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Antibiogram of *Klebsiella pneumoniae* among Pulmonary Tuberculosis Suspects Attending Infectious Diseases Hospital, Kano

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Authors' contributions

This work was carried out in collaboration between both authors. Author AM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SDS managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to determine the antibiogram of *Klebsiella pneumonia* (*K. pneumoniae*) among pulmonary tuberculosis (TB) suspected patients attending IDH Kano, Kano metropolis, Nigeria.
Study Design: The study is descriptive cross sectional study.
Place and Duration of Study: In this research of the 300 TB suspected patients referred to infectious diseases hospital Kano were used for the study. Sputum specimens collected were analysed at Medical Laboratory Science Department, School of Health Technology Kano, Kano state, Nigeria. Sputum samples were stored at 4ºC if not analysed at the same day. The work was carried out between January to June, 2017.
Methodology: Early morning sputum samples were examined microscopically and cultured on blood, chocolate and MacConkey's agars, followed by confirmation of presumptive colonies using different biochemical tests. Antibiotic susceptibility studies were done by the disc diffusion method

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using Ceftriaxone (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ofloxacin (5µg), Cloxacillin (5µg), Co-trimoxazole (25µg), Augumentin (30µg), Cefuroxime (30µg) and Ceftazidime (30µg).

Results: Of the 28 isolates Ceftriaxone 28(100.0%), Ciprofloxacin 20(71.5%), Gentamicin 12(85.7%) and Ofloxacin 11(78.6%) had significant ($P > 0.05$) activity against *K. pneumoniae*, followed by Cefuroxime 18(64.3%) and Ceftazidime 16(57.1%) with moderate activity while Augumentin 4(12.3%), Cloxacillin 0(0.0%), and Co-trimoxazole 0(0.0%) had poor activity. against *K. pneumoniae* isolates. Out of 224 Bacteria isolated 60 (26.8%) were *Streptococcus pneumoniae* (*S. pneumoniae*) 20(16.1%) were *Staphylococcus aureas* (*S. aureas*), 28(12.5%) were *K. pneumoniae*, 24(10.7%) were *Haemophillus influenzae* (*H. influenzae*), 24(8.9%) were *Streptococcus pyogenes* (*S. pyogenes*) and 56(25.0%) were other bacterial isolates. However, their relationship was not statistically significant ($P < 0.05$).

Conclusion: In conclusion, *Klebsiella pneumoniae* was recorded to be the most prevalent 28(12.5%) bacterial isolate among TB suspected patients. All the *K. pneumoniae* isolates resisted to co-trimoxazole and cloxacillin. While Ceftriaxone 28(100.0%), Gentamicin 24(85.7%), Ofloxacin 22(78.6%), Ciprofloxacin 20(71.5%), remained the most effective against *K. pneumoniae* isolates tested.

Keywords: Antibiogram; antibiotics; Klebsella pneumoniae; TB; IDH; Kano metropolis.

1. INTRODUCTION

Lower Respiratory Tract Infections (LRTIs) are some of the most prevalent diseases of humans worldwide [1]. These diseases directly result in about 7 million deaths annually in persons of all ages [2]. According to WHO, tuberculosis and acute lower respiratory tract infections are two among the 6 leading causes of death [3]. Of all the deaths caused by acute respiratory tract infections, LRTIs account for 20 – 24% [4]. Tuberculosis is one of the most debilitating bacterial pulmonary infections and it presents with similar signs and symptoms as LRTIs of other etiologies. Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of Mycobacteria, usually *Mycobacterium tuberculosis* in human [5]. Tuberculosis usually attacks the lung but can also affect other parts of the body. Its mode of transmission is respiratory aerosoles generated by patients cough, sneeze or spit [6].

The multi resistant phenotype emerges with a sequential acquisition of mutations in several loci of separate gene [6,7]. Knowledge of this mechanism of resistance permits the development of techniques for the early detection of resistance strains, thereby making proper control possible [6]. It has been found that tuberculosis patients whose immune system is compromised are prone to many secondary bacterial infections. Some of the bacteria capable of causing such infections were found to be resistant to many antibiotics such as cefazolin, ceftazidine, imipenem, carbenicillin by producing enzymes such as extended-spectrum-beta-lactamases (ESBLs). Such isolates may cause serious opportunistic infections especially in tuberculosis patients. Resistance to such enzymes in clinical isolate has lead to little or no treatment option which consequently leads to death. A third of the world's population is said to be infected with *Mycobacterium tuberculosis* [8] with about 95% of cases coming from developing countries [9].

