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Incidence of secondary *Klebsiella pneumoniae* infections associated with tuberculosis patients attending Aminu Kano Teaching Hospital, Nigeria

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ABSTRACT: One third of the world's populations are thought to be infected with *Mycobacterium tuberculosis*, and new infections occur at a rate of about one per second. Secondary by *Klebsiella pneumonia* resulting to secondary infection among TB patients which causes tissue destruction and ulceration which had already occurred as a result of primary infection. This study aimed at assessing the incidence of *Klebsiella pneumonia* infections associated with TB patients attending Aminu Kano Teaching Hospital (AKTH), Kano. A total of 206 sputum samples were collected from TB patients attending AKTH across age groups and sex. Using a sterile swab, a portion of the sputum sample was transferred to plates of Chocolate and McKonkey agar, streaked for isolation using sterilized inoculating wire loop. All the plates were incubated in anaerobic state at 36^0C for 24hrs. Plates were examined for growth and colonies were Gram stained and sub-cultured to appropriate media for biochemical identification. The result revealed higher *K. pneumoniae* incidence among the male subjects, 23 (19.33%), also highest incidence was observed among the age range of 0-14 years. This finding may assist in the early recognition and diagnosis of persons likely to be at an increased risk for TB complications.

Keywords: Incidence, *Klebsiella pneumoniae, Mycobacterium tuberculosi,s,* Secondary infection,

Introduction

Tuberculosis which is also referred to as phthisis pulmonalis is among leading infectious diseases, which causes death in humans among adults and youth (WHO, 2006). WHO estimates that active cases of TB afflict seven to sight million people annually leading up to three million deaths per year (WHO, 2006).
Tuberculosis or TB (short for *Tubercle bacillus*) is a common and often deadly infection disease caused by various strains of mycobateria, usually *mycobacterium tuberculosis* in humans (Boyd and Robert, 1984). Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have the disease cough, sneeze, or spit. Subsequent invasion of the secondary bacteria like *Klebsiella pneumonia, Aspegillus niger, streptococcus pneumonia, and Pseudomonas aeruginosa* resulting in the secondary infection which causes tissue destruction and ulceration which had already occurred as a result of primary infection (Jacquelyn, 2002).

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Klebsiella pneumonia is a Gram – negative, non – motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines (Selden *et al.,* 1971) is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae; it is closely related to *K. oxytoca* from which it is distinguished by being indole – negative. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic condition (Postgate, 1998).

This pneumonia attacks the very old, the very young, alcoholic, nursing home patients, those delibilatated by other diseases, and immunocompromised persons. Pneumonia causes by Gram-negative rods cause most of the deaths from nosocomial infection (Eugene *et al.,* 2007).

As a general rule, *Klebsiella* infections tend to occur in people with a weakened immune system. Many of these infections are obtained when a person is in the hospital for some other reason. The most common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia. Classically, *Klebsiella pneumonia* causes a severe, rapid-onset illness that often causes areas of destruction in the lung. The mucous (or sputum) that is coughed up is often thick and blood tinged and has often referred to as "current jelly" sputum due to its appearance (Ryan and Ray, 2004).

Mortality in *Klebsiella pneumonia* is around 50% due to the underlying disease that tends to be present in affected persons. While normal pneumonia frequently resolves without complication, *Klebsiella pneumonia* more frequently causes lung destruction and pockets of pus in the lung (known as abscesses). The mortality rate for untreated cases is around 90% (Ryan and Ray, 2004). Therefore it has become a necessity out to study the various pathogens involved.

The study is aimed at finding the incidence of *Klebsiella pneumonia* infections associated with Tuberculosis patient attending Aminu Kano Teaching Hospital.

Materials and Method

Study Site

The study was conducted at Aminu Kano Teaching Hospital (AKTH) Kano, at the Tuberculosis laboratory (TB lab) of the microbiology department in the AKTH from November – January, 2010.

