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Research Article

Prevalence and Antimicrobial Susceptibility Pattern of *Klebsiella Pneumoniae* in Sputum Samples of Patients Attending Aminu Kano Teaching Hospital of Kano State, Nigeria

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Abstract

Klebsiella pneumoniae is one of the commonest bacterial isolates in infectious respiratory tract conditions and also an important cause of infection in persons with an impaired immune system. It can spread rapidly between patients in healthcare settings and is a frequent cause of hospital outbreaks. The increasing prevalence of antimicrobial-resistant *K. pneumoniae* is a public health concern globally. However, data on this subject matter are scarce in the study area. This study was carried out to determine the prevalence and antimicrobial susceptibility pattern of *K. pneumoniae* isolated from sputum, causing respiratory tract infection in a tertiary care hospital in Kano, Kano State, Nigeria. A total of 150 sputum samples were collected and tested using standard microbiologic procedures. Antibiotic susceptibility test was by modified Kirby –Buaer disk diffusion method. Data were analysed using Open Epi Info statistical package version 2.0 and Chi-square test was used for statistical analysis. The present study reveals 30% frequency of isolation of *K. pneumoniae* from sputum samples and their significance antibiotic resistance. Females and patients above 60 years of age were more affected by the infections. *K. pneumoniae* was found to be resistant to Cotrimoxazole and penicillin derivatives but most sensitive to Levofloxacin, Ciprofloxacin, Gentamicin and Ofloxacin. There is need for increased enlightenment campaign about prevention/control of *K. pneumoniae* to checkmate the spread of associated infections, morbidity and mortality

Keywords: *Klebsiella pneumoniae*; Antimicrobial Susceptibility Testing; Respiratory Tract Infection; Antibiotic Resistance; Sputum

Introduction

Lower respiratory tract infection (LRTI) is an important cause of morbidity and mortality among humans affecting all age groups worldwide [1]. It describes a range of symptoms and signs, which vary in severity from non-pneumonic LRTI in young healthy adults to pneumonia or life threatening exacerbation in patients with severe, disabling chronic obstructive pulmonary disease (COPD)[2]. Lower respiratory tract infection, along with pneumonia, constitute a disease of poor-resourced countries, have an incidence of about 20–30% in those countries as compared to 3–4% in developed countries [1, 2]. Most common lower respiratory infections are acute tracheobronchitis, acute exacerbations of chronic bronchitis, pneumonia and common causative agents include Streptococcus pneumoniae, Haemophilus influenzae, Enterococcus spp, Klebsiella pneumoniae and many others [2].

Klebsiella pneumoniae is the second most popular member of the aerobic bacterial flora of the human intestine. It is the most commonest causative agent of healthcare-associated and community- acquired lower respiratory tract infections. In both community and hospital settings, the resistant strains are emerging as a worldwide threat even in common infections. It is also known to produce community- acquired pneumonia especially in chronic alcoholics, urinary tract infection, wound infections, blood infections (sepsis) and infections in the intensive care unit [3]. *Klebsiella pneumoniae* is known to have several virulence factors such as capsular polysaccharides, lipopolysaccharide (LPS) and iron-scavenging systems (siderophores). Healthcare-associated isolates are frequently resistant to numerous antibiotics as a result of the acquisition of multidrug resistance (MDR) plasmids [4].

Currently, drug resistance to human pathogenic bacteria is frequently being reported worldwide. However, the situation is alarming in both developing as well as developed countries due to increasing abuse and injudicious use of antibiotics in spite of pharmaceutical industries producing large number of newer antibiotics in the last three decades [5]. Microorganisms develop resistant to both older and newer antibiotics. Bacteria that are known to have an increased ability to transmit and acquire resistance to these therapeutic drugs can also transfer the resistance from one bacterium to other bacteria, thus posing a serious threat to public health by increasing morbidity and mortality [6]. Antibiotics were considered to be the most effective therapeutic agents to combat microbial infections. But as a result of the overuse and irrational use of antibiotics, there have been an emergence and spread of multidrug resistant strains among different groups of microorganisms. Infections resistant bacteria are emerging threat all over the world both as hospital-acquired as well as community-acquired microorganisms resulting in treatment failure and poor prognosis [7].

