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Isolation, Identification and Determination of Antibiotic Susceptibility Pattern of Some Bacteria Associated with Upper Respiratory Tract Infections

Muhammad Ali[1]*, Lurwan Mu'azu[2], Muhammad S. Abdallah[3]

[1]*Department of Microbiology, Federal University Gusau, Nigeria.
[2]Department of Biological Sciences, Federal University Gusau, Nigeria.
[3]Desert Research Monitoring and Control Centre, Yobe State University Damaturu, Nigeria.

**Corresponding Author: Muhammad Ali, Department of Microbiology, Federal University Gusau, Nigeria.*

Abstract

Infection of the respiratory tract is regarded as the most common infection among humans worldwide. The present study was conducted to isolate, identify and determine the antibiotic susceptibility pattern of some bacteria associated with upper respiratory infections in Kano, Northern Nigeria. Nasal swabs were collected from a total of 45 patients including both male (n=26) and females (n=19), attending Yan-awaki Hospital, Kano and exhibiting upper respiratory infection manifestations. The samples collected were inoculated onto freshly prepared different bacteriological media such as Nutrient agar (NA), MacConkey Agar (MAC), blood agar, Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA) (Life save Biotech, USA) and incubated at 37 °C for 24 hours to obtain pure culture of the bacteria. Identification of bacteria was performed on the basis of colony morphology, Gram staining and biochemical test. The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method. A total of 78 bacteria were isolated and 7 genera were identified as follows; Klebsiella 18 (23.2%), followed by S. aureus 15 (19.2%), Pseudomonas aeruginosa 14 (17.9%), E. coli 11 (14.1%), CoNS 9 (11.5%), and least number was recorded by Proteus 6 (7.7%) and Streptococcus 5 (6.4%). Most of the isolates showed susceptibility to Ciprofloxacin Vancomycin, Gentamicin, Tobramycin and Amoxicillin. It is recommended that bacteria are one of the etiological agents of upper respiratory tracts infection.

Keywords: *Bacteria, Identification, Kano, Respiratory tract infection, Susceptibility pattern*

INTRODUCTION

The respiratory tract can be classified into two parts: the upper respiratory tract (URT) consists of a nose, a nasal cavity, sinuses, pharynx, epiglottis, larynx and the lower respiratory tract (LRT) consists of trachea, bronchi, and lungs [1]. The upper respiratory tract infections are more common, but lower respiratory tract infections are generally more severe. RTIs represent the most common acute illness evaluated in the outpatient setting. Respiratory tract infections are the most frequently reported of all human infections, which are mostly mild, transient lasting and sometimes self-limiting [2]. The most of respiratory tract infections (RTIs) are viral; however, bacterial infections are also responsible for considerable morbidity and have potential for life-threatening consequences [3]. Respiratory tract infections account for more than 40% of days of disability secondary to acute illness and pneumonia and influenza are among ten leading causes of death in general population, and 80-90% of the deaths occur in the elderly (persons >65 years of age) [4,5]. According to Ndip *et al*. [6] the Respiratory Tracts Infections are more common in developing countries in which, after diarrhea, RTIs are the second cause of death in children due to pneumonia

Upper respiratory tract infections are one of the common diseases which can be found in the individuals of all age groups. Pharyngitis, nasopharyngitis, tonsillitis, otitis media and sinusitis are the major infections of upper respiratory tract. These major problems are generally caused by viruses (rhinovirus, adenovirus, parainfluenza virus, human metapneumovirus, and influenza virus. After the viral invasion, the secondary infection is caused by various types of bacteria resulting in chronic obstructive lung disease and high fever. The different types of bacteria which are involved in upper respiratory tract infections (RTIs) are *Haemophilus influenzae, Streptococcus pyogenes, Moraxella catarrhalis, Staphylococcus aureus,* and *Streptococcus pneumonia* [7].