Aminu Kano Teaching Hospital is situated along New Court Road in Kano metropolis. The state capital is located on latitude 12.000N and longitude 8.300E. It is within the semi-arid Sudan Savannah zone of West Africa about 840 kilometers from the edge of the Sahara desert. Kano has a mean height of about 472.45m above sea level. Aminu Kano Teaching Hospital was established in August 1988 as the teaching hospital for Bayero University Medical School.

Targets Groups and Sample Size

Five age groups were selected for this study, which were 0-14, 15-25, 26 – 35, 36 – 45 and 46 – above years of age respectively. A total of two hundred and six (206) sputum samples were collected from patients cutting across all age groups and of both sexes were surveyed with suspected respiratory tract infections. Their informed oral consents were obtained with the assistance of the laboratory personnel, after approval from the ethical committee of the hospital.

Sample Collection and Handling

A total of 206 sputum sample were collected from patient with TB infection at the DOTS clinic of the Virology centre. Patients that fall within the age range of between 0-69 years were examined. Each patient was given a sterile sample bottle and asked to cough deeply so that sputum was produced. The containers (sample bottle) after sample collection were labeled appropriately indicating the name, age and sex of the concerned patient. However, specimens intended for the detection of isolation of *Klebsiella pneumonia* were not refrigerated neither were they allowed to stay for long because of the possible death of the expectedly-implicated organism. The period of sampling was between November, 2010 and January, 2011.

Inoculation and Detection of *Klebsiella pneumoniae*

This was carried out in accordance with the method described by (Cheesbrough, 2000).

Culture Medium and Preparation of Innoculums

MacConkey and Chocolate agar were used for the isolation and identification of *Klebsiella pneumonia.* The media were prepared as directed by the manufacture following king aseptic procedure.
The prepared media were labeled according to the labeled sputum samples, a Bunsen burner was used to sterilized wire loop and a purulent portion of the sputum was used (taken) to make a primary inoculums on both the chocolate and MacConkey agar. The wire loop was re-sterilized and used to streak the primary inoculums to have distinct colonies of any organism that will grow.

The inoculated media plates were then incubated in a carbon dioxide-enriched atmosphere (incubator) at a temperature range of 35 – 37^{o}c for 24 hours. The inoculation was done aseptically (near a flame) to reduced microbial contamination from the surrounding.

After overnight incubation, organisms that grew as both MacConkey and Chocolate agar plates yielding large mucoid colonies (were found to be *Klebsiella*) and were then set for microscopy and biochemical tests. Interpretation of results was done using WHO guidelines (WHO, 2000).

Gram's Stain Reaction of *Klebsiella pneumonia*

This was also carried out in accordance with the method described by (WHO, 2001). The slide was finally observed microscopically using low-power objectives lens under immersion oil. Gram – positive cells were observed to have retained the blue or violent colour after final decolonization with alcohol, where as those been decolorized were designated as the gram – negative ones. Microscopically, the test organism appeared to be gram negative, non – motile – capsulated rod after gram staining and cannot be differentiated from other gram-negative rod. Results were accordingly recorded.

Biochemical Reaction of *Klebesella pneumoniae*

Urease Test: The urease test is used to determine the ability of an organism to split urea through the production of the enzyme urease. Two units of ammonia are formed with resulting alkalinity in the presence of the enzymes, and the increased pH is detected by a pH indicator 2. Christensen's urea medium contains the pH indicator phenol red which under acid condition (pH 6.8) is yellow. In alkaline condition (pH 8.4) the indicator turns the medium rose pink. To carry out the test, the urease slope is inoculated with the bacterial isolate and then incubated at 35^{o}C and then examined after about 4 hours. This was also carried out in accordance with the method described by (Cheesbrough, 1984).

The media were prepared base on the manufacturer's instruction and dispensed in bijour bottle and kept on a slanted position, then the test organism was inoculated greatly on the surface of the entire slope. The strains that are urease producers breakdown the urea (By hydrolysis) to give ammonia and carbon dioxide. The presence of ammonia creates an alkaline environment the medium turned to pink. This indicate positive reaction, failure of pink color to develop indicate a negative reaction (Cheesbrough, 2000).