Therefore, it is essential to understand the antimicrobial susceptibility pattern of *K. pneumoniae* which shows variations in different geographical settings, in order to implement effective control measures and prevent rapid spread of drug resistance strains among the populace [4].

The present study was carried out to assess the prevalence of *K. pneumoniae* in sputum of patients (as there has been increasing outbreak of infection caused by this organism at this center) from this tertiary hospital and also to carry out a rational selection of antimicrobials useful in the treatment of *K. pneumoniae* –associated infections in our hospital to enhance good prognosis.

Materials and Methods

Study population

Subjects in this study included 150 patients aged 10 years and above (age range, 10-78 years; mean age, 32+-1.4years) presenting with respiratory tract infection and attending the Aminu Kano Teaching Hospital (AKTH), Kano, a tertiary healthcare center located in the northwestern region of Nigeria.

Ethical consideration

Ethical clearance was obtained from the medical ethics committee of the hospital (AKTH) before the commencement of the study. Signed or thumb printed of written informed consent was obtained from each patient before taking the specimen. This was interpreted into local languages of Hausa, Fulani, Yoruba and Ibo etc. for easy communication.

Materials

These include sterile universal containers, incubators, autoclave, Bunsen burner, Conical flasks, Petri-dishes, wire loop, weighing balance, nutrient agar powder, MacConkey agar powder, Brain heart infusion broth, Thioglycollate infusion broth, sterile blood, deionized and distilled water, peptone water, Nutrient broth, 0.5 MacFarland standard, urea, citrate, Kovac's reagent, Oxidase reagent, multiple antibiotic discs, Gram stain solutions, glass slides etc; these were utilized during the study [2].

Sample collection

Early morning sputum specimens "before eating" were aseptically collected in to sterile containers from 150 patients attending Aminu Kano Teaching Hospital, Kano, Nigeria between January and June, 2017. These samples were immediately transported to Microbiology laboratory unit of the hospital for analysis.

Sample processing

The purulent samples collected were aseptically, and first, washed in 5ml sterile physiologic saline and qualitatively cultured on Blood agar (Oxoid, England) and Chocolate agar in duplicates. Optoching sensitivity disc (5micogram, Merck, USA) was placed on inoculated Blood agar to detect the growth of *K. pneumoniae* on it. Bile solubility test was also performed on the organism.

In addition, each of the purulent sputum sample was inoculated on to the surface of prepared Blood and Chocolate (heated blood) agar plates using sterilized wire loop. The Blood and Chocolate agar plates were then incubated aerobically and in carbondioxide enriched atmosphere at 370C for 24hours. After incubation, the plates were observed for the following colonial morphological characteristics; growth of the pathogens, size, shape and of the colony, elevation, odor, pigmentation and hemolysis mucoid appearance. Gram reaction was carried out in order to differentiate the bacteria into gram –positive and gram –negative and also to identify the shape of the organisms [8].

Sensitivity screening

Antibiotic susceptibility of the organism was determined using modified Kirby Bauer disc diffusion method as proposed by CLSI (2016) [14]. Plates were inoculated by dipping sterile cotton swabs into the suspension of the overnight growth of the organism prepared to a density of 0.5 McFarland's standard. The excess liquid from the swab was expressed; and swab was then used to inoculate the surface of Mueller-Hinton agar by spread method. Antibiotic discs (Oxoid, England) were aseptically placed on the inoculated plates and were incubated overnight at 37^0C for 18-24 hours. Sensitivity and resistance pattern of bacterial isolates were evaluated by measuring the zone of inhibition (mm) of the antibiotics via underneath the plates. Sensitive and resistant bacteria were recorded as prescribed by CLSI (2016)[14].

