Phenotypic evaluation of antibacterial resistance in the bacteria isolated from sputum of healthy human subjects in the rural community

Accepted 15th January, 2019

ABSTRACT

Respiratory Tract Infections constitute high morbidity in human population both in young age, pregnant women, neonates, aged and immune-compromised individuals. It is generally caused by bacteria and other group of micro-organisms. Sputum samples were collected from healthy individuals in the rural community with symptoms of Upper respiratory tract infection using sterile swab stick. Bacteria isolation and identification were carried out using pour plating technique with distinctive morphological and biochemical characteristics. Subsequently, antibiotic susceptibility assay was carried out and interpreted using CLSI, 2014. The frequent symptoms observed common to human subjects are cough, phlegm and disturbed sleep. Knowledge, attitude and practice of antibiotic showed poor knowledge towards the concepts and abuse of the common antibiotics. Bacteria diversities were observed in the study with extensive antibiotic resistance. The extensive level of antibiotic resistance raised an alarm on the recurrent cases of respiratory infections despite the rate at which antibiotics are being consumed. The knowledge of the rural dwellers on the proper use of antibiotics is very shallow which called for intervention programme to educate the rural community on the need for the proper usage of antibiotics.

Keywords: Respiratory tract infections, sputum, antibacterial resistance,

INTRODUCTION

Respiratory tract infections are one of the common human diseases, world-wide with global mortality rates estimated at about 7 million (Ozyilmaz et al., 2005). The human respiratory tract, particularly, the upper respiratory tract is usually exposed to colonization by diverse organisms especially anaerobes with a ratio of up to 1: 100 aerobes to anaerobes (Murray, 1998). It is one of most frequently reported of all human diseases. Some of these diseases are sometimes mild, transient lasting and sometimes self-limiting. Due to this, many infected persons tend to ignore the infection (Ndip et al., 2008).

In developed countries like USA, greater than 62 million people suffer from cold annually (National Institute of Allergy and Infectious Diseases, 2010), while in the United Kingdom, about 8 million persons are infected by some forms of chronic lung diseases which now kills one in every five persons (British Lung Foundation, 2010). However, in most developing countries, the situation is more complicated and management is often difficult due to the problem associated with the identification of the etiological agents and administration of appropriate treatment in cases requiring antibiotic therapy (Alter et al., 2011) and lack of adequate case records.

Acute lower respiratory tract infection is a common cause of hospital admission in Nigeria; however, no comprehensive study on the prevalence of pneumonia and other respiratory infections in adults is readily available. There are very scanty data on the bacterial aetiology of
respiratory diseases like pneumonia among children in Nigeria. Also, seasonal variation in acute respiratory infections among children and teenagers in Nigeria is with higher occurrence during the rainy season (Fabule et al., 1994; Johnson et al., 1996).

Upper respiratory tract infections (URIs) are mostly caused by viruses, however, Group A beta hemolytic Streptococci (GABHS) was reported to cause 5 to 10% cases of pharyngitis in adults (Nichol et al., 2005). Streptococcus pneumonia and Haemophilus influenzae are the most common organisms causing bacterial super infection of respiratory tracts. Thus, transmission occurs more commonly in crowded conditions.

Respiratory tract infections (RTIs) are usually handled with antibiotics and in most cases, there is need to start treatment before the causative agent is isolated or confirmed. Recently, treatment was complicated by the emergence of multi-antibiotic resistant bacteria. This has called for a definitive bacterial diagnosis and susceptibility assay so as to ensure effective management of the disease (Aydemir et al., 2006; Sahm et al., 2008). Issues of serious concern are the reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among micro-organisms associated with respiratory tract infections (Tenever and McGowan, 1996; Hryniewicz et al., 2001; Kurutepe et al., 2005). Nevertheless, the kind of antimicrobial therapy to use becomes easier when the causative agents and their susceptibility patterns are known (Zafar et al., 2008).

In Nigeria, just as in the other parts of the developing countries, most RTIs are treated empirically, possibly due to higher cost of laboratory services or illiteracy among antibiotic users. The concept of emergence of antibiotic resistance in the management of respiratory tract infections is a serious public health problem, particularly in the developing countries which could be as a result of high level of poverty, ignorance, poor hygienic practices and high prevalence of drugs of questionable quality in circulation. The global problem of antibiotic resistance is of serious concern and this necessitates the need for identification of the etiological agents of RTIs and their antibiotic sensitivities (El-Astal, 2004).

