results were expressed as tables and figures.

Results and Discussion

In this study, 2,510 clinical specimen submitted to the microbiology laboratory in Federal Medical Centre Umuahia (FMCU) for microscopy, culture, and sensitivity that included wound, urine, sputum, urogenital, blood, and aspirate between January 2022 and June 2022 were cultured on appropriate culture media.

Of the 2,510 clinical samples, 84 gave positive results for Klebsiella associated infections. The isolates were identified using standard microbiological procedure and biochemical panel tests. Fifty-four (54; 64.3%) of the 84 Klebsiella spp, were identified as K. pneumoniae. They produced gas from lactose at 44.50C, showed the positive test to glucose, sucrose, inositol, mannitol, adonitol, Vogues Proskauer, citrate, urease, catalase, KCN, but were negative to Indole, methyl red, and oxidase. Thirty (30; 35.7%) were identified as K. oxytoca. These had similar morphologic and biochemical characteristics to K. pneumoniae except that K. oxytoca was positive for Indole and did not produce gas from lactose at 44.50C. Both species were Gram-negative rods, capsulated and non-motile.

The true burden of Klebsiella infections remain an issue especially in the hospital community where majority of Klebsiella infections have been associated with hospitalization. Hospital acquired Klebsiella infections are caused mainly by Klebsiella pneumoniae said to be the most medically important specie of the Klebsiella genus. However, according to Podschun and Ullmann [16], a lower percentage of K. oxytoca has been isolated from clinical specimens when compared to K. pneumoniae. This agrees with the findings of this study where 54 (64.3%) of K. pneumoniae and 30 (35.7%) of K. oxytoca species were isolated from clinical samples respectively. Podschun and colleague opined that 8% of all nosocomial bacterial infections are caused by Klebsiella spp. in the United States and Europe.

The emergence of extended-spectrum beta-lactam (ESBL) producing Klebsiella according to Jain et.al, is a cause for concern for the management of Klebsiella infections which are resistant to ESBL antibiotics, aminoglycosides, and fluoroquinolones [6].

The distribution of the isolates according to clinical specimen is shown in Table 1. Majority of the Klebsiella isolates 30 (35.7%) were isolated from urine samples followed by wound swab 25 (29.8%), sputum 16 (19.0%), urogenital 7(8.3%), blood 3 (3.6%), and aspirate 3 (3.6%) respectively. This result is consistent with other studies by Riaz et. al.[17] and Akter et.al. [18], who reported urine sample as the most prevalent source of Klebsiella isolates, while Sah, et. al.[19] and Varghese et. al. [20] reported Klebsiella pneumoniae as the second most frequently isolated species from urine, after Escherichia coli. The special features of capsule, adhesin, siderophore production by Klebsiella that allow the organism to survive and cause pathogenicity could be considered the reason for the high prevalence of Klebsiella infections.

The age distribution of specimen of subjects is shown in Figure 1. The age range was between 1and 95years with a mean of 41±20.21years. Majority of the samples were from the age group 21-30 years (18) followed by the age group 51-60 years (17) and then the age group 41-50 years (14),

Table 1: *Distribution of Klebsiella spp by Clinical Specimen (n=84)*

Source of Isolate	*K. pneumoniae*	*K.oxytoca*	Frequency	Percentage Total
Urine	17	13	30	35.7
Wound	18	7	25	29.8
Sputum	13	3	16	19.0
Urogenital	1	6	7	8.3
Blood	3	0	3	3.6
Aspirate	2	1	3	3.6
Total	54	30	84	100