There has however been such a great emphasis on tuberculosis almost suppressing the amount of attention given to other lower respiratory tract infections which are also major health challenges both among communities and in hospitalised patients [4]. As populations evolve and immunocompromising conditions become more prevalent many more people become at risk of LRTIs [1]. The HIV pandemic has even worsened morbidity and mortality due to LRTIs. The lungs are said to still be the number one infection site for people living with HIV [10]. LRTIs are reported to cause about 70% of illnesses in AIDS patients. The outcome of other non-mycobacterial bacterial infections especially in HIV immunecompromised patients may be fatal if not properly diagnosed and treated. During the time taken to rule out a TB diagnosis, the patient invariably deteriorates with an infection which may have been adequately treated if bacterial culture and sensitivity was done. Different studies have shown different bacterial pathogens to be implicated to diverse extents but generally the major pathogens encountered are *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,*

Staphylococcus aureus, Moraxella catarrhalis, Streptococcus pyogenes and some other enteric gram negative rods [10].

The objectives of this research are, to isolate and identify *Klebsiella pneumoniae* among Pulmonary TB suspects, to perform the susceptibility test of the commercially available antibiotics against clinically isolated *Klebsiella pneumoniae*, and investigate the major risk factors for developing lower respiratory tract infections among patients

2. MATERIALS AND METHODS

2.1 Hospitals

The infectious diseases hospital (IDH) is situated within Kano metropolis. The selected hospital is reference hospitals in the state where people from various parts of the state and neighboring states of various occupations attend. The study hospital gave more than 70% treatment of pulmonary tuberculosis in the state at large.

2.2 Specimens

Sputum specimen collected from each patient suspected with pulmonary tuberculosis infections in Kano, North-West, Nigeria were used as samples for the study. However, three hundred (300) sputum specimens were collected and processed in this research.

2.3 Test Organisms

Klebsilla pneumoniae isolated in sputum specimens collected among patients suspected with pulmonary tuberculosis infections in infectious diseases hospital Kano, North-West, Nigeria were used as the test organisms.

2.4 Criterion for Selection of Patients

2.4.1 Inclusion criterion of patients

Patients (in and out) who patronised the infectious diseases hospital, Kano with some or all clinical sign and symptoms of pulmonary tuberculosis such as cough lasting more than two weeks after commencing antibiotics, fever, night sweats, chest pain and other symptoms suggestive of lower respiratory tract infections were used for the study.

Any patient (in and out) who brought his sputum specimen to the laboratory reception infectious diseases hospital, Kano for pulmonary tuberculosis test was used for the study.

2.4.2 Exclusion criterion of patients

Patients (in and out) who patronised the infectious diseases hospital, Kano with without clinical sign and symptoms of pulmonary tuberculosis such as cough lasting more than two weeks after commencing antibiotics, fever, night sweats, chest pain and other symptoms suggestive of lower respiratory tract infections were excluded in this study.

Any patient (in and out) who brought other specimen (i.e. not sputum) to the laboratory reception infectious diseases hospital, Kano not for pulmonary tuberculosis test was excluded in this study.

2.5 Isolation and Identification of Bacterial Pathogens in Sputum Specimens

2.5.1 Presumptive isolation of the bacterial pathogens

Sputum specimens were inoculated onto blood, chocolate and MacConkey's agars. The inoculated plates were incubated at 37°C for 24-48 hours aerobically, except for chocolate agar plates which were incubated for 24-48 hours at 37°C under reduced atmospheric condition of (5-10% CO_2.) The Clinical isolates of *Klebsiella pneumoniae* were identified by morphological characteristics on MacConkey agar media. The large mucoid colonies obtained from MacConkey's agar plate were processed for Gram staining. In Gram staining, all the isolates were negative and rod shaped [11]. Standard microbiological techniques and biochemical tests were used to confirm the pathogenic bacteria. Gram staining was done to investigate bacterial pathogens [12].

2.5.2 Biochemical characterisation of *Klebsilla pneumoniae*

Isolation and identification of organisms was carried out as described by ISO [13]; Habtamu et al. [14]; OIE [15]. A 24 h pure culture of each isolate was used to determine their gram stain reaction. The biochemical tests employed were IMViC (Indole, Methyl red, Voges Proskeaur, Citrate), H_2S production on TSI agar, Motility test, Oxidase test, Urease test, fermentation of Lactose and Mannitol. For biochemical tests standard procedures were used [11].

