

Original Research Article

Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolated from sputum samples amongst hospitalized adults in parts of Edo State, South-South, Nigeria

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Abstract

This study aimed at the identification, isolation and determination of antibiogram of *Klebsiella pneumoniae* isolates from other pathogens in sputum samples of hospitalized adults in five primary healthcare centers (PHCs) in parts of Edo North district - Nigeria over a period of twelve (12) months. *K. pneumoniae* has become one of the common causes of infections in hospitalized individuals with the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases. A total number of 70 pathogens were isolated from the 500 samples collected. Out of the 70 pathogens, 31(44.3%) were *Klebsiella pneumoniae*. *K. pneumoniae* was identified by standard microbiological techniques and API20E. Statistically analysis was based on $p < 0.05$, the isolation rate was higher in males (54.8%) than females (45.2%) and in 20-29yrs old subjects (35.5%), there was no statistically significance of *K. pneumoniae* occurrence among the different sex at $p > 0.05$ ($p = 0.306$) or age group $p = 0.141$. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and interpreted as per CLSI guidelines. High level susceptibility of *Klebsiella pneumoniae* was observed with levofloxacin (100%), (96.8%) to imipenem, (93.5%) to ciprofloxacin and gentamicin (87.1%). Isolates showed high intermediate resistance to Streptomycin (35.5%), high antibiotic resistance to ampicillin (80.6%), amoxicillin (74.2%) and streptomycin (64.5%) was reported. The present study reveals the frequency of isolation of *Klebsiella* from sputum samples and their tendency towards antibiotic resistance. The data from this study reveals the incidence of *klebsiella pneumoniae* strains in hospitalized patients and their tendency towards antibiotic resistance.

Keywords: *Klebsiella pneumoniae*, Antibiotic susceptibility, Sputum, Ekpoma

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INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium and of the family *Enterobacteriaceae*. It is a close relative of many familiar genera, such as *Citrobacter*, *Escherichia*, *Enterobacter*, and *Salmon-*

ella (Kumar *et al.*, 2011). It appears as a mucoid lactose fermenter on MacConkey agar.

Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated (inhaled), specific

ally to the alveoli (in the lungs) resulting in bloody sputum (Kumar *et al.*, 2011). In the clinical setting, it is the most significant member of the *Klebsiella* genus of the *Enterobacteriaceae* (From Wikipedia, the free encyclopedia). The increasing clinical incidence of antibiotic-resistant bacteria is a major global health care issue (Kumar *et al.*, 2011). Especially this is true when the infections with multidrug-resistant (MDR) pathogens impose a significant and increasing burden on both patients and healthcare providers (Braykov *et al.*, 2013). Among MDR pathogens, *Klebsiella pneumoniae* (*K. pneumoniae* or *KP*) is one of the world's most dangerous superbugs; and becoming resistant to virtually every antibiotic available today (Braykov *et al.*, 2013).

Klebsiella is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics (Carpenter, 1990) and showing characteristic radiographic abnormalities (Favre-Bonte *et al.*, 1995) due to a severe pyogenic infection which has a high fatality rate if untreated.

Antimicrobial resistance developed by microbes against antibiotics open serious debates and recognized as a serious problem by global medicinal and research community (Finch 2004; Kumari *et al.*, 2007). Many factors play in the emergence of resistance (WHO, 2012) from poor utilization of antimicrobial agents, to the transmission of resistant bacteria from patient to patient and from healthcare workers to patients and vice versa, to a lack of guidelines for a appropriate and judicious use of antimicrobial agents, to lack of easy-to-use auditing tools for restriction (Mahmoud Aly and Hanan H Balkhy., 2012).

The vast majority of *Klebsiella* infections, however, are associated with hospitalization. As opportunistic pathogens, *Klebsiella* spp. primarily attack immune-compromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. Sputum specimen is a sample of mucous secretion from the bronchi and lungs. It is essential that the specimen obtained be mucus from the lungs and bronchi and not saliva (Kumar *et al.*, 2011).

