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Isolation and Antimicrobial Susceptibility Patterns of Bacterial Pathogens Causing Respiratory Tract Infections in Children

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Abstract

This study aimed to isolate bacteria from children infected with upper and lower respiratory bacterial infections and study antibiotics sensitivity patterns of isolated bacteria. This cross-sectional study involved 120 pediatric participants, 71 males and 49 females T. These individuals divided into three groups :severe ,moderate and mild diagnosed by pediatricians. Acute infections of the nose, throat, and larynx characterized upper respiratory infections; on the other hand, chest pains, a protracted cough, copious sputum, dyspnea, fever, and weight loss were the hallmarks of lower respiratory infections. pediatric who were clinically suspected of having respiratory infections had their throat and sputum samples taken aseptically. The samples were then cultured in blood agar, MacConkey agar, and chocolate agar. Colony morphology and Gram stain were used to identify bacterial isolates, and biochemical testing was used to corroborate the results. The agar disc diffusion method was used to determine the antimicrobial susceptibility profile. Results of current study showed that lower respiratory tract infections 71.7%, is more prevalence in investigated children than upper respiratory tract infections 28.3%. Bacteria could cause severe respiratory diseases, such as *Pseudomonas aeruginosa* 26(21.6%) and *Klebsiella pneumonia* 24(20%), wheras *strep. Pneumonia* 18(15%), *Haemophilus influenza* 20(21.6%), were more repeated bacteria associated with moderate respiratory diseases. However, in mild cases, *streptococcus pyogen* is the most prevalent bacterium. The largest proportion (30%) of the children who took part belonged to the age group of 10-14 years, with 27.5% falling within the 1-4 years category, 21.7% within the 5-9 years group, and 20.8% under the age of 1 year.

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1. INTRODUCTION

Respiratory tract infections is one of the most prevalent and varied types of infections that has consistently been a significant contributor to morbidity and mortality in clinical medicine [1]. Any infectious condition of the upper or lower respiratory tract is referred to as a respiratory tract infection. Although they are primarily preventable causes of disease and death, lower respiratory tract infections (LRTIs) are the world's most common cause of infectious disease-related deaths, the fifth overall cause of death, and the second general cause of disability-adjusted life years (DALYs). The epidemiology of long-term viral infections has changed over the past ten years due to a decrease in cases among children under five, a rise in infections among older individuals, and an increase in viral infections.

From the perspective of epidemiology, the majority of definitions of long-term respiratory infections (LRTIs) list influenza, pneumonia, bronchitis (including acute exacerbations of chronic obstructive pulmonary disease [COPD] [AECOPD]), and bronchiolitis as significant illnesses. *M. catarrhalis*, *Streptococcus pneumoniae,* and, *Haemophilus influenza*,are the three most significant bacterial respiratory pathogens. Regrettably, the prevalence of these factors is rising globally, speeding up the development of antibiotic resistance [2]. Preschool-aged children experience six to eight upper respiratory tract infections (URTIs) a year on average, making them the most frequent infection in children. More days missed from work, school, and day care are attributed to URTIs than to any other ailment. A URTI can cause nasal congestion, overall irritability, eating difficulties, nausea, and occasionally fever in young children. Older children typically have lower grade fevers and have milder, primarily local symptoms. There is no cure for the common cold since it is a self-limiting viral infection [3].

Antimicrobial resistance is an increasing public health concern which is solely linked to the usage of antibiotics [4]. The following methods could lead to bacterial resistance to antibiotics: active antibiotic efflux, reduced cell membrane permeability, altered drug target, or antibiotic inactivation. Poor antimicrobial agent use, the spread of resistant bacteria within patients, between patients and healthcare workers, and between patients and healthcare workers are some of the factors linked to the emergence of resistant bacteria. Another factor is inadequate guidelines for the administration of antimicrobial agents [5]. Antibiotics are not necessary for the self-treatment of the majority of URTIs because they are viral, self-limiting infections [6]. Given the diminishing availability of novel antibiotic medications, the overuse of antibiotics has led to bacterial resistance to these treatments, posing a serious threat to public health [7] .One successful strategy to prevent further antibiotic resistance is to decrease the demand for antibiotics in self-limiting settings[8]. The objects of this study were to isolation bacteria from children infected with upper and lower respiratory bacterial infections and study antibiotics sensitivity patterns of isolated bacteria.

