

Isolation and Diagnosis of Microbial Types in Nasal Tract of School Aged Students, and their Antibiotic Sensitivity Pattern

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Abstract

Bacterial colonization in nasopharyngeal duct is referred to it as normal flora in order to obtain information about it in school aged students, ninety five of them were taken randomly from both sexes (aged 7-18 years) for this work, the total number of isolates was (561) among them found in (210 aerobic, 43 anaerobic and 303 facultative anaerobic) bacteria, also *Candida albicans* (5) isolates was isolated from both genders.

Staphylococcus aureus and *Morexella catarrhalis* (23.6%), and facultative anaerobic (15.2%) respectively while *Veillonella* were dominant anaerobic isolated bacteria.

It is also found that *Staphylococcus aureus* is more spreading in both genders while *Streptococcus pyogenes* and *Morexella catarrhalis* obtained second level in male isolates, in female *M. catarrhalis* in second stage. The least aerobic bacteria were represented by *Corynebacterium* in both males (1.5%) and females (1.2%). *Fusobacterium* was least isolated genus (0.5%) in both sexes and *Candida albicans* (0.8%) from both genders.

The antibiotic Ciprofloxacin (5ug/disk) was found most effective antimicrobial agent while Oxacillin (1ug/disk) was the least one.

These findings demonstrated that aerobic and anaerobic microbial flora were present in school aged students, the aerobic microflora outnumbered of anaerobic bacteria species, also showed that the number of bacteria effected in creases with age.

Keywords: *candida albicans* , *staphylococcus* , *moraxell catarrhalis* , *Veillonella corynebacteria* spp.

عزل وتشخيص الأنواع المايكروبيه في التجويف الأنفي لطلاب المدارس ومدى استجابتها للمضادات الحيوية

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المستخلص

لغرض دراسة الفلورا المجهرية في المخاط الأنفي تم انتخاب (٩٥) طالب مدرسه من كلا الجنسين وبصوره عشوائية تتراوح أعمارهم بين (٧-١٨) سنة، حيث تم عزل (٥٦١) عزله كان منها (٢١٠) عزله هوائيه و(٤٣) لاهوائيه و(٣٠٣) لا هوائيه اختياريه لذلك تم عزل (٥) عزلات تعودل *Candida albicans* وكانت *Staphylococcus aureus* و *Moraxella catarrhalis* هي أكثر البكتريا الهوائية (23.6%) اللاهوائيه الاختياريه (15.2%) بينما كانت *Veillonella* (3.9%) أكثر البكتريا اللاهوائيه عزلا ، أيضا *Candida albicans* كانت (0.8%) .
وقد وجد بان *Staphylococcus aureus* هي أكثر الأنواع سيادة في كلا الجنسين في حين كانت *Streptococcus pyogenes* و *M. catarrhalis* ثاني أكثر الأنواع عزلا من الذكور بينما كانت *M. catarrhalis* النوع الثاني المعزول من الإناث لذلك كانت ال *Corynebacteria spp* من العزلات الهوائية ا لانتشار بنسبه 1.5% بالذكور ونسبة 1.2% في الإناث بينما حصلت *Fusobacterium* على نسبة 0.5% في كلا الجنسين بذلك كانت اقل الأنواع عزلا.

وجد أن المضاد الحيوي Ciprofloxacin(5ug/disk) أكثر المضادات فعالية ضد جميع عزلات البكتريا الهوائية بينما اظهر Oxacillin(1ug/disk) بأنه اقل العوامل فعالية .
أن هذه النتائج تبين إن البكتريا الهوائية و اللاهوائيه موجوده في أطفال المدارس و انها تتناسب طرديا مع العمر، كذلك كانت البكتريا الهوائية أكثر انتشارا من اللاهوائيه .

Introduction

Normal microflora are organisms that live in the body but don't cause any disease two categories can be distinguished, the resident microflora and transient microflora. Resident microflora contain microorganisms like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* often found in the nasopharynx. Children were susceptible to bacterial infections within or soon after upper respiratory tract infections(1). Upper respiratory tract is mostly infected by these four bacteria alone or in combination with other bacteria, there is no association between infection with *Streptococcus pneumoniae* and *Haemophilus influenzae*, also there was competitive interaction when *H. influenzae* and *M. catarrhalis* infected together the same site of human body(1). On the other hand negative association between colonization of *Streptococcus pneumoniae*, and *Staphylococcus aureus* was found.(1) .