2.6 Test of Antibacterial Sensitivity of the *Klebsilla pneumoniae* Isolates

In-vitro susceptibility of *Klebsilla pneumoniae* isolates to various nine (9) routine antibiotics selected were tested by the standard disc diffusion technique [16].

2.6.1 Standardisation of inoculum

This was done as described by CLSI guidelines [16]. Pure culture of identified *Klebsilla pneumoniae* isolate (s) from an 18-hour plate culture was selected. Sterile wire loop was used to pick 2 to 3colonies of each *Klebsilla pneumoniae* and emulsified in 5 ml of sterile normal saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic systems, UK) after calibration. Adjustment was made with extra inoculum or diluents, if necessary, until 0.5 McFarland standards were obtained. Fifty microliter of the broth was further transferred into 5 ml of Mueller-Hinton broth (Oxoid, UK) in a tube [16].

2.6.2 Inoculation of test plates

Optimally, within 5 to 10 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the standardised suspension in Mueller-Hinton broth. The dried surface of a 20 ml Mueller-Hinton agar plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking with the cotton swab over the entire sterile agar surface. The inoculated plates were air dried at 37ºC to allow for any excess surface moisture to be absorbed before applying the antibiotic discs [16].

2.6.3 Application of discs to inoculated agar plates

All positive cultures of *Klebsilla pneumoniae* isolated from sputum samples were tested *in vitro* for susceptibility to different antibiotics by agar diffusion technique as described by Kirby-Bauer [17] and WHO [18]. This was carried out according to WHO protocol [19]. The susceptibility testing of *Klebsilla pneumoniae* isolates were carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to nine (9) routine antibiotics selected namely: Ceftriaxone (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ofloxacin (5µg), Cloxacillin (5µg), Co-trimoxazole (25µg), Augumentin (30µg), Cefuroxime (30µg) and Ceftazidime (30µg)

2.7 Statistical Analysis of Results

Statistical Package for Social Science (SPSS) version 14 was used [20]. Descriptive statistics were used to categorical (frequency percentages) variables. Chi-square test analysis was use to determined association between the susceptibility/resistant rate of *Klebsiella pneumoniae* isolates and antibiotics activities.

3. RESULTS

Table 1 shows the numbers of each *K. pneumoniae* and other bacterial isolated from TB suspects in according to months (January to June, 2017). A total of 228 bacterial isolates were isolated from 300 patients. Out of 224 Bacteria isolated 60 (26.8%) were *S. pneumoniae*, 36(16.1%) were *S. aureas*, 28(12.5%) were *K. pneumoniae*, 24(10.7%) were *H. influenzae*, 20(9.0%) were *S. pyogenes* and 56(17.0%) were other bacterial isolates. However, their relationship was not statistically significant ($P < 0.05$) (Table 1).

Table 2 indicated the occurrences of *K. pneumoniae* and other bacterial isolated from TB suspects. *S. pneumoniae* had the highest occurrence rate 60(26.8%) followed by *S. aureus* 36(16.1) then *K. pneumoniae* 28(12.5%). The pathogen with lowest occurrence rate is *S. pyogenes* 20(9.0%). The relationship between bacterial isolates was not statistically significant ($P < 0.05$) (Table 2).

Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years were found to be more susceptible to *Klebsiella pneumoniae* infections with 12 representing 4.0% followed in that order by 11-20 years (7), 31-40 years (4), ≤10 years (3) and >40 years (2) age groups, representing 2.3%, 1.3%, 1.0% and 0.7% respectively (Table 3).

When the 28 infected patients were assed in relation to sex, the prevalence rate of *Klebsiella pneumoniae* infections was significantly higher ($P < 0.05$) in males than the females patients with 16(5.3%) and 12(4.0%) respectively (Table 5).

Table 5 exhibited in-vitro Antibiotic Sensitivity and Resistance Pattern of *K. pneumoniae* Isolated from TB Suspects. Ceftriaxone

28(100.0%), Gentamicin 24(85.7%), Ofloxacin 22(78.6%) and Ciprofloxacin 20(71.4%) had significant ($P > 0.05$) activity against *K. pneumoniae* isolates. Cefuroxime 18(64.3%) and Ceftazidime 16(57.1%) had moderate activity against *K. pneumoniae.* while Cloxacillin 0(0.0%), Co-trimoxazole 0(0.0%) had no activity against all *K. pneumoniae* tested.

