Original article

Unreported Case Incidence of Klebsiella pneumonia among Patients Suspected of Respiratory Infections at Muritala Mohammed Specialist Hospital, Kano, Nigeria: A Retrospective review study

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ABSTRACT

Background: Klebsiella pneumoniae is one of the most important pathogenic bacteria in healthcare. It is a gram-negative, bacilli, nonmotile, and causative agent of many infectious diseases, such as pneumonia, sepsis, burns, wound infections, pyogenic liver abscesses, and urinary tract infections. Klebsiella pneumoniae affects mainly patients who have predisposing debilitating backgrounds. In Nigeria, Klebsiella pneumoniae is among the most common etiological agents of lower respiratory tract infections. This bacterium has been reported to be the second most common cause of urinary tract infections, with an increasing rate of drug resistance to many commonly used antibiotics.

Methods: Specimen isolation was carried out between the period of 7th September to 9th October 2016 by cultural techniques on McConkey and blood agar, microscopy, and a series of biochemical tests and antimicrobial susceptibility profiles of the isolates.

Results: A total of 96 bacterial colonies of 6 bacterial species were isolated. Staphylococcus aureus has the highest incidence of 27; Klebsiella pneumoniae with 18 incidences; Streptococcus pneumoniae 17; E. coli 15; staphylococcus epidermidis 10 incidence, and viridians streptococci 9 incidences respectively. Klebsiella pneumoniae has an 18% incidence. Ciprofloxacin, streptomycin, pefloxacin, and Tarivid have antimicrobial susceptibility on Klebsiella pneumoniae isolates, and ciprofloxacin has the highest antimicrobial activity on all the isolates.

Conclusion: Klebsiella pneumoniae is a gram-negative bacillus, non-motile and causative agent of lower respiratory infection among patients hospitalized at Muritala Mohammed specialist Hospital, Kano with incidence of 18%. Klebsiella pneumoniae isolates are highly sensitive to ciprofloxacin, compared with streptomycin, pefloxacin and tarivid which also showed a mild antimicrobial activity. Novel mitigating strategies and new antibiotics are needed in combating emerging threat of Klebsiella pneumoniae. Furthermore, epidemiological surveillance and control can reduce incidence and emergence of Klebsiella pneumoniae among hospitalized patients.

Keywords: *Klebsiella Pneumoniae, nosocomial infection and antimicrobial susceptibility.*

BACKGROUND

Klebsiella pneumoniae is a Gram-negative opportunistic nosocomial bacterial pathogen. It is involved in several localized and disseminated hospital-acquired infections such as burns infections, sepsis, respiratory and gastrointestinal tract infections, urinary tract infections, pyogenic liver abscesses, soft tissue and wound infection [1]. Also, the nonmotile baccili is a causative agent of many other infectious diseases, such as pneumonia, sepsis and urinary tract infections [2]. K. pneumoniae affects mainly patients who have predisposing debilitating backgrounds. In Nigeria, Klebsiella pneumoniae is among the most common etiological agents of lower respiratory tract infections [2]. This bacterium has been reported to be the second most common cause of urinary tract infections, with an increasing rate of drug resistance to many commonly used antibiotics. The emergence of carbapenem-resistant K. pneumoniae (CRKP) strains has become a critical challenge for public health worldwide due to their capacity to disseminate rapidly in the hospital environment. The rapid spread of carbapenem-resistant Klebsiella pneumoniae (CRKP), listed by the WHO as a critical priority pathogen, has become a global threat to human health due to high morbidity and mortality [2]. In Nigeria, Klebsiella pneumoniae was among the most common causes of lower respiratory tract infections [1].

Klebsiella pneumoniae, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections [3]. The principal pathogenic reservoirs for transmission of Klebsiella are the gastrointestinal tract and the hands of hospital personnel. These bacteria tend to cause nosocomial outbreaks because of their ability to spread rapidly in the hospital environment. These organisms have been isolated from abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum, and throat cultures. Klebsiella pneumoniae is an important nosocomial pathogen that has the potential to cause severe morbidity and mortality [3].