Statistical analysis

The data generated from this study were organized into contingency tables and were analyzed using Open Epi Info statistical package Version 2.0. The proportions and ratios were compared using the Pearson's Chi square (χ^2) test. A p- value equals to or less than 0.05 ($p \le 0.05$), was taken as statistically significant. Tables and charts were used where necessary.

Results

During the 6 months period, a total of 150 sputum samples were processed for culture and sensitivity testing.

Sputum samples of patients between the age groups (10 years to >60years) both sexes were processed. A total of 45 Klebsiella pneumoniae were isolated thus culture positivity was 30.0% (Table 1).

S/no	Pathogen	Total no. of samples (%)	Positive samples (%)	Negative samples (%)
1	*Klebsiella pneumoniae*	150 (100)	45 (30.0)	105 (70.0)

Table 1: The distribution of *Klebsiella pneumoniae* isolated from the sputum samples (n=150).

Of the 45 *K. pneumoniae* isolates, 17 (37.8%) were from males and 28 (62.2%) were from female patients (Table 2). Isolation rate was highest in patients aged above 60 years (33.3%) followed by 50-59 years (20.0%). The lowest rate of isolation (2.2%) was observed among patients aged 10-19 years. Only 30.0% of the isolates were *K. pneumoniae*, while 70.0% were non-*K.pneumoniae* isolates.

Age group	Total (%) 150 (100)	Positive (%)Samples n=45 (30%)	Negative (%)Samples n=105 (70%)
10-19	7 (4.7)	1 (2.2)	6 (5.7)
20-29	27 (18.0)	5 (11.1)	22 (21.0)
30-39	37 (24.7)	8 (17.8)	29 (27.6)
40-49	28 (18.7)	7 (15.6)	21 (20.0)
50-59	29 (19.3)	9 (20.0)	20 (19.0)
>60	22 (14.6)	15 (33.3)	7 (6.7)
Total	150 (100)	45 (100)	105 (100)
Chi-square (χ^2) = 19.38; p-value= 0.0016 ($p<0.05$)			
Sex			
Male	82 (55.0)	17 (37.8)	65 (61.9)
Female	68 (45.0)	28 (62.2)	40 (38.1)
Total	150 (100)	45 (100)	105 (100)
Chi-square (χ^2) = 7.399; p-value= 0.00653 ($p<0.05$)			

Table 2: The Distribution of the Isolates by Age Groups and Gender (n=150).

Patients who had cough and produced sputum had the highest proportion (15.6%) of isolation of *K. pneumoniae*, while patients who presented with tachypnea had the lowest isolation proportion (6.7%) (Table 3).

The isolates were most susceptible to Levofloxacin (91.1%) and Ciprofloxacin (82.2%); but were most resistant to Amoxicillin- (82.2%) and Cotrimoxazole (75.6%) (Table 4).

The most probable useful drugs for treatment include: Levofloxacin (91.1%), Ciprofloxacin (82.2%), Gentamycin (71.1%), Ceftriazone (64.4%), and Ofloxacin (64.4%) (Table 5).

Clinical Features	Total (%) n=150 (100)	Positive (%) Samples n=45 (30%)	Negative (%) Samples n=105 (70%)
Fever	8 (17.8)	6 (13.3)	2 (4.4)
Chills	7 (15.6)	5 (11.1)	2 (4.4)
Rigors	6 (13.3)	3 (6.7)	3 (6.7)
Cough	9 (20.0)	7 (15.6)	2 (4.4)
Sputum	9 (20.0)	7 (15.6)	2 (4.4)
Tachypnea	6 (13.3)	3 (6.7)	3 (6.7)
Total	45 (100)	31 (68.9)	14 (31.1)
Chi-square (χ^2) = 2.822; p-value= 5.24e-5($p>0.05$)			

Table 3: Distribution of Isolates Showing Relationship with Observed Clinical Features (n=150).