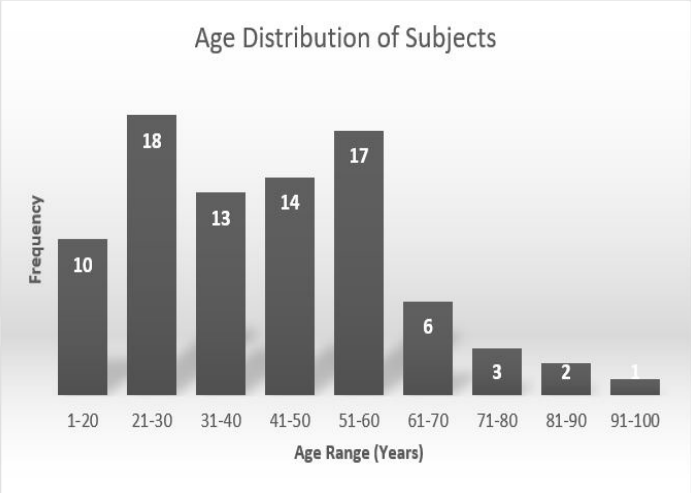

Figure 1: *Age-wise distribution of Subjects.*

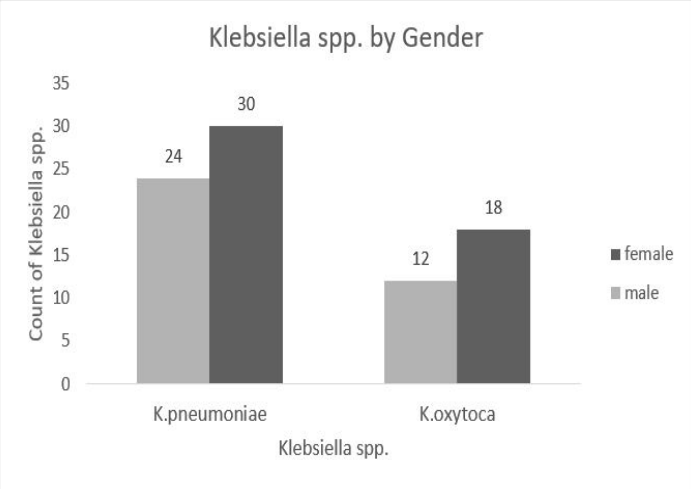

Figure 2: *Distribution of Klebsiella Isolates by Gender of subjects.*

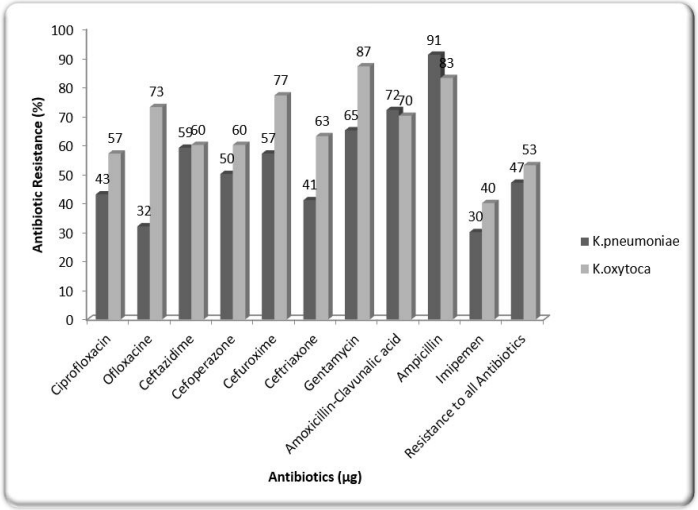

Figure 3: *Antibiotic Resistance pattern of Klebsiella Isolates of Clinical Specimen in FMC Umuahia.*

Table 2: *Prevalence of MDR- Klebsiella by Specimen.*

Clinical specimen Of Isolate	Klebsiella Isolates N (%) MDR *K. pneumonia* n=54	MDR *K. oxytoca* n=30	Prevalence of MDR-*Klebsiella* N (%)
Wound n=25 (29.8%)	10 (40.0%)	6 (24.0%)	16 (64.0%)
Urine n=30(35.7%)	11(36.7%)	10 (33.3%)	21 (70.0%)
Sputum n=16(19.0%)	4 (25.0%)	0 (0.0%)	4 (25.0%)
Urogenital n=7(8,3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Blood n=3(3.6%)	2 (67.0%)	0 (0.0%)	2 (67.0%)
Aspirate n=3(3.6%)	2 (66.7%)	1(33.3%)	3 (100.0%)
Overall Prevalence	29 (63.0%)	17 (37.0%)	46(55.0%)

while the age group 91-100 had only 1 specimen.

The distribution of Klebsiella isolates according to gender of subjects is shown in Figure 2. Our study showed that Klebsiella isolates from females 48 (52.0%) were higher compared to males 36 (43.0%) (Figure 2). Similar findings have been reported by Sah, et. al. 2015 (85.1% women, 14.89% men); Rana et. at. 2016 (females=298, males=280). However, other studies by Akter et. al. 2014; Sharanya et. al. 2018 reported contrary reports where males were more vulnerable to Klebsiella infections than females (males 57%, females 42%); (males 58.9%, females 41.03%) respectively.