Klebsiella pneumoniae utilizes different resistance mechanisms to counteract the effects of antibiotics, such as the production of destructive enzymes, target alteration, efflux pumps, and porin loss. Therefore, hospital-associated infections with multidrug-resistant (MDR) strains of K. pneumoniae occur with high morbidity and mortality [3]. The emergence of extended-spectrum beta-lactamases (ESBL)-producing organisms was considered to be from the dissemination of clones of some epidemic strains along with the horizontal transmission of resistance gene-carrying plasmids among bacteria. The development and selection of multiple drug-resistant bacteria, such as ESBL producers, have also been attributed to the rise in the use of second and third-generation cephalosporins to treat K. pneumonia infections [3].

Carbapenemases are enzymes that are capable of hydrolyzing the newer carbapenem antibiotics used in the t reatment of MDRbacterial infections. Among these, Klebsiella pneumoniae carbapenemase (KPC), metallo-β-lactamases (VIM, IMP, NDM), and OXA-48 types of enzymes are the most common. Mobile genetic elements, including plasmids, transposons, and integrons, are involved in disseminating related encoding genes. ESBL and carbapenemase-producing organisms often acquire resistance to non-β-lactam antibiotics, including aminoglycosides and fluoroquinolones, resulting in multi-drug resistant properties [3].

OBJECTIVES

1. To investigate incidence of Klebsiella pneumoniae and antimicrobial susceptibility among patients suspected of respiratory infections at Muritala Mohammed specialist hospital, Kano

2. To determine antibiotic susceptibility on every organism isolated using 10 antibiotics on a multipurpose disc, containing ciprofloxacin (CPX) lOµg, streptomycin (S) 10µg, peflacine (pef) 10µg. Ampicilin (PN) 30µg, Tarivid (Ofx) 10µg, Gentamycin (CN) 10µg, Augmentin (AU) 30µg, Nadixic acid (NA) 30 µg, Ceporex (cep) 10µg, Septrin (SXT) 30µg.

METHODOLOGY

STUDY AREA

The study was conducted at the microbiology laboratory of the pathology department of Murtala Muhammad Specialist Hospital Kano, Nigeria, between 7th September to 9th October 2016.

STUDY POPULATION

A total number of 100 sputum samples were collected from patients with suspected respiratory infections at Murtala Muhammad Specialist Hospital Kano, Nigeria.

STUDY DESIGN

Sample Collection

The patients were given a clean dry, wide necked leak proof, container and requested to cough deeply to produce sputum specimen, preferably 'first morning sputum', and then transported to Microbiology and parasitology laboratory of the same hospital for microbiological and bioassay analysis.

Isolation and Identification of Bacteria

Sample were cultured by inoculating into blood and MacConkey agar and incubated at 37°C for 18-24 hrs. Growth on blood agar and MacConkey agar was identified by cultural, morphological appearance and biochemical tests such as (Catalase, Coagulase, Urease, citrate and Tripple iron Agar test) to confirmed Klebsiella Pneumoniae isolates.

Antimicrobial Susceptibility testing

All confirmed Klebsiella pneumoniae strains were tested against ten antibiotics by both disc diffusion and microdilution methods according to the CLSI guidelines [4]. The following antibiotic discs were used: ciprofloxacin (CPX) lOµg, streptomycin (S) 10µg, peflacine (pef) 10µg. Ampicilin (PN) 30µg, Tarivid (Ofx) 10µg, Gentamycin (CN) 10µg, Augmentin (AU) 30µg, Nadixic acid (NA) 30 µg, Ceporex (cep) 10µg, Septrin (SXT) 30µg.

