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# RESPIRATORY TRACT BACTERIAL INFECTION ETIOLOGICAL AGENTS AND SUSCEPTIBILITY TESTING

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**Abstract**

Respiratory tract infections are believed to be one of the main reasons peoplestill visits their GP or pharmacist.Respiratory tract infections (RTIs) are the leading cause of Death in USA. Thus, lower respiratory tract infections(LRIs) are generally more serious than upper respiratory infections. Specifically, the LRIs have been themajor cause of death among all infectious diseases. However, the two most common LRIs are bronchitis and pneumonia. Typical infections of the upper respiratory tract include tonsillitis, pharyngitis, laryngitis, sinusitisandotitis media. The aim of this study is to strengthen the surveys of the cases and the causes of therespiratory tract infection. This study also aims at evaluating the number of persons whohad negative result and the reason for the lack of screening of all pathogenic virus and search for bacterial infection only. In addition,this study finally carries out culture characterization and antibiotic susceptibility testing of the isolated pathogenic bacteria. In order to attain these goals, we carried out a survey of 635 throat swabsand sputum sample between January 2013 to December 2014 using throat and sputumin the Department of Microbiology at the Central Laboratory ofthe Ministry of Health in Amman- the capital of Jordan. In addition, culture and biochemical test and antiserawere also carried out. Atotal of 635individuals (275 males and 360 females)swabs and sputumwere tested for bacterial infection, a total 55 throat swabs showed positive results for Group A beta-hemolytic streptococci with an overall prevalence of 8.7 %.Also, a total of 23 sputum samplesshowed positive results for different types of bacterial infection with an overall prevalence of 10 %. After then, the result about their antibiotic susceptibility was reported. In conclusions, Group A beta-hemolytic *streptococci* isthe most common cause of the upper respiratory tract infection, while*K. pneumonia*is the most common cause of lower respiratory tract infection.

**Keywords:** Respiratory tract infections, Lower respiratory tract infections, throat swab, sputum sampleand Antibiotic Susceptibility testing