Statistical Analysis

OpenEpi version 2.3 statistical software was utilized in the Chi-square test to find any significant variations on the incidence between age group and sex of the subjects at $P \leq 0.05$.

Result and Discussion

Table 1 shows demographic characteristics in which highest percent distribution of 65 (31.56%) of the TB patients were within 26-35 year age group. This is in conformity with the report of Rothel and Andersen (2005) that most chest infections are more implicative of individuals within the age range of 15-35 years. The result also agrees

with the observations of Imam and Oyeyi (2008) in which highest percent distribution of those patients attending TB clinic are within 15-29 year age group.

Table 2 illustrates highest incidence of 2 (28.6%) among male subjects within 0-14 year age group. This result is worrisome in that despite the scanty number of paediatric patients with TB infection, they ha the highest *K. pneumoniae* infection. This could be due to their relatively weak immunity status as well as poor personal hygiene of the affected subjects. Although transmission of both TB and *K. pneumoniae* is unusual among the age group, it has been reported in association with the presence of chest infection among their parents and/or guadians (Lawrence, 1996).

Table 3 shows the incidence of *K. pneumoniae* among the female subjects with TB, where highest incidence of 1(25%) and 6 (25%) were observed within the 0-14 years and 15-25 years age groups respectively. This is in agreement with the report of Lawrence (1996). It was observed that the overall incidence of *K. pneumoniae* inection was higher among the male subjects with 23 (19.37%) out of 119 as compared with the incidence among the female subjects of 14 (16.09%) out of 87. This disparity could be due to the fact that male subjects are more exposed to risk factors of TB and consequently *K. pneumoniae* infection such as smoking, nature of occupation etc. which can make them more susceptible (Imam and Oyeyi, 2008). It is important to emphasize that most of the severe chest infections are endogenous infections by opportunistic pathogen like *K. pneumoniae.* They develop when respiratory mucosal resistance is lowered by antecedent bacterial or viral infections or form social and environmental causes (Imam and Oyeyi, 2008).

Table 1: Demographic Characteristics of Sampled Subjects

Age Groups (Years)	Male	Female	Total
0-14	7 (63.64)	4 (36.36)	11 (5.34)
15-25	25 (51.02)	24 (48.98)	49 (23.8)
26-35	36 (55.4)	29 (44.6)	65 (31.56)
36-45	29 (69)	13 (31)	42 (21.84)
46-above	22 (56.4)	17 (43.6)	39 (18.9)
Total	119 (57.8)	87 (42.2)	206 (100)

NB: Numbers in parentheses are percent distribution.

Table 2: Total percentage prevalence of *Klebsiella pneumoniae* (Male patients) by age group

Age Group (Years)	Number Examined	Number Positive	%Incidence
0 – 14	7	2	28.6*
15 – 25	25	7	28*
26 – 35	36	5	13.9*
36 – 45	29	6	20.7*
46 – above	22	3	13.6*
Total	119	23	19.33*

*There was no significant difference between age group at P≤0.05.

Table 3: Total percentage prevalence of *Klebsiella pneumoniae* (female patients) by age group

Age Group (Years)	Number Examined	Number Positive	% Prevalence
0 – 14	4	1	25*
15 – 25	24	6	25*
26 – 35	29	3	10.34*
36 – 45	13	1	7.69*
46 – above	17	3	17.64*
Total	87	14	16.09*

*There was no significant difference between age group at P≤0.05.

Conclusion and Recommendations

Incidence of *K. pneumoniae* infection among the TB patients was high in regardless of age and sex of the subjects. It is obvious that *k. pneumoniae* is an important opportunistic pathogen causing secondary infection among TB as the current study has observed. It can therefore be concluded that the isolation of *K. pneumoniae* from the sputum of patients with TB is an one of the effective control strategies which need to be carried out in all the compromised patients.

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