A colony of the test organism was picked using a sterilized wire loop and streaked the surface of the medium in three directions rotating 16 plates approximately 60° to ensure even distribution. With the aid of sterilized forceps, a multipurpose disc was placed on the streaked organisms. The plate was inverted and incubated aerobically at 35-37°C for 24 hours. A ruler was used to measure the diameter. The diameters of inhibition zones were measured to the nearest millimeter using a ruler. Control strain K. pneumoniae ATCC 700603 was used in the testing to validate the results of disc diffusion.

Ethical Consideration

Ethical approval for this study was sought and obtained from ministry of Health, Kano state..

Informed written consent was obtained from the respondents after adequate explanation on the nature, purpose and benefit of the study before samples were collected. Confidentiality of respondents' information was assured by keeping the data in a secured place. Respondents were given the right to decline or withdraw from the study at any time.

respondents. Confidentiality and anonymity of the data were ensured throughout the study.

Data Analysis:

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics were used to summarize the socio-demographic characteristics of the participants. The mean and standard deviation of respondents' knowledge and attitudes were calculated. Inferential techniques, including chi-square and logistic regression, were used to determine relationships between variables. A probability value of $P < 0.05$ was considered statistically significant.

RESULT

A total of 100 samples were obtained from 56 male and 44 female, the age range of the patients was between 1 and 95 and the mean age was 36.15± 16.57. Majority of the patients 87 (87%) were Hausa, 5 (5.0%) were Yoruba, 3 (3.0%) were Igbo while the remaining 8(8.0%) belong to other ethnic groups such as Fulani and Nupe. Islam was the religion practiced by majority 92 (92.0%) of the patients while the remaining 8(8.0%) were Christians.

The incidence of klebsiella pneumoniae among elderly was 40%. Staphylococcus aureus were most isolated from the sample collected (27) while Viridians Streptococci have the lowest isolates.

Ciprofloxacin, Streptomycin, pefloxacin, and tarivid show high antimicrobial activity on the klebsiella pneumoniae isolates with 83.3% susceptibility each. Gentamicin, Ceporex, and Augumetin show a moderate activity of klebsiella pneumoniae with 61.1% susceptibility each. Septrin and nalidixic acid showed the least activity with 50% each.

It can be shown that klebsiella pneumoniae has the highest susceptibility pattern to all antibiotics followed by viridians streptococci followed by E coli. The majority of

Streptococcus pneumoniae isolates were resistant to antibiotics followed by Staphylococcus aureus. Streptococcus pneumoniae shows the highest resistance to antibiotics.

Ciprofloxacin shows the highest activity with 64% susceptible isolates then Gentamicin and Tarivid with 63% Susceptible isolates each, Ceporex showed 60% susceptible isolate, Nalidixic acid showed 53% susceptible isolate, Augumetin and Septrin shows 47% and 46% susceptibility each respectively.

Incidence of klebsiella pneumoniae among elderly

The incidence of klebsiella pneumoniae among elderly will be:-

Number of isolates × 100 = 18 × 100 = 40%

Number of elderly persons 45

Variables	Frequency	Percentage
Age group		
1 – 15	6	6.0
16 – 25	23	23.0
26 – 35	26	26.0
36 – 45	16	16.0
46 – 55	19	19.0
56- 65	4	4
66-75	4	4
76-85	1	1
86-95	1	1
Mean + SD	36.15± 16.57	
Gender		
Male	56	56
Female	44	44
Tribe		
Yoruba	5	5.0
Hausa	87	87
Igbo	3	3.0
Others	8	8.0
Religion		
Islam	92	92.0
Christianity	8	8.0

Table 1: Socio - Demography distribution of studied patients

Bacteria	Total number of samples	Frequency	Percentage (%)
Staphylococcus aureus	100	27	27
Klebsiella pneumoniae	100	18	18
Escherichia Coli	100	15	15
Streptococcus pneumoniae	100	17	17
Viridians Streptococci	100	9	9
Staphylococcus epidermidis	100	10	10
No growth	100	4	4
Total			100

Table 2 : The cumulative frequency of organisms isolated.