A person placed in a hospital as a patient for observation, treatment and management can be referred to as a hospitalized individual (Braykov *et al.*, 2013). Bartlett and Mundy (1995) suggested that hospitalized patients should be treated with second- and third-generation cephalosporin, with or without erythromycin. With the development of bacterial antibiotic resistance, as noted in Canada, where *H. influenzae* was found to be resistant to b-lactams (Hoban *etal.*,1995) and *S. pneumoniae* resistant to penicillin and macrolides (Davidson,1999), the need for careful selection of antimicrobial therapy has become essential. Moreover, it is estimated that as many as 30% of avoidable deaths

from pneumonia are due to incorrect selection of antimicrobial agents (Dubois and Brooks, 1988). Bacterial sputum cultures detect the presence of disease causing bacteria in people who are suspected of having bacterial pneumonia or other respiratory tract infections.

Aims/Objectives

To screen for antibiotic of choice for *K.pneumoniae* infection among hospitalized patients.

Area of study

Five (5) Primary health centers (PHCs), classified as high priority facilities (WHO surveillance standard) with a client flow of 40-45 patients/day in parts of Edo State, South-South, Nigeria.

Sample size

A total of 70 positive samples collected from 500 subjects (ages 20-65years) randomly selected were subjected to laboratory analysis.

Data analysis

Statistical analysis was carried out using Chi-square to compare the means of the male and female. One-way analysis of variance (ANOVA) was used to compare the means of the patients under the various age groups against the pathogens. A p-value<0.05 was considered as significant.

MATERIALS AND METHOD

Sample collection

Sputum samples were collected in the morning into dry, wide-necked leak proof sterile containers. The collection was done over a 12 month period (April 2016-April 2017).

Sample analysis

Samples collected were transported in geostyles containing frozen icepacks to the laboratory for analysis. They were examined macroscopically and microscopically, a smear was prepared from the most purulent material in the sputum and the slide was left to dry in the biosafety cabinet and then heat-fixed. A gram stain was done using standard methods, samples with >25 WBCs and <10 SECs were deemed appropriate for

Table 1. Types of Respiratory Pathogens Isolated

Age (Yrs)	SAMPLES (>25WBCs)	Number of Pathogens			
		<i>K.pneumoniae</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>Acinetobacter spp</i>
20-29	20	11	3	3	3
30-39	14	6	4	3	1
40-49	16	8	5	2	1
50-59	12	3	3	4	2
≥60	8	3	4	0	1
TOTAL	70	31	19	12	8

Table 2. Distribution of *Klebsiella pneumoniae* in Relation to Sex

Age (Yrs)	SEX		TOTAL N (%)
	MALE N (%)	FEMALE N (%)	
20-29	6 (35.3)	5 (35.7)	11 (35.5)
30-39	4 (23.5)	4 (28.6)	8 (25.8)
40-49	2 (11.8)	3 (21.8)	5 (16.1)
50-59	2 (11.8)	1 (7.1)	3 (9.7)
≥60	3 (17.4)	1 (7.1)	4 (12.9)
TOTAL	17 (54.8)	14 (45.2)	31 (100)

$\chi^2 = 0.581$, P-value = 0.306

Table 3: Distribution of *Klebsiella pneumoniae* in Relation to Age

Age (Yrs)	No. of subjects N (n)			OCCURRENCE (%)
	MALE	FEMALE	TOTAL N (n)	
20-29	10 (6)	10(5)	20 (11)	55.0
30-39	7 (2)	7 (4)	14 (6)	42.9
40-49	7 (4)	9 (4)	16 (8)	50.0
50-59	8 (2)	4 (1)	12 (3)	25.0
≥60	5 (3)	3 (0)	8 (3)	37.5
TOTAL	37 (17)	33 (14)	70 (31)	44.3

$\chi^2 = 6.903$, P-value = 0.071, (n)= number of positive *K.pneumoniae* isolates