In this cross-sectional study, a wide range of antibiotics which included the broad-spectrum penicillin, second and third-generation cephalosporin, fluoroquinolones, aminoglycosides, and carbapenems that are useful in the treatment of Klebsiella infections were tested against the Klebsiella isolates.

A high prevalence of multidrug resistance with Klebsiella pathogens were observed. Of the 84 Klebsiella isolates, 55.0 % were confirmed to be MDR (Table 2). K. pneumoniae had MDR prevalence of 63% while the MDR prevalence for K. oxytoca was 37%. This result is comparable to the results obtained by Sikarwar and Batra [5] where they reported multidrug resistant prevalence of 54% among the K. pneumoniae isolates that were all resistant to carbenicillin in their study in India on the Prevalence of Antimicrobial Drug Resistance of Klebsiella pneumoniae. Ferreira, et. al, recorded a higher value of 88% in their study [21]. Though their value was higher (88% prevalence). Another similar result was reported by Akter and colleagues in their study on Prevalence and Antibiotic Resistance Pattern of Klebsiella Isolated from Clinical Samples in South East Region of Bangladesh [18]. However, their figures were lower (20%) than the figures obtained in this study. Baraj et.al, also reported a lower percentage of MDR isolates (38.2%) in their study [22].

The high prevalence recorded in this study could be due to the high consumption of broad spectrum antibiotics and the indiscriminate use of antibiotics in both humans and animals (World Health Organization (2020). Furthermore, the large volume of antibiotic consumption in our communities may play a role in the development of antibiotic resistance. According to Sheth et. al., the development of antibiotic resistance is directly proportional to the volume of antibiotics consumed [23].

The antimicrobial resistance and pattern of K. pneumoniae and K. oxytoca strains according to CLSI breakpoint values are shown in Figure 3. Majority of MDR Klebsiella isolates showed high level of resistance to the different classes of antibiotics applied. Our results showed high prevalence of total resistance of K. pneumoniae and K. oxytoca to all the five classes of antibiotic tested (47% and 53%) respectively (Figure 3). High prevalence of antimicrobial cross-resistance was observed more with K. oxytoca compared to K. pneumonia with the exception of Ampicillin and Amoxicillin-Clavunalic acid where resistance was observed more with K.pneumoniae.(Figure 3). The highest antimicrobial resistance of the isolates was observed with Ampicillin (91%), followed by Gentamycin (87%), Cefuroxime (77%), then Ofloxacine (73%), and Amoxicillin-Clavulanic acid (72%). The two Klebsiella species were almost equally resistant to Ceftazidime and Amoxicillin-Clavulanic acid. The results also showed that 30% of K.pneumoniae and 40% of K.oxytoca were resistant to Imipenem (carbapenems). According to Okoche et al. [24], Carbapenems are most times the drug of choice for the treatment of infections caused by extended-spectrum beta-lactamase (ESBL)-producing bacteria such as K. pneumoniae. In as much as the Carbapenems are considered the last resort for managing life-threatening Klebsiella associated infections, it is sad to know that bacterial multi-drug resistance to carbapenems is on the increase [25].

The association between Klebsiella isolates and clinical specimen (source of specimen) is shown in Table 3. The result showed that there was an association between clinical specimen and Klebsiella spp (p-value = 0.018 <0.05).

Table 3: *Association Between Klebsiella spp and Clinical Specimen.*

	Chi Square (χ2)	*p*-value	Significance
Clinical Specimen Vs Klebsiella	21.473	0.018	Sig

Conclusion

Our findings indicated a high prevalence of MDR Klebsiella. This emerging challenge of MDR Klebsiella pose a significant threat to public health in Federal Medical Centre Umuahia and the communities in southeast Nigeria in general. These findings help as justifiable reasons for constant surveillance and necessary actions to address the challenges of MDR Klebsiella. The observations call for strict policy on the regulation of antibiotic use and inappropriate prescription of antibiotics as antibiotic stewardship will reduce the emergence of antibiotic resistance.

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