The objective of this study was to determine the bacteria associated with community healthy people presenting signs and symptoms of respiratory tract infections and their antibiotic susceptibility.

**MATERIALS AND METHODS**

**Design of questionnaire**

Informed consent from the subjects were duly sought using consent form after the subjects were educated on the concept of the study. Structural questionnaire was designed to measure and quantify the attitude and practice of antibiotic abuse among the people with symptoms of upper respiratory tract infection and to evaluate symptoms including frequent cough, breathlessness and wheezing, etc. The questionnaire was administered to patients prior to sample collection.

**Sample collection**

Sputum samples were collected from healthy individuals in the rural community with symptoms of Upper Respiratory Tract infection especially cough. The sputum collection was carried out according to the Standard Manual for Sputum Collection, 2006. The sputum was collected in the early hours of the morning before the patients eats or takes medication. Patients were encouraged to take several deep breaths and cough deeply to produce sputum into wide neck-sterile containers with screw cap tops. A total of 50 sputum samples were collected from both males and females and transported in ice pack to the laboratory for analysis.

**Bacteria isolation and identification**

The sputum samples were streaked onto prepared blood agar (Difco, Becton Dickson and company, sparks MD USA) and nutrient agar plate using wire loop. The blood agar plate was incubated anaerobically with a carbon dioxide enriched candle jar at 35±2°C for 18 to 24 h while nutrient agar plate was incubated aerobically at 35±2°C for 18 to 24 h. After incubation, the plates were observed for the distinctive characteristics of haemolysis and swarming. The bacteria isolates were identified based on the morphological and biochemical characteristics.

**Antibiogram of bacterial isolates**

Multiple antibiotic disc (Abtek, Biologicals, Ltd) was used for the antibiotic susceptibility of the bacteria isolated from sputum samples. The disc contained Ceftriaxime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ceftraxone (30 µg), Erythromycin (30 µg), Cloxicillin (5 µg), Ofloxacín (5 µg), Amoxycillin/Clavulinate (30 µg) (Falegan and Akere, 2014).

**Preparation of bacterial suspension**

The inoculum density standardization for susceptibility test was based on BaSO₄ turbidity standard equivalent to a 0.5 McFarland standard which was strictly used as the standard for the preparation and standardization of the bacteria. The bacteria suspension was swabbed on Mueller Hinton agar (Oxoid, England) and the multiple antibiotic
discs placed aseptically on the plates and incubated aerobically at 35±2°C for 24 h. Diameter of the zones of inhibition (millimetre) was measured with a ruler and interpreted using Clinical and Laboratory Standard Institute (CLSI, 2014). Multiple antibiotic resistances were defined as resistance of the bacterial isolates to more than three antibiotics investigated in the study.

**RESULTS**

**Questionnaire investigation**

It was observed in this study that there were frequent cough, phlegm and disturbed sleep among the healthy human subjects that participated in the study. However, the frequency of antibiotic usage showed that Ampicillin and Ampiclox were the commonly used with 74 and 71% respectively. However, the frequency of Erythromycin and Amoxycillin use are 54 and 65% respectively. The knowledge, Attitude and Practice (KAP) of antibiotic use in the rural setting of our study showed that only 36% had the knowledge of antibiotic prescription and usage. It was also observed that 20% of the subjects have the knowledge of side effects associated with antibiotic usage.

**Bacterial identification**

A total number of 100 sputum samples from both males and females were examined. Bacteria occurrence was observed to be as follows: *S. pneumoniae* (18.6%), *Staphylococcus* spp. (23.2%), *H. influenzae* (7%), *Micrococcus* spp. (30.2%), *Klebsiella* spp. (18.6%), and *Corynebacterium* spp. (2.3%) from sputum. Table 1 shows that *Staphylococcus* sp and *Streptococcus* sp. were found to have high levels of occurrence in the study. Some of these bacteria isolated in this study haemolyzed red blood cells in the blood agar plate by showing clear zones that appeared greenish around the area of growth on the culture plate (Figure 1).