Antibiotics (µg)	Sensitive (%)	Resistance (%)
Amoxiclav(10)	8 (17.8)	37 (82.2)
Ofloxacin (10)	29 (64.4)	16 (35.6)
Ciprofloxacin (30)	37 (82.2)	8 (17.8)
Ceftriaxone (10)	29 (64.4)	16 (35.4)
Levofloxacin (10)	41 (91.1)	4 (8.9)
Erythromycin (10)	26 (57.8)	19 (42.2)
Chloramphenicol (30)	16 (35.6)	29 (64.4)
Gentamicin (10)	32 (71.1)	13 (28.9)
Cotrimoxazole (25)	11 (24.4)	34 (75.6)
Tetracycline (30)	25 (55.6)	20 (44.4)

Table 4: Distribution of Isolates Showing the Antibiotics Susceptibility Pattern (n=45).

S/N	Name of Drug	Sensitivity (%)
1.	Levofloxacin	91.1
2.	Ciprofloxacin	82.2
3.	Gentamicin	71.1
4.	Ceftriazone	64.4
5.	Ofloxacin	64.4

Table 5: Probable Drug of Choice for *Klebsiella pneumoniae* Isolated from Sputum Samples.

Discussion

The prevalence of *K. pneumoniae* in the present study was 30.0%. This finding is higher than the result of study carried out by Shilpa *et al*. [8] who also reported a prevalence of 23.0%. These variations in prevalence may be due to differences in the geographical locations and sampled populations [9, 10] as well as other vari-

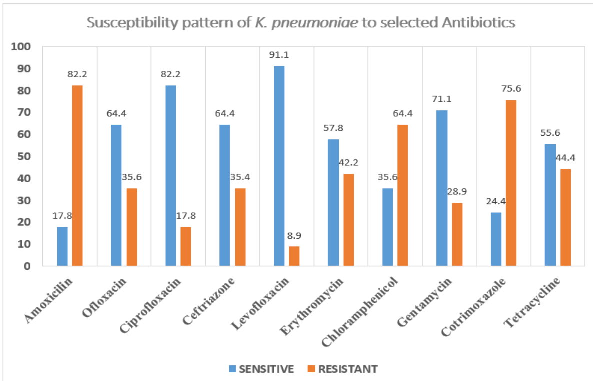

Figure 1: Percentage Susceptibility of *Klebsiella pneumoniae* to Selected Antibiotics.

ables and category of organisms sought for during the studies. For instance, detection of viral agents was included in other investigations as against only bacteria in the present study.

Klebsiella pneumoniae was found most prevalent in the age group of 50-59 year (21.3%) while Sharma et al. (2004) reported it in the age group of 51-60 year. This high incidence among the elderly group can be explained by a compromised or weakened immunity; since immune system in older patients become less effective owing to either malnutrition or underlying degenerative diseases such as diabetes mellitus, emphysema, uraemia [2, 9].

It is noteworthy that viruses like adenovirus, respiratory syncytial virus, parainfluenza virus and rhino virus are significant contributors to LRTI and were not looked for in our study due to limitation of resources and the predefined scope of the study. Likewise, for same reasons, other pulmonary pathogens such as *Mycobacterium tuberculosis, Mycoplasma* spp., *Chlamydia pneumocystis*, Fungi, *Legionella* spp and anaerobes which could not be cultured by routine methods, were not also included in this study.

It has been established that age and gender affect the prevalence of lower respiratory tract infections [11]. Humphrey *et al.* [12], in their study of prevalence of pneumonia and LRTI, reported a high prevalence in males than females. According to the report by Gauchan *et al*. [9] and Akingbade *et al*. [10], the reason for the high risk in males of LRTI is attributable to smoking, use of tobacco, alcohol consumption, etc., causing decreased local immunity in the respiratory tract due to defective mucociliary clearance, mucous plugging, airway collapse, respiratory muscle fatigue and the effect of medications used [1]. This finding is consistent with the report from a similar study by Akingbade *et al*. [10], in which 25.37% males were positive for LRTI, while 23.47% females were positive for LRTI.