## Introduction

Respiratory tract infection refers to any number of infectious diseases involving the respiratory tract. An infection of this type is normally further classified as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). Lower respiratory infections, such as pneumonia tends to be in a far more serious condition than upper respiratory infections, such as the common cold(Antibiotic, 2006).Although some disagreement exists on the exact boundary between the upper and lower respiratory tracts, the upper respiratory tract is generally considered to be the airway above the glottis or vocal cords. However, this includes the nose, sinuses, pharynx, and larynx. The lower respiratory tract consists of the trachea (wind pipe), bronchial tubes, the bronchioles and the lungs. Lower respiratory tract infections are generally more serious than upper respiratory infections. LRIs have been the leading cause of death among all infectious diseases (Antibiotic, 2006).Therefore, the two most common LRIs are bronchitis and pneumonia(Musher, 2003).Influenza affects both the upper and lower respiratory tracts, but more dangerous strains such as the highly pernicious H5N1 tend to bind tothe receptors deep in the lungs (VanRid, et al 2006).Furthermore, the upper respiratory tract infection (URI) is a nonspecific term used to describe acute infections involving the nose, paranasal sinuses(Wilson, 2010), pharynx, larynx, trachea, and bronchi. The prototype is the illness known as *common cold*, which was discussed in this study in addition to pharyngitis, sinusitis and tracheobronchitis. Influenza is a systemic illness that involves the upper respiratory tract and should be differentiated from other URIs(Fendrick, et al 2003).Pharyngitis is an inflammation of the throat caused by a respiratory virus (rhinovirus, coronavirus, adenovirus, influenza virus, parainfluenza viruses, respiratory syncytial virus), Epstein–Barr virus or coxsackievirus(Kistler, et al 2007).Also, bacterial pharyngitis is less commonand its single most frequent cause is S. pyogenes(Wessels, 2011).Other rare bacterial causes include Neisseriameningitidis, Mycoplasma pneumoniae, C. diphtheriae andArcanobacteriumhaemolyticum. Peak incidence is between autumn and spring in temperate climates, and during the rainy season in the tropics. However, transmission is more rapid among groups sharing crowded living quarters and can be by droplet spread or direct transmission. The investigation most frequently requested for pharyngitis is the detection of S. pyogenes(Kistler, et al 2007).This species is detected either by culture on blood agar and subsequent latex agglutination reaction for group-specific polysaccharide, or by direct antigen detection. Therefore, neither method can distinguish oropharyngealcolonization from true infection, but only culture allows antibiotic susceptibility testing(Antibiotic, 2006). Suspicion of infection with N. gonorrhoea, Mycoplasma spp., Arcanobacterium sp. or Corynebacterium spp. should be communicated to the laboratory so that specialist non-routine culture media can be used. An oral penicillin or erythromycin is used to treat streptococcal pharyngitis(Bauer, 2002).Treatment might not alter the course of the primary pharyngeal infection, but it should reduce the risk of major non-infective sequelae such as rheumatic heart disease, poststreptococcalglomeru- lonephritis and Sydenham’s chorea(Robert, et al 2004).The need for antibiotic treatment of streptococcal pharyngitis has been questioned in developed countries, since the non-infective sequelae of streptococcal infection are all rare. Nevertheless, the recent increase in streptococcal infection in Europe and North America may change this view(Antibiotic, 2006). The other complications of streptococcal pharyngitis includes scarlet fever (less common than in the past in developed countries), streptococcal toxic shock syndrome (both caused by toxin) and quinsy (paratonsillar abscess). In quinsy, there may be secondary infection with oral anaerobic bacteria, but these are often penicillin sensitive. In addition, the drainage of purulent foci is required.In order for the pathogens (viruses and bacteria) to invade the mucus membrane of the upper airways, they have to fight through several physical and immunologic barriers. Common lower RTIs include flu (this can affect either the upper or lower respiratory tract),bronchitis (infection of the airways),pneumonia (infection of the lungs),bronchiolitis (an infection of the small airways that affects babies and children younger than two) and tuberculosis (persistent bacterial infection of the lungs)(Wenzel, 2006). The main symptom of a lower RTI is cough, although it is usually more severe and you may bring up phlegm and mucus. Other possible symptoms are a tight feeling in your chest, increased rate of breathing, breathlessness and wheezing. RTIs can spread in several ways. If you have an infection such as a cold, tiny droplets of fluid containing the cold virus are launched into the air whenever you sneeze or cough. Thus, if someone else breaths these, they might also become infected. Infections can also be spread through indirect contact. For example, if you have a cold and you touch your nose or eyes before touching an object or surface, the virus may be passed to someone else when they touch that object or surface. The best way to prevent the spread of infection is to practice good hygiene, such as regularly washing your hands with soap and warm water. This is because most RTIs will pass out without the need for treatment, and you would not need to see your GP. However, you can treat your symptoms at home by taking over-the-counter painkillers such as paracetamol or ibuprofen[13], drinking plenty of fluids and resting. Antibiotics are not recommended for most RTIs because they are only effective if the infection is caused by bacteria.

## Aim of the Study

The aim of this study is to strengthen the surveys of the cases and the causes of therespiratory tract infection.This study also aims at evaluatingthe number of persons who had negative result and the reason for the lack of screening for all pathogenic virus and search for bacterial infection only. However, this study finally conducted culture characterization and antibiotic susceptibility testing of the isolated pathogenic bacteria.

## Methods

From January 2013 to December 2014, a total of 635 individual (275 males and 360females) swabs and sputum sampleswere testedat the Department of Microbiology at Central Laboratory of the Ministry of Health in Amman- the capital of Jordan. Swabs were tested for Group A beta-hemolytic streptococci. Throat swabs were collected and sent to the laboratory for processing and analyses; and were processed following standard guidelines. Briefly, a loopful of each specimen was taken from the throat swab and inoculated on blood agar. The plates were incubated under aerobic conditions at 37 for 24-48 hours. Microscopic and macroscopic examinations of the growing colonies on the plates were performed. Suspicious colonies were then subcultured on a blood agar for purification. Preliminary identification was performed based on morphology and cultural characteristics of the pure cultures on selective and differential media as described by Cheesbrough. Also, it was described by subsequent latex agglutination reaction for group-specific polysaccharide, or by direct antigen detection and differential media as described by Cheesbrough. The API 20E kit (Biomerieux, France) was then used for the final confirmation. Furthermore, antibacterial susceptibility testing was performed using the Kirby-Bauer disc diffusion method as described by Bauer and co-workers(Bauer, 2002).Briefly, for each isolate, a small inoculum was emulsified in 3 mL sterile normal saline. The density was then compared with a barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used for evenly inoculating Mueller-Hinton agar plates (Oxoid, USA) and were allowed to dry. Next, antibiotic discs with the following drug contents-ampicillin (10 g), cefuroxime (30 g), ceftriaxon gentamicin (10 g), t and penicillin (10 IU) (Oxoid, England) were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37 for 24 hours, and the diameters of the zone of inhibition were compared with recorded diameters of thereference isolate.