Table 1. Isolation bacterial agents based on months from tuberculosis suspects

Isolates	January-February	March- April	May – June	Total
Klebsiella pneumoniae	12(5.4%)	10(4.5%)	6(2.7%)	28(12.5%)
Streptococcus pneumoniae	24(10.8%)	20(9.0%)	16(7.2%)	60(26.8%)
Staphylococcus aureus	14(6.3%)	12(5.4%)	10(4.5%)	36(16.1%)
Haemophillus influenzae	10(4.5%)	8(3.6%)	6(2.7%)	24(10.7%)
Streptococcus pyogenes	8(3.6%)	8(3.6%)	4(1.8%)	20(9.0%)
Other Bacterial isolates	30(13.4%)	20(8.9%)	12(2.7%)	56(17.0%)
Total	98(43.8%)	78(34.8%)	48(214%)	224(100.0%)

% = Percentage of total number of Klebsiella pneumoniae and other bacterial isolates (224)

Table 2. Occurrences of *Klebsiella pneumoniae* and other bacterial isolates from tuberculosis suspects

Isolates	Occurrences	Occurrences rate (%)
Klebsiella pneumoniae	28	12.5
Streptococcus pneumoniae	60	26.8
Staphylocucus aureus	36	16.1
Haemophillus influenzae	24	10.7
Streptococcus pyogens	20	9.0
Other Bacterial isolates	56	7.2
Total	224	100.0

% = Percentage of total number of Klebsiella pneumoniae and other bacterial isolates (224)

Table 3. The distribution of *Klebsiella pneumoniae* infections in relation to age among tuberculosis suspects

Age group (Years)	No. examined	No. (%) infected
≤10	21	3(1.0)
11-20	88	7(2.3)
21-30	113	12(4.0)
31-40	42	4(1.3)
≥40	36	2(0.7)
Total	**300**	**28(9.3)**

Key: ≥ = Greater than or equal to; ≤ = Less than or equal to; No. = Number, % = Percentage of total number of Sputum samples collected and processed (300)

Table 4. The distribution of *Klebsiella pneumoniae* infections in relation to sex among tuberculosis suspects

Sex	No. infected (%)	No. and % not infected	Total (%)
Males	16(5.3)	171(57.0)	187(62.3)
Females	12(4.0)	101(33.7)	113(37.7)
Total	**28(9.3)**	**272(90.7)**	**300(100)**

Key: NO. = Number; % = Percentage of total number of Sputum samples collected and processed (300)

Table 5. *In-vitro* antibiotic sensitivity and resistance pattern of *Klebsiella pneumoniae* isolated from TB suspects

Antibitics	Conc of disc (µg)	No. sensitive	% sensitive	No. resistance	% Resistance
Ceftriaxone	30µg	28	100.0	0	0.0
Ciprofloxacin	5µg	20	71.4	8	28.6
Co-trimoxazole	25µg	0	0.0	28	100.0
Augumentin	30µg	4	12..3	24	85.7
Cefuroxime	30µg	18	64.3	10	35.7
Ceftazidime	30µg	16	57.1	12	42.9
Gentamicin	10µg	24	85.7	4	14.3
Ofloxacin	5µg	22	78.6	6	21.4
Cloxacillin	5µg	0	0.0	28	100.0

No. = Number; % = Percent; µg = microgram; conc. = concentration;
% = Percentage of total number of Klebsiella pneumoniae isolates tested (28)

4. DISCUSSION

The comprehensive data analysis of *Klebsiella* infected patients in different months suggested the prevalence of multidrug resistant *Klebsiella* is increasing sharply. The prevalence of *Klebsiella* infections varies in different geographical regions which create an alarming situation nowadays [21]. From the screened cases, *Klebsiella* was found in 28(12.5%). Similar results were found earlier by [21,22,23] were *Klebsiella* was mostly the etiologic agent. High infection recorded among males between 21-30 years age group, could be due to socio-cultural behaviour of males such as swimming, bathing, fishing and laundry services, and other risk factors like smoking, alcoholism which facilitate the transmission of the diseases [23]. Females in the area are usually restricted from swimming and bathing in the rivers on religious and socio-cultural grounds.