Haemophilus influenzae, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Staphylococcus aureus* are asymptotically found in the nasopharynx of young children and also may be associated with important diseases which infected them(2,3). *Streptococcus pneumoniae*, were always causing pneumonia, meningitis and sepsis and these mainly infect young children(4). The ratio of young children infected by any of these three species may be >50% in certain population(5-7). Young people are colonized by many bacteria like *Staphylococcus aureus* found in more than 35% of them and responsible for wide range of diseases like soft tissue infections, pneumonia and sepsis(8,9). Antimicrobial therapy and vaccination strategies could kill or inhibit many microbial species but may alter the normal flora with unforeseen ways(9). Increased ratio of infection with methicillin-resistant *S. aureus* causes great concern(10).

Many hosting factors like age, gender, daycare, breastfeeding, race, exposure to tobacco smokers and personal immunity effect colonization of Haemophilus influenza, Streptococcus pneumonia, Morexella catarrhalis and Staphylococcus aureus(11).

Materials and methods

Sample

The study included 95 school aged students were taken randomly from both genders (aged 6-18 years) attending schools in Al-Kut/Wassit governorate. All persons don't receive any antimicrobial therapy, at least one week before sampling.

Specimen Collection and Processing

Microbiologically, infection was evaluated by culture on different media which is used for isolation and cultivation of various aerobic and anaerobic microorganisms and these included(Alternative thioglycolate broth, blood agar base, chocolate agar, MacConkey agar, Nutrient agar, Schaedler broth, Brain heart infusion agar media, sabouraud's glucose agar), after that microscopic examination was done. The nasal specimen were collected according to Vandepitte et al. (12). Nasal specimens was collected by nasal swabs and cultured immediately after collection stained with (Gram's and capsule) stain. this was done during the beginning of January 2014 to the middle of January 2016.

Samples were cultured immediately over mentioned media. The plates were incubated at 37°C for 24-48 hours at different aerobic and anaerobic conditions. Only those samples that gave significant growth were considered as infection.

Identification of the Isolates

Many biochemical tests were done amino acids decarboxylation, carbohydrate fermentation, catalase, coagulase, IMVIC, phenylalanine deaminase, ONPG(O-Nitrophenyl-B-D-Galactopyranoside), Oxidase, Staphylase, Urease and Utilization of nitrate and nitrite tests.

Antibiotic Sensitivity Test

It was carried out using agar diffusion method (13).

Results and Discussion

Microscopical examination with Gram's stain of nasopharyngeal specimens showed the presence of Gram's positive and negative cocci as well as bacilli and yeast cells (Table 1). In addition to that numbers of male, female (table 2), and also percentages for each type of microbial isolation by using different biochemical and physiological tests, found the following microorganisms (Figure 1):

Staphylococcus aureus gave the following results: positive catalase and coagulase but negative oxidase enzyme also, appear bet-hemolysis when growing on blood agar media, ferment glucose, fructose, lactose, maltose, manitole and sucrose.

Moraxella catarrhalis showed positive reaction to catalase and oxidase, don't ferment glucose, fructose, maltose, sucrose and mannitol also no hemolysis on blood agar media, other bacteria were isolated such as *Streptococcus pneumonia*.

Table 1: Numbers and percentages of microbial isolates recovered from the nose of school aged students in Al-Kut city of Wassit government.

Serial no.	Isolated bacteria	Male	Female	Total number of isolates	Percentage %
1	<i>Staphylococcus aureus</i>	40	32	72	12.8
2	<i>Moraxella catarrhalis</i>	39	27	66	11.8
3	<i>Streptococcus pneumoniae</i>	40	19	59	10.6
4	<i>Escherichia coli</i>	39	19	58	10.4
5	<i>Streptococcus pyogenes</i>	40	18	58	10.4
6	<i>Klebsiella spp</i>	37	19	56	10.1
7	<i>Staphylococcus epidermidis</i>	23	28	51	9.2
8	<i>Neisseria meningitides</i>	30	10	40	7.2
9	<i>Micrococcus spp</i>	20	14	34	6.0
10	<i>Veillonella</i>	14	8	22	3.9
11	<i>Corynebacterium spp</i>	11	5	16	2.9
12	<i>Bacteroides</i>	4	7	11	1.9
13	<i>Peptostreptococcus spp</i>	4	4	8	1.4
14	<i>Candida albicans</i>	2	3	5	0.8
15	<i>Fusobacterium spp</i>	1	2	3	0.5

Table 2: Numbers of Gram's positive and negative microbial organisms in each age group and also the total numbers of microbial organisms in each age group.