Table 4. Antibiogram of *K.pneumoniae* isolates

ANTIBIOTICS	Conc (µg/ml)	N = 31		
		Susceptible N(%)	Intermediate N(%)	Resistant N(%)
Ampicilin	10	1(3.2)	5(16.1)	25(80.6)
Amoxycilin	25	5(22.6)	3(9.7)	23(74.2)
Cefotamine	30	22(71.0)	0(0.00)	9(29.0)
Ceftriaxone	30	24(77.4)	2(6.5)	5(16.1)
Ciprofloxacin	30	29(93.5)	0(0.00)	2(6.5)
Chloramphenicol	30	17(54.8)	4(12.9)	10(32.3)
Ertapenem	10	28(90.3)	3(9.7)	0(0.00)
Erythromycin	25	25(80.6)	1(3.2)	5(16.1)
Gentamycin	10	27(87.1)	0(0.00)	4(12.9)
Imipenem	10	30(96.8)	0(0.00)	1(3.2)
levofloxacin	5	31(100.0)	0(0.00)	0(0.00)
Streptomycin	20	14(45.2)	11(35.5)	20(64.5)
Tetracycline	30	12(38.7)	4(38.7)	11(35.5)

culture (Clinical Microbiology Procedures Handbook (CMPH)). Those that did not meet these criteria were discarded.

Blood chocolate, MacConkey agar and a selective medium: m-FC agar (*K.pneumoniae*) was used for isolation. Visible growth was observed after 48hrs for *K.pneumoniae*, all other organisms grew within 24hrs incubation. Further identification to specie level was achieved by the use of API20E strip.

Determination of Antibiogram of Isolates

Antibiogram of *K.pneumoniae* was determined using the disc diffusion method (NCCLS). The drugs and concentrations, chosen based on the frequency of prescription by doctors to pneumonia patients, the zone sizes were interpreted for strains that were sensitive, intermediate and resistance, using published guidelines (NCCLS, BBL,1990). Antibiotic sensitivity pattern was determined by the Kirby-Bauer disc diffusion method.

RESULTS

The data in table1 shows that out of the 70 confirmed

pathogens, *K.pneumoniae* was the most isolated 31(44.3%), followed by *S.aureus* 19(27.1%), *P. aeruginosa* 12(17.1%) the least was *Acinetobacter* spp 8(11.4%). There was no statistically significance in the isolation of the pathogens at $p > 0.05$ (0.071). Table 2 shows the incidence of *K.pneumoniae* occurrence amongst male and female subjects, there was a 54.8% (17) occurrence in males which was higher compared to females, 45.2% (14), this was not statistically significant at $p > 0.05$ (0.306). In Table 3, the age group 20-29yrs had the highest number of confirmed pathogens 20(28.6%) with *K.pneumoniae* accounting for the most isolated pathogen 11(55%), this was closely followed by age 40-49yrs with 16(22.9%) confirmed pathogens where 8(50%) were *K.pneumoniae* strains. The age ≥ 60 yrs had the least number of pathogens 8(11.4%), *K.pneumoniae* 3(37.5%), this was not statistically significant with a p-value of 0.141 ($p > 0.05$). The results of antimicrobial susceptibility testing of 31 *K.pneumoniae* strains isolated from patients to 13antibiotics are shown in Table 4. The most prevalent susceptible profile was levofloxacin (31 isolates, 100 %), followed by imipenem (30 isolates, 96.8 %) and ciprofloxacin (29 isolates, 93.5%). The highest resistance profile was recorded in ampicillin 25(80.6%) and amoxicillin 23(74.2%) closely followed by Streptomycin 20 (64.5%) which also exhibited the highest

intermediate pattern 11(35.5%) next was tetracycline 11 (35.5%) and chloramphenicol 10 (32.3%). Levofloxacin (a quinolone) and imipenem (carbapenem) were found to be effective antibiotics against *K.pneumoniae*.