Antibiotic		Total number of susceptible isolates	The total number of klebsiella pneumoniae isolate	Percentage (%)
Ciprofloxacin (CPX)	10	15	18	83.3
Streptomycin (S)	30	15	18	83.3
Peflacine (PEF)	10	15	18	83.3
Ampicillin (PN)	30	11	18	61.1
Septrin (SXT)	10	9	18	50
Tarivid (OFX)	10	15	18	83.3
Gentamicin (CN)	30	15	18	83.3
Augumetin (AU)	30	11	18	61.1
Nalidixic acid (NA)	30	9	18	50

Table 3 : antimicrobial activity on the klebsiella pneumoniae isolates

Antibiotic	K pneumoniae	S aureus	E Coli	S epidermidis	Viridians Streptococci	Streptococcus pneumoniae
CPX	83.3	51.9	66.7	80	66.7	35.3
S	83.3	59.3	66.7	80	66.7	11.8
PEF	83.3	59.3	60	80	66.7	29.4
PN	61.1	59.3	46.7	30	66.7	23.5
SXT	50	55.6	26.7	60	66.7	17.6
OFX	83.3	63	73.3	80	66.7	29.4
CN	83.3	55.6	66.7	80	66.7	23.5
AU	61.1	51.9	40	30	66.7	35.3
NA	50	59.3	33.3	60	66.7	35.3
CEP	61.1	59.3	46.7	70	66.7	29.4

Table 4: The percentage susceptibility of each isolate to a particular antibiotic.

Key : Ciprofloxacin (CPX), Streptomycin (S), Peflacine (PEF), Ampicillan (PN), Septrin (SXT), Tarivid (OFX), Gentamicin (CN), Augumetin (AU), Nalidixic acid (NA) and Ceporex (CEP).

Antibiotics	Number of the susceptible organism	Total number of tested organisms	Percentage (%)
CPX	61	96	64
S	45	96	47
PEF	60	96	63
PN	58	96	60
SXT	57	96	59
OFX	48	96	50
CN	51	96	53
AU	57	96	59
NA	60	96	63
CEP	44	96	46

Table 5: The cumulative percentage of activity of each antibiotic on isolates.

Key : Ciprofloxacin (CPX), Streptomycin (S), Peflacine (PEF), Ampicillan (PN), Septrin (SXT), Tarivid (OFX), Gentamicin (CN), Augumetin (AU), Nalidixic acid (NA) and Ceporex (CEP).

DISCUSSION

In this study, klebsiella pneumoniae and other bacteria were isolated from the mucous samples. 18% incidence klebsiella pneumoniae was isolated as one of the etiological agents of respiratory and nosocomial infections. This is similar to a finding at Ilorin, North central Nigeria where 14.7% klebsiella pneumoniae incidence was reported [5]. However, lower than the current findings in this report. The high incidence rate of klebsiella pneumoniae can be attributed to the fact that all isolates were collected from patients hospitalized, which indicates low or lack of prevention

measures against nosocomial infection. In the same vein, the result of this study shows the incidence rate of klebsiella pneumoniae to be 18% and 40% among elderly persons, this is also similar to the preponderance of community acquire pneumonia in individuals older than 65years [5]. This can be explained by the high risk of micro aspiration in elderly patients during sleep from depressed cough and glottis reflexes, the less efficient airway muscularly clearance, the presence of comorbidities which serves as risk factors for pneumonia as well as waning immunity in the unique population. Contrarily, the incidence of klebsiella pneumoniae is low in children population (1-15) years age group (6%) compared to elderly population (>65) years age group. This corroborates report from Claassen-Weitz et al, where pneumococcus number of Hib associated lower respiratory tract infection deaths among young children has significantly decreased with implementation of Hib-conjugate vaccines [6].