Age group (years)	Gram's-positive microbial isolates	Gram's-negative microbial isolates	Total numbers
7-9	57	19	76
10-12	80	50	130
13-15	101	57	158
16-18	132	65	197

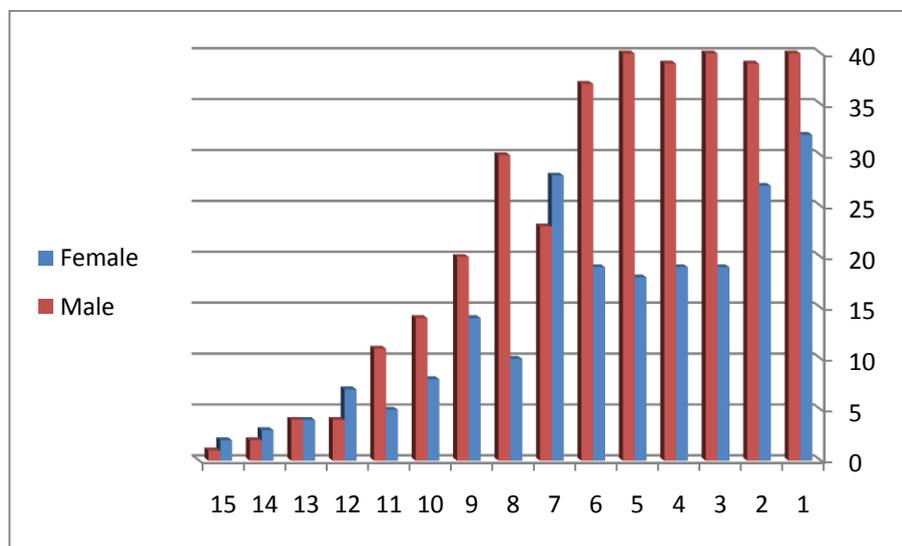


Figure 1: Comparison in numbers of infected bacteria between males and females, the horizontal line refers to the name of microorganism as in table 1 and vertical line refers to numbers of infected persons.

The fourth bacteria which showed positive reactions for catalase, decarboxylation of lysine and ornithine, they don't

produce H₂S, phenylalanine, urease enzyme and arginine decarboxylation test these properties found in *Escherichia coli*.

Streptococcus pyogenes were also diagnosed as related to the following results: negative reaction for catalase, oxidase and bile solubility tests but have ability to ferment some carbohydrates like glucose, fructose, lactose, maltose and sucrose but no mannitol, it gives clear zone of hemolysis if growing on blood agar.

Klebsiella genus differentiated from *Escherichia coli* on the basis of many biochemical tests, they appeared showed negative oxidase, phenylalanine deaminase, hydrogen sulfide, arginine and ornithine decarboxylation. Also they were able to ferment glucose, fructose, lactose, maltose, mannitol and sucrose in addition to that they have no ability to hemolysis of blood.

Staphylococcus epidermidis give positive results for catalase, ferment glucose, fructose, lactose, maltose and sucrose with no ability to ferment mannitol carbohydrate, oxidase and coagulase production enzymes.

Neisseria meningitides had positive results for catalase, oxidase and hemolysin enzymes, ferment glucose, lactose, maltose, fructose but don't fermented to mannitol and sucrose.

Other isolate associated genus *Micrococcus* which give positive reaction for catalase but negative for oxidase and nitrate and unstable result both urease and hemolysis with no ability to ferment glucose, fructose, maltose, mannitol and sucrose.

Veillonella genus were isolated according to the following characteristics: anaerobic, Gram-negative cocci arranged in clusters, non-capsulated and no blood hemolysis, these isolates appeared in clusters consisted 10, 14, 17, 22 and 31 bacterial cells, in addition to that *Corynebacterium* spp. was isolated in this study.

Bacteroides spp. Also discovered in this study depends to following results, Grams negative rods clustered in 3,4 and 6 cells, presence of capsule and many biochemical tests, other bacteria isolated such as *Peptidostreptococcus*.

Last bacteria included *Fusobacterium* spp. in which Gram-negative rods mostly occurred in pairs with tapered ends, anaerobic, no capsule exhibited beta-hemolysis on blood agar media.

Other microorganisms were isolated in this study *Candida albicans* which appeared after culturing on sabourauds glucose agar media, producing pseudohyphae and have the ability to ferment glucose, maltose and sucrose with no ability to ferment lactose.