In the present study however, more females (62.2%) than males (37.8%) presented with symptoms of LRTI in contrast with the previous studies. This, however, may be due to constant exposure to environmental source of infection as significant number of females now engages in outdoor socio-economic activities in this environment just like their male counterparts.

Klebsiella pneumoniae infection was more commonly seen in persons above 60 years (33.3%) and in persons aged 50-59 (20.0%). The least proportion (2.2%) was among the age 10-19 years. This finding is consistent with the reports of Shresth *et al*.(2013) [2] in which majority of infected patients belonged to age group of 61-75 years, and the findings of Serchan *et al*. (2007) who noticed that LRTI.

In the present study, patients who had cough and produced sputum had the highest culture positivity (15.6%) for *K. pneumoniae*, while patients who presented with tachypnea had the lowest (6.7%). There was a significant correlation (P<0.05) between patients that had cough and produced sputum and the culture positivity of *K. pneumoniae*. This outcome is possibly because of the close linkage of these clinical features to the infection with *K. pneumoniae* and thus could be useful in the characterization of the illness, and can also aid diagnosis.

The present study revealed significant resistance to diverse antibiotics. However, among the Fluoroquinolones tested, Levofloxacin (91.1%) was the most effective antibiotic against *K. pneumoniae*, followed by Ciprofloxacin (82.2%). Aminoglycosides such as Gentamycin (71.1%) also was equally effective against the isolates. A high resistance to penicillin such as Amoxicillin (82.2%) was recorded, followed by an equally high resistance to an Antifolate such as Cotrimoxazole (75.6%) and to Chloramphenicol (64.4%). The chromosomally encoded β- lactamases could be responsible for this intrinsic resistance to Amoxicillin. This finding is consistent with the report of a similar study by Shilpa *et al*. [8] in India, but is contrary to another similar study by Asati [5] which reported that Chloramphenicol was more effective (58.5%) against *K. pneumoniae* isolated from a tertiary hospital in Bhopal, India. This difference could be because of level of abuse of class, geographical and seasonal variations. It is also widely believed that the etiologic agents of LRTIs vary from area to area [11], so the susceptibility profile will also differ between geographical locations. Knowing the local susceptibility profile is important, as antimicrobial therapies for LRTIs are frequently empirical and presumptive [11].

There are reports covering high levels of resistance of *K. pne moniae* towards these antibiotics in other countries also. This may be due to the production of β-lactamase enzymes which cause the hydrolysis of β-lactam ring resulting in inactivation of β-lactam antibiotics [13]. Bacteria are known to have an increased ability to transmit and acquire resistance to therapeutic drugs and also transferring the resistance from one bacterium to another, and resistant bacteria pose a serious threat to public health thereby increasing morbidity and mortality [6].

Conclusion

Analysis of data generated in this study has indicated that the rate of isolation of *K. pneumoniae* observed among all age groups in this study was not significantly different. The culture positivity of *K. pneumoniae* observed among females in this study was significantly higher than that observed among their male counterparts. The culture positivity of *K. pneumoniae* was significantly different among the observed clinical features of the studied patients. The most probable drugs that may be used for the treatment *K. pneumoniae*-associated LRTIs as shown in this study were Levofloxacin, Ciprofloxacin, Gentamycin, Ceftriazone, and Ofloxacin.

Recommendations

We therefore recommend that Levofloxacin, Ciprofloxacin, Gentamycin, Ceftriazone, and Ofloxacin should be considered for use in the treatment of respiratory infection caused by *Klebsiella pneumoniae*; and the administration of the antibiotics should be guided by proper laboratory diagnosis to determine the most suitable among these probable drugs. There is need for public awareness campaign about prevention/control of *K. pneumoniae* to checkmate the spread of associated infections, morbidity and mortality.

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