The highest significant resistance rate ($P > 0.05$) of *Klebsiella pneumoniae* isolates to Cloxacillin and Co-trimoxazole (0.0%) could probably be due to antibiotics usage in the study area which is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine [24,25]. However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant *Klebsiella pneumoniae* constitute a global problem [26]. It has been observed that antibiotics susceptibility of *Klebsiella pneumoniae* isolates is not constant but dynamic and varies with time and environment. This, therefore, demands the need for periodic screening of common bacterial pathogens for their antibacterial drug susceptibility profiles in different communities. There is strong evidence that the use of antibiotics can lead to the emergence and dissemination of resistant *Klebsiella pneumoniae*, which can then be passed onto people via food or through direct contact with animals. During recent years the wide spread use of antibiotics in the field of veterinary medicine has resulted in the development of increasing number of bacterial strains possessing resistance to many antibiotics [27]. The property of multiple antibiotics resistance could be transferred through conjugation from resistant strains of salmonellae, to another by means of plasmid, which occur in the cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA. Thus a new bacterium with resistance factor emerges that is resistant to one or more antibiotics. In another instant the high resistance of *Klebsiella pneumoniae* isolates to commonly used antibiotics is probably due to some factors ranging from the use of fake antibiotics, abuse and misuse of those antibiotics found commonly in circulation among the general populace and health resources centers [26,28].

Abdullahi [29] reported that, acquired antibiotics resistance is a growing worldwide problem due to the increasing use of antibiotics in humans, animals, and agriculture. In developing countries the situation is particularly serious for the following reasons: In many countries, antibiotics can be obtained outside of recognised treatment centres, and taken without medical authorisation or supervision. This leads to inappropriate use of antibiotics and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibacterial drug, results in an incomplete course being purchased,

sufficient only to alleviate symptoms. Patients are not sufficiently informed about antibiotics and their use [29,30]. Problems also arise when antibiotics sold in local markets are sub-standard or expired. Guidelines regarding the selection of antibiotics, correct prescription, and information about antibacterial drug resistance and how to minimise its spread are not communicated to those purchasing the antibiotics. Antibiotics are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections [29,30].

Broad spectrum antibiotics are frequently used prophylactically, e.g. tetracycline. Laboratory facilities for accurate diagnosis and isolation of pathogens are often not available, resulting in an overuse and inappropriate use of antibiotics [31]. Many countries do not have effective surveillance of important antibacterial drug-resistant bacteria. Training and facilities for performing standardised antibiotics sensitivity tests are often lacking. Developing countries are often unable to afford costly second-line antibiotics to treat infections due to resistant organisms. This results in prolonged illness with longer periods of infectivity and to the further spread of resistant strains [30].

5. CONCLUSION AND RECOMMENDATIONS

In this study, high infection recorded among males between 21-30 years age group. The result of *in vitro* – antibacterial drug susceptibility testing of *Klebsiella pneumoniae* isolates using disc diffusion test demonstrated that of the nine (9) antibiotics tested, all the isolates was significantly ($P > 0.05$) resistant to Cloxacillin and Co-trimoxazole (0.0%). And were significantly ($P > 0.05$) sensitive to Ceftriaxone 28(100.0%), Gentamicin 24(85.7%), Ofloxacin 22(78.6%) and Ciprofloxacin 20(71.4%) in decreasing order. However, the relationship between *Klebsiella pneumoniae* and antibiotics tested was not statistically significant ($P > 0.05$).

There is a relatively high prevalence of drug resistant *K. pneumoniae* isolates in Kano. Thus, a high degree of awareness among physicians and microbiologists, active infection control committee, appropriate antimicrobial therapy, improvement of hygiene condition and monitoring of drug resistant isolates are urgently needed in order to better control the emergence and spread of drug-resistant *K. pneumoniae* isolates in hospital settings. To reduce this antimicrobial resistance, public health reference laboratory with a tool to produce standardised antimicrobial susceptibility test results of antimicrobial susceptibility tests are important for clinical treatment plans; adequate information must be provided to the health care providers. In addition, susceptibility testing help on providing guidance and monitor of treatment, narrower the spectrum of its antimicrobial (the more proffered is it's use when one knows specifically the organism being treated), degree of susceptibility of organism can assist in determining the length of therapy (but not the only factor) and choice of cheaper antimicrobial agents with less side effects [32,33].

CONSENT

Author declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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