The total microbial isolates were 561 among them 216 aerobic 43 anaerobic and 302 facultative anaerobic. According to this study *Staphylococcus aureus* were isolated from 15% from total nasal discharges isolates, *Moraxella catarrhalis* and *Streptococcus pneumoniae* were the next most common isolated species which showed (51) isolates of *Streptococcus pneumoniae* were diagnosed and this was higher than those isolated by Ismaeel and daghistani(2013), *Staphylococcus aureus* was the predominant aerobic bacteria discovered from nasal discharge followed by *Moraxella catarrhalis*. On the other hand *Veillonella* was detected as the most distributed anaerobic pathogen in females while *Fusobacterium* spp was the least one in both genders.

The results of this study recovered numerous numbers of aerobic and anaerobic microorganisms isolated from school aged children. After birth, the neonates rapidly acquire commensal bacteria that colonize the skin and mucous membranes. High incidence of bacteria appeared in children with age ranging 7-8 years old while few bacteria were recovered in six year old children. The host defense

mechanisms were not well developed at this stage and commensals may become opportunistic pathogens, some of the bacteria that make the normal flora are pathogens which rarely caused diseases unless when introduced into another region of the body. The human normal flora depends upon various factors including age, sex, stress, nutrition and diet of individual. The importance of normal flora for protection against infection in an anatomical site has been shown in upper respiratory tract, acquisition of predominant bacterial flora other than alpha-hemolytic streptococci is a risk factor for the development of invasive infection by the strain in children (Ismaeel and Daghistani, 2013).

The antibiotic sensitivity test showed that Ciprofloxacin (5ug/disk) was the most effective antimicrobial agent compared with Oxacillin (1ug/disk) had lowest worked against bacteria, while other antibacterial agents showed different activity as cleared in (Table 3), which organized in it gradually from up to down depending on the activity of antibiotics against bacteria.

Many factors influence the outcome of antimicrobial therapy of infection including pharmacokinetic factors (i.e. absorption, distribution and excretion of the antimicrobial agents), pharmacodynamics (i.e. interactions between antimicrobial and pathogens) and patient factors such as immune function, site of infection and the presence of prosthetic devices (Ismaeel and Daghistani, 2013).

Table 3: Antibiotic sensitivity test to bacteria isolated from the nose tract of school aged students.

Antibiotic	Sensitive bacteria	Resistant bacteria
Ciprofloxacin	A	-
Nalidixic acid	A	-
Vancomycin	A	-
Amoxicillin	A	-
Cefoperazone	A	-
Carbenicillin	A	-
Nitrofurantoin	A	-
Erythromil	A	-
Bakatrin	A	-
Ampicillin	A	-
Ultracillin	A	-
Cefuroxim	<i>Corynebacterium</i> spp, <i>E.coli</i> , <i>N. meningitis</i> , <i>Staph. aureus</i> , <i>Staph. epidermidis</i> , <i>Strep.</i> <i>Peumoniae</i> , <i>Strept. Pyogenes</i>	<i>Branhamella catarrhalis</i> , <i>Klebsiella</i> spp, <i>Micrococcus</i> spp, <i>Streptococcus pyogenes</i> ,
Oxacillin	<i>Branhamella catarrhalis</i> , <i>Strep. pyogens</i>	<i>Corynebacterium</i> spp, <i>E.coli</i> , <i>N. meningitis</i> , <i>Staph. aureus</i> , <i>Staph.</i> <i>epidermidis</i> , <i>Strep.</i> <i>Peumoniae</i> , <i>Micrococcus</i> spp, <i>Klebsiella</i> spp.
Ceftazidime	<i>Corynebacterium</i> spp, <i>E.coli</i> , <i>N. meningitis</i> , <i>Staph. aureus</i> , <i>Staph. epidermidis</i> , <i>Strep.</i> <i>Peumoniae</i> , <i>Strep. Pyogenes</i> , <i>Micrococcus</i> spp, <i>Klebsiella</i> spp.	<i>Branhamella catarrhalis</i>
Ultragent	<i>Corynebacterium</i> spp, <i>E.coli</i> , <i>N. meningitis</i> , <i>Staph. aureus</i> , <i>Staph. epidermidis</i> , <i>Strep.</i> <i>Peumoniae</i> , <i>Strep. Pyogenes</i> , <i>Klebsiella</i> spp.	<i>Micrococcus</i> spp.
Ultracloxam	<i>Corynebacterium</i> spp, <i>E.coli</i> , <i>N. meningitis</i> , <i>Staph. aureus</i> , <i>Staph. epidermidis</i> , <i>Strep.</i> <i>Peumoniae</i> , <i>Strep. Pyogenes</i> , <i>Micrococcus</i> spp.	<i>E.coli</i> , <i>Klebsiella</i> spp.
Amphotericin-B, 5-fluorocytocin for yeast isolates	A	-

A: sensitive to all antibiotics,(-): no resistance

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