## Results

Atotal of 635 individuals (275 males and 360 females) swabs and sputumwere tested for bacterial infection. Also, a total of 55 throat swabs gave positive results for Group A beta-hemolytic streptococci with an overall prevalence of 8.7 % (Table 2). Furthermore, a total of 23 sputum samplesgavepositive results for different types of bacterial infection with an overall prevalence of 10 %. The seroprevalence in males was approximately double of that of females (10.9% vs. 6.9%)(Table 1).Thus, five different types of bacteria were recovered: *Pseudomonaseearuginosa*(*P. earuginosa*), *Klebsiellapneumoniae* (*K. pneumoniae*), *Streptococcus*pneumoniae(S. pneumonia),*PseudomonasEnterobacter*, *Acinetobacter* and other bacteria like candida spp(Table 3). Also, the resistance patterns of the recovered bacteria isolates from respiratory tract infections(Table 4).

Table 1: The Seroprevalence in males was approximately doublefor that of females (10.9% vs. 6.9%)

| **Months** | **Female** | **Male** |
|---|---|---|
| January | **2** | **2** |
| February | **1** | **3** |
| March | **3** | **4** |
| April | **4** | **1** |
| May | **0** | **2** |
| June | **2** | **3** |
| July | **3** | **1** |
| August | **1** | **0** |
| September | **3** | **1** |
| October | **2** | **4** |
| November | **2** | **3** |
| December | **2** | **2** |
| Total | **25** | **30** |
| Prevalence | **6.9** | **10.9** |

Table 2: The number of isolates of the upper respiratory bacteria pathogen

| **Months** | Number of throat swab | Group A beta-hemolytic *streptococci* |
|---|---|---|
| January | 52 | 4 |
| February | 63 | 4 |
| March | 64 | 7 |
| April | 46 | 5 |
| May | 55 | 2 |
| June | 40 | 5 |
| July | 68 | 8 |
| August | 32 | 1 |
| September | 90 | 4 |
| October | 35 | 6 |
| November | 60 | 5 |
| December | 50 | 4 |
| Total | 635 | 55 |
| Prevalence | | 8.7 |

Table 3: The number of isolates of the lower respiratory bacteria pathogen

| **Months** | **Number of sputum samples** | ***K. pneumoniae*** | ***S. pneumonia*** | ***Pseudomonas*** | ***Enterobacter*** | ***Acinetobacter*** |
|---|---|---|---|---|---|---|
| January | 50 | 4 | 1 | 1 | 1 | 2 |
| February | 35 | 6 | 2 | 1 | 2 | 1 |
| March | 32 | 5 | 1 | 2 | 1 | 1 |
| April | 40 | 3 | 3 | 1 | 1 | 1 |
| May | 52 | 4 | 1 | 1 | 1 | 1 |
| June | 60 | 2 | 2 | 1 | 0 | 0 |
| July | 90 | 1 | 1 | 0 | 0 | 1 |
| August | 64 | 1 | 4 | 0 | 1 | 0 |
| September | 68 | 2 | 0 | 1 | 0 | 0 |
| October | 64 | 0 | 1 | 0 | 0 | 1 |
| November | 55 | 2 | 2 | 1 | 1 | 0 |
| December | 50 | 1 | 0 | 0 | 0 | 1 |
| November | 60 | 1 | 1 | 0 | 0 | 0 |
| December | 47 | 3 | 0 | 0 | 0 | 0 |
| Total | 635 | 35 | 19 | 9 | 8 | 9 |
| Prevalence | | 5.5 | 3 | 1.4 | 1.3 | 1.4 |