Nowadays, the resistance of bacteria against existing antibiotics has been at alarming stage throughout the world with high mortality rate due to acute infections by various bacteria. The main reason for increase in resistance from antibiotics in bacteria is due to the mobile genes present on the plasmids which spread and transfer through bacterial populations [8]. The overuse and misuse of antibiotics for the treatment of respiratory tract infection has been considered one of the major reasons for the emergence of resistance of bacteria against antibiotics [9]. Another reason for antibiotic resistance of bacteria has been considered to be the production of biofilms during quorum-sensing-regulated mechanism which releases beta-lactamase responsible for degradation of various antibiotics [10]. The correct identification of the etiological agents is important for proper treatment, in particular for appropriate prescribing of antibiotics. The aim of this study was the isolation and identification and determination of antibiotic susceptibility pattern of some bacteria associated with the respiratory tract infections among patients visiting Yan awaki hospital in Kano, Nigeria.

MATERIALS AND METHODS

Ethical Approval

The ethical approval for the study was obtained from Department of health, Kano Municipal Local Government based on the consent of Yan-awaki maternity ethical committee

Study Area

The study was conducted at Yan-awaki Maternity in Kano, Northern Nigeria. Kano State is located in the North-west Nigeria located at latitude 11030N and longitude 8030E. It share borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the North. It has a total area of 20,131 km^2 and estimated population of 13.4 million [11].

Study Population

In the present study, nasal swab samples from a total number of 45 patients (26 from males and 19 from females) were collected. All the subjects included in the study were having symptoms of sore throat, cough and running nose. The study was conducted for a period of 6 month (February 2017 – July, 2017) at Yan Awaki Hospital in Kano Metropolis. A simple structured questionnaire was administered to the patients as part of the study for the collection of demographic data such as age, sex and residential area of the patients.

Table1. *Sex and age distribution of the Patients with Percentage Frequency*

Parameter	Frequency (n)	Percentage (%)
Age (Years)		
Less than 20	03	06.7
21 – 40	11	24.4
41 – 60	19	42.2
61 – Above	12	26.7
Sex		
Male	26	57.8
Female	19	42.2
Settlement		
Rural	14	31.1
Urban	31	68.9

Specimen Collection

The nasal swab samples were aseptically collected early in the morning in to appropriate sterile containers from 45 patients attending Yan Awaki Hospital in Kano Metropolis. Each container was clearly labeled with sex, age and residential area of the patient and transported to the Department of Microbiology laboratory of Kano University of Science and Technology Wudil for bacterial isolation and identification.

Isolation of Bacteria

The samples collected were inoculated onto freshly prepared different bacteriological media such as Nutrient agar (NA), MacConkey Agar (MAC), blood agar and Mannitol Salt Agar (MSA) (Life save Biotech, USA) and incubated at 37°C for 24 hours to obtain pure culture of the bacteria.

Identification of Bacteria

Identification of bacteria was performed on the basis of colony morphology (colour, size, shape, texture, and edge elevation), Gram staining, motility and biochemical tests. Gram staining was conducted according to method of Holt *et al*. [12]. Motility test was performed to differentiate motile bacteria from non-motile one [13]. Isolated bacteria from each sample were biochemically identified by sugar fermentation test, indole test, Methyl red (MR) test, Voges-Proskauer (VP) test, oxidase, catalase and coagulase tests as described by Cheesbrough [14].

Antibiotic Susceptibility Test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method as described by Bauer *et al*. [15]. Mueller Hilton agar (MHA) (Life save Biotech, USA) plates were inoculated with overnight culture of each isolate by streak plating method. The standard antibiotic sensitivity discs (Abtek pharmaceutical limited, India) were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hour. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [16].

RESULTS

Isolation and Identification of Bacterial Isolates

The number and percentage occurrence of identified bacteria isolated from 45 nasal swab samples of the patients is represented in Table 2 below. The result showed that a total of 78 isolates were obtained. The isolates were identified into 7 different bacteria namely; *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Proteus sp, coagulase negative Staphylococcus* (CoNS) *aureus* and *Streptococcus sp. Klebsiella* has the highest number of appearance among the isolates 18 (23.2%), followed by *S. aureus* 15 (19.2%), *Pseudomonas aeruginosa* 14 (17.9%), *E. coli* 11 (14.1%), CoNS 9 (11.5%), and least number was recorded by *Proteus* 6 (7.7%) and *Streptococcus* 5 (6.4%).