To addresses critical knowledge gaps regarding the characteristics of K. pneumoniae in Nigeria and presents 5 Klebsiella quasipneumoniae genomes, which without whole genome sequence (WGS) cannot be differentiated from K. pneumoniae in our setting, even at the reference laboratory level [7]. Although the VITEK2 identification system is innovative and is currently used in reference microbiology laboratories worldwide, the accuracy of its identification is dependent on the isolate's phenotypic variability (e.g, variation in colony morphology) and the completeness of the system's identification database [8] The misidentification of K. quasipneumoniae and Klebsiella variicola as K. pneumoniae has been reported previously, and thus is not unique to our setting [8]. Afolayan AO. et al, and other data revealed that multiple Klebsiella quasipneumoniae lineages, including resistant ones, circulate in Nigeria [7,9].

The result of this study revealed that Klebsiella pneumoniae infection was seen more in males than females. Contrarily, previous study reported that klebsiella pneumoniae causes infection in female than male [7]. This can be determined by virulent nature of microorganism, immunity status of individual and comorbidity factors.

Microbiological findings revealed that staphylococcus aureus (27%) was the highest etiological pathogen isolated from sputum samples, while viridians streptococci (9%) have the lowest isolates. However, this is contrary to report by Ojuawo et al, where streptococcus pneumoniae was the main isolates. The ascendancy of staphylococcus aureus in this study stand in need of further investigation into epidemiological trends of etiological agents of respiratory and nosocomial infection in hospitalized patients [5].

Klebsiella pneumoniae isolates were generally susceptible to Streptomycin, Ciprofloxacin, tarivid among the antibiotics that show highest activity on klebsiella pneumoniae isolates. Ciprofloxacin shows the highest activity with 64% susceptible isolates then Gentamicin and Tarivid with 63% Susceptible isolates each, Ceporex showed 60% susceptible isolate, Nalidixic acid showed 53% susceptible isolate, Augumetin and Septrin shows 47% and 46% susceptibility each respectively. This contradicts findings of Ayandele et al, where high resistance rates of strains of Escherichia coli and Klebsiella sp. to commonly used antibiotics, such as streptomycin (98%), cloxacillin and oxacillin (96%), and colistin (84%), with relatively lower resistance rates to ciprofloxacin (66%) [10]. This study also contradict report from Kenya where the percentage of resistance to the antibiotics differed among the Salmonella isolates, 50% of the isolates were resistant to amoxicillin.

Resistance to co-trimoxazole, tetracycline and streptomycin was 28%, 11% and 6% respectively [11]. None of the isolates were resistant to gentamicin, nalidixic acid, ciprofloxacin and chloramphenicol. It is common knowledge that some antibiotics were active in vitro but did not show any activity in vivo or some showed high activity in vivo but did not show any in vitro. Similarly, Most of the Gram-negative isolates from neonates with sepsis were resistant to at least one β-lactam and one aminoglycoside (597/885; 67%), as has been reported previously for cohorts in India [12] and 26 countries in Africa [13].

World Health Organization guidelines stipulate ampicillin plus gentamicin as the first line of empirical treatment for neonatal sepsis and third-generation cephalosporins as the second line of treatment. Of note, many of the cultured isolates from this report were resistant to ampicillin treatment, meaning that treatment options are unlikely to be curative [14].

CONCLUSION AND RECOMMENDATION

Conclusion: Klebsiella pneumoniae is a gram-negative bacillus, non-motile and causative agent of lower respiratory

infection among patients hospitalized at Muritala Mohammed specialist Hospital, Kano with incidence of 18%. Klebsiella pneumoniae isolates are highly sensitive to ciprofloxacin, compared with streptomycin, pefloxacin and tarvid which also showed a mild antimicrobial activity.

To improve Klebsiella pneumoniae prevention efforts, the following recommendations are proposed:

Novel mitigating strategies and new antibiotics are needed in combating emerging threat of Klebsiella pneumoniae.

Epidemiological surveillance and control can reduce incidence and emergence of Klebsiella pneumoniae among hospitalized patients.

Improved Accessibility to Healthcare Services: Expanding healthcare infrastructure, providing better road networks, and ensuring the availability of Klebsiella pneumoniae prevention drugs will mitigate it incidence.

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