**Antibiotic susceptibility of bacteria isolates**

The *in vitro* antibiotic susceptibility pattern showed that there was high rate of antibiotic resistance pattern. The break-point for antibiotic susceptibility was taken to be 50%. *Klebsiella* spp. isolates were 88.8 and 77.7% sensitive to Gentamicin and Ofloxacin respectively but showed 11.1% sensitivity to Ceftazidime, Erythromycin and Amoxycillin. *Corynebacterium* species was only susceptible to Ofloxacin at a rate of 100% but highly resistance to other antibiotics investigated in this study. *Micrococcus* spp. was 78.6% susceptible to Gentamicin and 92.8% to Ofloxacin but 7.14% sensitive to Ceftriaxone and 21.4% sensitive to Erythromycin in this study. Overall antibiotic evaluation in this study revealed that no organism showed resistance to Ofloxacin and Gentamicin except *Corynebacterium* spp. that showed resistance to Gentamicin. *S. pneumoniae and H. influenzae* were 65 and 96% susceptible to only Gentamicin and Ofloxacin respectively. *Staphylococcus* spp. was observed to be 100% susceptible to Ofloxacin and Gentamicin but 25% to Ceftriaxone (Table 2).

**DISCUSSION**

It was discovered through structured questions and subsequent analysis that people investigated in this study were found with symptoms of upper respiratory tract
infection. Some of the symptoms observed were cough, runny nose, high body temperature and other feverish conditions. The concepts of antibiotic abuse through self-medication were also looked into in this study due to self-treatment of upper respiratory infections. The common drugs frequently consumed by the people in the rural community are Ampicillin, Erythromycin, Amoxycillin and Ampiclox based on our questionnaire study.

The antibiotics are mostly purchased on the counter and this enhances abuse of antibiotics capable of creating pressure for the antibiotics resistant strains. This practice contributed greatly to the emergence of multi-drug resistant strains which can threaten the management of RTIs. The findings in our questionnaire study showed that people in the rural community had poor knowledge on the proper use of antibiotics and possible side effect associated with each antibiotic drug. The need for antibiotic prescription is not well practiced in the rural setting either due to lack of awareness on the need for antibiotic prescription or inability to afford the cost of laboratory test before embarking on the use of antibiotics.

The bacteria were isolated from sputum of health community people with symptoms of upper respiratory tract infection and the bacteria are *S. pneumonia* (18.6%), *Staphylococcus* spp. (23.2%), *H. influenza* (7%), *Corynebacterium* spp. (2.1%), *Micrococcus* spp. (30.2%)
and *Klebsiella pneumonia* (18.6%). *Micrococcus* spp. (30.2%) had the highest occurrence followed by *Staphylococcus* spp. (23.2%) however, *Klebsiella* spp. and *S. pneumonia* had occurrence of 18.6%. This report is similar to that of El-Mahmood et al. (2010) in the study carried out in Yola, Nigeria in which *S. pneumoniae*, *H. influenzae* and *K. pneumonia* were isolated from the sputum of patients. The high occurrence of *Micrococcus* spp. followed by *Staphylococcus* spp. is slightly different from the report of Egbe et al. (2011) who recorded high distribution rate of *K. pneumonia* and *S. pneumoniae*. The occurrence of *S. pneumoniae* and other bacteria associated with sputum of the healthy community people with signs and symptoms of RTIs was observed to be very low indicating that age could be a determining factor in the prevalence of the aetiology of respiratory diseases among the studied population. Previous report showed that pneumococcal infections are more common in children, aged individuals and the immuno-deficient individuals.

*In vitro* antibiotic study revealed that *Micrococcus* spp., *Klebsiella* spp. and *H. influenzae* were susceptible to Gentamicin and Ofloxacin. Only Ofloxacin was found effective against all the bacteria investigated in this study which is similar to the result of El-Mahmood et al. (2010) that reported high level of susceptibility to Ofloxacin exhibited by bacteria isolated from sputum. Extensive antibiotic resistance was found in this studied area because resistance was observed in the following antibiotics: Ceftazidime, Cefuroxime Erythromycin, Cloxacillin, Ceftriaxone and Amoxycillin. This finding raised alarm that respiratory diseases may prove difficult to treat with convectional antibiotics in the circulation.

This study also confirmed the reason for the recurrent episodes of respiratory associated symptoms in humans despite the usage of antibiotics that might not be prescribed due to high cost of medical investigation or poverty among the people in the developing countries in Africa like Nigeria.

**Conclusion**

The magnitude of antibiotic resistance earlier called for serious concerns especially the need for urgent public health intervention in educating the rural dwellers on the need to abstain from antibiotic abuse and ensure antibiotics are prescribed before use.

**REFERENCES**


Academia Journal of Microbiology Research; Olayiwola et al. 019


Cite this article as:

Submit your manuscript at http://www.academiapublishing.org/journals/ajmr