Table2. *The number and percentage occurrence of identified bacteria isolated from 45 nasal swab samples*

Isolates	Frequency (n)	Percentage occurrence (%)
Staphylococcus aureus	15	19.2
Klebsiella pneumonia	18	23.2
Escherichia coli	11	14.1
Pseudomonas aeruginosa	14	17.9
Proteus sp	6	7.7
CoNS	9	11.5
Streptococcus sp	5	6.4
Total	**78**	**100**

Gram Positive Bacteria

The result of antibiotic susceptibility test of Gram positive bacteria isolated from the samples tested is presented in Table 3. The result showed that *S. aureus* showed maximum susceptibility to Ciprofloxacin and Vancomycin. It showed high susceptibility to Gentamicin (73.4%). CoNS showed high susceptibility to Amoxicillin (88.8%), Amoxicillin/Clavulanic acid and Doxycycline (77.7%) each. *Streptococcus* sp showed maximum susceptibility to Vancomycin. About 80% of *Streptococcus* sp showed susceptibility to erythromycin, Ceftriaxone, Amoxicillin/Clavulanic acid and Doxycycline

Table3. *Antibiotic Susceptibility Test for Gram positive bacteria isolates*

	Number of sensitive isolates (percentage)		
Antibiotics	***S. aureus* (15)**	**CoNS (9)**	***Streptococcus sp* (5)**
Erythromycin	5 (33.4%)	4 (44.4%)	4 (80%)
Levofloxacin	6 (40%)	4 (44.4%)	3 (60%)
Ofloxacin	7 (46.6 %)	6 (66.7%)	2 (40%)
Ciprofloxacin	15 (100%)	3 (33.4%)	2 (40%)
Gentamicin	11 (73.4%)	3 (33.4%)	0 (00%)
Amoxicillin	4 (26.6%)	8 (88.8%)	3 (60%)
Ceftriaxone	6 (40%)	5 (55.5%)	4 (80%)
Vancomycin	15 (100%)	6 (66.7%)	5 (100%)
Amoxicillin/Clavulanic acid	6 (40%)	7 (77.7%)	4 (80%)
Doxycycline	2 (13.3%)	7 (77.7%)	4 (80%)

Gram Negative Bacteria

The result of antibiotic susceptibility test of Gram negative bacteria isolated from the samples tested is presented in Table 4. The result showed that *Pseudomonas aeruginosa* showed maximum susceptibility to Amoxicillin, and also highly susceptible to Gentamicin and Tobramycin 66.7 and 61.1% respectively. *Klebsiella* showed high susceptibility to levofloxacin, Ciprofloxacin (83.3%) each, Gentamicin, Ofloxacin and Ceftriaxone (75%) each and relatively sensitive to Tobramycin (66.7%). More than 69% of *Escherichia coli* were susceptible to Septrin but showed average susceptibility to levofloxacin, Gentamicin and Tobramycin (46.1%) each. *Proteus* showed higher sensitivity to Amoxicillin (83.4%). More than 66.7% of *Proteus* spp were susceptible to chloramphenicol, levofloxacin, Ceftriaxone and Tobramycin.