Table 4:The resistance patterns of the recovered bacteria isolates from respiratory tract infections

| Antimicrobial agent | s. pyogenes | K. pneumoniae | S. pneumoniae | *Pseudomonas* | *Enterobacter* | *Acinetobacter* |
|---|---|---|---|---|---|---|
| Cefuroxime | 7.4( 5) | 10.4 (6) | 16.1(7) | 35.5(9) | 37.8(9) | 30.1(6) |
| Gentamycin | 11.4(5) | 7.9(5) | 12.1(3) | 20.6(5) | 19.3(4) | 15.7(5) |
| Ampicillin | 65.7 (29) | 50.3(35) | 56.8(19) | 56.8(19) | 65.6(8) | 58.5(9) |
| Cotrimoxazole | 13.5(6) | 60.0 (21) | 65.8(10) | 80.2(12) | 82.3(9) | 77.5(8) |
| Erythromycin | 40.1(15) | 28.6 (10) | 35.4(8) | 45.3(6) | 43.8(7) | 32.1(6) |
| vancomycin | 9.1(10) | 6.7(4) | 11.5(4) | 19.6(6) | 17.5(6) | 14.2(7) |
| Penicillin | 87.9(30) | 85.2(32) | 80.2(10) | 90.1(7) | 88.1(8) | 85.4(9) |
| Ciprofloxacin | 14.5(22) | 19.9(9) | 17.2(4) | 20.9(8) | 21.3(7) | 17.5(5) |

## Discussion

The aim of this study is to strengthenthe surveys of the cases and the causes of the respiratory tract infection.This study aims at evaluating the number of persons who have negative result. Thus,it finally culture characterization and antibiotic susceptibility testing of the isolated pathogenic bacteria. In order to reach those goals, we carried out a survey of 635 throat swabs and sputum sample between January 2013 to December 2014 using throat and sputumin the Department of Microbiology at Central Laboratory of the Ministry of Health in Amman - the capital of Jordan. In addition, culture and biochemical test and antisera were also carried out.

Atotal of 635 individuals (275 males and 360 females) swabs and sputumwere tested for bacterial infection, and a total of 55 throat swabs gave positive results for Group A beta-hemolytic streptococci with an overall prevalence of 8.7 %.In addition, a total of 23 sputum samples gave positive results for different types of bacterial infection with an overall prevalence of 10 %.However, when reporting the information about their antibiotic susceptibilityfrom our results, we noted that males are more affected than females. This is because the males are more exposed to Environment pollution atwork. Also, we noted that the prevalence of bacterial is very low despite the patient is suffering from respiratory tract infection. However, this isthe reason for the lack of screening for all pathogenic virus and search for bacterial infection only. GroupA beta-hemolytic *streptococci* is themost common cause of the upper respiratory tract infection. However, different types of bacteria cause lower respiratory tract infection such as*Pseudomonaseearuginosa*(*P. earuginosa*), *Klebsiellapneumoniae* (*K. pneumoniae*), *Streptococcus*pneumonia (S. pneumonia ) ,*PseudomonasEnterobacter*, *Acinetobacter.* Therefore, *K. pneumonia*is the most common cause of lower respiratory tract infection (table 3). Also, our study showed that the bacterial isolates were most sensitive to vancomycin followed by Cefuroxime and gentamicin. The bacteria isolates showed lower resistant to Ampicillin and penicillin because theantibiotics areabused without control.

## Conclusion

Group A beta-hemolytic *streptococci* is the most common causeofthe upper respiratory tract infection.But*K. pneumonia*is the most common cause of the lower respiratory tract infection . The prevalence of bacterial is very low despite in the patient suffer from respiratory tract infection. Consequently, this is the reason for the lack of screening for all pathogenic virus and search for bacterial infection only. The Susceptibility data obtained in our study provided information to the clinician when they want to make decisions on therapeutic options. In addition, the bacteria isolates showed lower resistant to Antibiotic,and the reason for that is that the antibiotics areabused without control.

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