DISCUSSION

A total of 70 pathogenic sputum samples from 500 patients were analyzed for *Klebsiella pneumoniae* isolate out of which a total number of 31 strains of *K.pneumoniae* were confirmed. Other pathogens obtained from this study were *S.aureus* (19), *P.aeruginosa* (12) and *Acinetobacter* spp (8) These isolates clearly represented clinically significant pathogens which were similar to the results obtained in India (Kumari *et al.*,2007) and also falls within the range of frequencies reported in other countries such as Nigeria (Okesola and Oni, 2009) and Turkey (EzgiOzilmaz *et al.*, 2005). The high incidence of *K.pneumoniae* isolation compared to other identified pathogens shows the involvement of the organism in nosocomial infections as well as in underlying respiratory infections where they could go undetected in sputum of such patients.

The high incidence of *K.pneumoniae* in males was attributed to their social life style such as smoking and alcoholism as earlier reported by Meatherall *et al.*, 2009 who equally observed an increased *K.pneumoniae* isolation rate amongst male smokers and alcoholics (45%). Increased outdoor activities (farming, commercial cyclists) as reflected in their medical history was suggestive of a high incidence in males in parts of Edo State. The age group 20-29 years showed the highest confirmed cases of *K.pneumoniae* isolation (55%), this group of subjects was found to be involved in social life styles and occupations that exposed them to adverse weather and environmental conditions as well as living in congested hostel rooms. The second highest (50%) was age 40-49yrs, some subjects in this group had history of long hospitalization while others had weight and dietary challenges. The least percentage occurrence was recorded in age 50-59years (25%), there was a high level of awareness on personal hygiene, health and diet management as well as dangers of self- medication among this age group. This probably accounted for the low incidence rate. This age group stratification of *K.pneumoniae* isolates is in agreement with the work of Badura *et al.*, 2016 who recorded a high incidence in ages 16-30 but our data was at variance with the findings of Shilpa *et al.*, 2016 that had the highest incidence in >60yrs and the lowest in 16-30years.

The finding that *K.pneumoniae* (GNEB) showed a 100% and 96.3% susceptibility to levofloxacin and imipenem respectively was not unexpected, considering that the drug target GNEB, this is similar to result obtained in the work of Mahmoud and Balkhy, 2012; Okesola and Oni, 2009. There was an observed high

resistant antibiotic profile to ampicillin (80.6%) and amoxicillin (74.2%). All *K. pneumoniae* isolates are naturally resistant to amoxicillin and ampicillin, due to a constitutively expressed chromosomal class-A β -lactamase (Livermore 1995).

The evidence that the fluoroquinolones are effective against these respiratory pathogens may make these drugs an alternative to the more widely-used macrolides and cephalosporin order to avoid the development of drug resistance. Also, the availability of drugs as once-daily treatments and the feasibility of intravenous-to-oral sequential therapy are advantageous (Mandell *et al.*, 2000). Often, this therapy is used when the laboratory findings are inconclusive or treatment is needed immediately, before laboratory diagnoses are available, forcing clinicians to implement "blind therapy". In our study, the frequency of antimicrobial resistance to various antibiotics was high in the strains. The high incidence of antibiotic-resistant *K. pneumoniae* isolates may be due to the widespread use of antibiotics. The transfer of resistance genes that may occur between species could lead to the construction of diverse resistance to usual antibiotics (Aslani *et al.*, 2011).

CONCLUSION

In conclusion, high antibiotic resistance of *K. pneumoniae* towards commonly used antibiotics are the major reasons for prolonged infections, increased hospitalization, increased cost of therapy and enhanced morbidity and mortality rates. Regular surveillance of antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics a major cause of emergence of drug resistance among pathogens. The data of this study may be used to determine trends in antimicrobial susceptibilities to formulate local antibiotic policies and assist clinicians in the rational choice of antibiotic therapy. The present data represents the effective firstline antibiotic agents against *Klebsiella pneumoniae* to be levofloxacin, imipenem and ciprofloxacin irrespective of age or gender.

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