Table4. *Antibiotic Susceptibility Test for Gram negative bacteria isolates*

	Number of sensitive isolates (percentage)			
Antibiotics	***Pseudomonas* (12)**	***Klebsiella* (18)**	***E. coli* (13)**	***Proteus* (6)**
Chloramphenicol	8 (44.4%)	6 (50%)	5 (38.5%)	4 (66.7%)
Levofloxacin	9 (50%)	10 (83.3%)	6 (46.1%)	4 (66.7%)
Ofloxacin	9 (50%)	9 (75%)	4 (30.7%)	3 (50%)
Ciprofloxacin	10 (55.5%)	10 (83.3%)	5 (38.5%)	2 (33.4%)
Gentamicin	12 (66.7%)	9 (75%)	6 (46.1%)	0 (00%)
Amoxicillin	18 (100%)	2 (16.7%)	3 (23.1%)	5 (83.4%)
Ceftriaxone	2 (16.7%)	9 (75%)	4 (30.7%)	4 (66.7%)
Tobramycin	11 (61.1%)	8 (66.7%)	6 (46.1%)	4 (66.7%)
Septrin	6 (33.4%)	3 (25%)	9 (69.2%)	3 (50%)
Streptomycin	6 (33.4%)	4 (33.4%)	5 (38.5%)	3 (50%)

Discussion

From the result of the present study, a total 78 isolates of microorganisms have been classified through various methods of identification. Gram positive bacteria accounted for 29 (37.2) and Gram-negative bacteria accounted for 49 (62.8%). The predominant isolates of Gram positive bacteria are *Staphylococcus aureus* (19.2%), CoNS (11.5%) and *Streptococcus sp* (6.4%) while the isolated Gram Gram-negative bacteria were *Klebsiella pneumoniae* (23.2%), *Pseudomonas* (17.9 %), *Escherichia coli* (14.1%) and *Proteus* sp (7.7%). Higher prevalence of *Klebsiella pneumoniae* in this study was conformity to the earlier findings conducted by other authors [2,17]. On the other hand, the results of our study disagree with the findings of Richard *et al*., [18], where *Staphylococcus aureus* was the most frequently identified isolate (17%) followed by *Pseudomonas aeruginosa* (15.66%), *Enterobacter* species (10.9%) and *Klebsiella pneumoniae* (7.0%).

The high percentage of *K. pneumoniae* infection could be explained by the fact that many of patients have been taking inappropriate antibiotics. It is known that *K. pneumoniae* is naturally resistant to penicillin such as amoxicillin, an antibiotic that is commonly used as the first line antibiotic therapy in Respiratory tracts infections in many developing countries. The problem is complicated by the fact that the etiological agents of Respiratory tracts infections must be more precisely defined before treatment. Notable, many studies carried out in different countries demonstrate that Gram-negative bacteria including *K. pneumoniae* species were the common microorganisms isolated from patients with Respiratory tracts infections [17].

Most of the Gram positive isolates in this study showed maximum susceptibility to Ciprofloxacin Vancomycin, Gentamicin and Amoxicillin. On the other hand, *Pseudomonas aeruginosa* showed susceptibility to Amoxicillin, Gentamicin and Tobramycin. *Klebsiella* showed high susceptibility to levofloxacin and Ciprofloxacin. More *Escherichia coli* were susceptible to Septrin. *Proteus* showed higher sensitivity to Amoxicillin,chloramphenicol,levofloxacin,Ceftriaxone and Tobramycin. This result was in line with the finding of Ali and Butt [19] who reported sensitivity of most Gram positive bacteria and *Pseudomonas* to Amoxicillin. The misuse of antibiotics is considered as a direct cause of antibiotic resistance worldwide. Half of the dispensed antibiotics are not truly needed [20]. The increased use of over the counter antibiotics not only produces resistance at the individual level but can also threaten the whole community [21]. Gram-negative organisms produce β-lactamases, and this is a major factor in the resistance of the semi synthetic broad-spectrum β-lactam antibiotics such penicillins [22].

Conclusion

Finding of the present showed that bacteria are one of the etiological agents of upper respiratory tracts infection. *Klebsiella* emerged as the most prevalent bacteria. Others include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus sp, coagulase negative Staphylococcus* (CoNS) *aureus* and *Streptococcus sp.* Most of the isolates showed susceptibility to Ciprofloxacin Vancomycin, Gentamicin, Tobramycin and Amoxicillin. It is recommended that Guided prescription of antimicrobial therapy should be implemented to limit the fast spread of antimicrobial resistance. Public awareness should also be raised to prohibit the widespread antibiotics misuse and to highlight the importance of hygienic practices.

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