2. METHODS

2.1 Collection of data

A cross-sectional study was carried out in the period from November 2023 to April 2024 at Babil Teaching Hospital for Maternity and Children and AL Nour hospital for children to detection about bacteria isolates in children suffering from respiratory infections. The study group consisted 120 patients with respiratory tract infection were diagnosed by pediatricians as mild, moderate and severe condition according to the severity of disease [9]. They were between the ages of one and fourteen years. The number of volunteers in the current study was divided into three groups as the following: severe groups :37, moderate groups :40, mild group :43.

The samples were collected from upper and lower respiratory tract .Sputum samples were collected into sterile container which was labeled and throat swab samples were collected using sterile cotton swab from all members of the study groups to identified type of bacteria that causes infection .All samples were transported immediately into the microbiology laboratory in the hospital for analysis and streaked into chocolate medium, blood medium, MacConkey medium and mannitol salt agar with the help of sterilized loop for inoculation of bacteria this media prepared according to[10] . Gram staining was done on all the plates examination of bacterial identification into two groups based on their Gram characteristics gram positive and negative. Then purified colonies were obtained by repeated streaking of the single colony on fresh nutrient agar plates. Standard enzymatic and biochemical tests were used to identify the species of the pure colonies of the bacterial isolates. *Strep. pyogenes* isolates were identified by small colonies, Gram-positive cocci organized in a chain, total hemolysis on blood agar, and both coagulase and catalase negative results. Gram positive alpha-hemolytic tiny colonies on blood agar were used to identify *S.pneumoniae* isolates, which were also optochin-susceptible. Gram-positive clusters that formed glistering golden yellow colonies on blood agar and Mannitol Salt Agar (MSA) and were positive for coagulase, catalase, and oxidase allowed the identification of *S.aureus* isolates. Large kidney-shaped diplococci that were both catalase and oxidase positive, exhibiting a gray to white hemispheric colony on blood agar were used to identify *M. catarrhalis*. Using routine manual biochemical testing, the isolates of *K.pneumoniae* and *P.aeruginosia* were identified. As described on Muller Hinton agar, the Kirby-Bauer disc diffusion method was used to test for antibacterial susceptibility. For 24 hours, the plates were incubated at 37ºC. Initially, we questioned the attending physicians about the antimicrobials they regularly recommended to treat the bacteria that induces RTI infections. The Clinical and Laboratory Standards Institute's (CLSI) standards were then compared to those prescriptions. Utilizing a ruler, the zone of inhibition was evaluated in order to compare the medication sensitivity or resistance of the drug susceptibility or resistance of samples containing isolated bacteria.

Results were reported as either Susceptible (S), Intermediate (I), or Resistant (R) following interpretation according to the CLSI guidelines. antibiotic that used in this study were:
Gentamycin, Cefuroxime, Amikacin, Cephalexin, Ceftazidime, Amoxicillin, Ampicillin, Piperacillin, Ciprofloxacin, Azithromycin Levofloxacin, Cefotaxime, Erythromycin.

Inclusion criteria

- Patients aged (less than 1-14 yrs.).
- Patients suffering from upper and lower respiratory tract infection.
- Patients with complete data.

Exclusion criteria

- Patients with incomplete data.
- Patients with tumors.
- Patients with autoimmune diseases.
- Patients who took antibiotics.
- Sputum samples were positive to acid fast bacilli.

Ethical considerations

In the present study it was ensured that all the specimens were collected from children only after obtaining verbal approvals from the parents. Both parents of the patient agreed in order to take samples of this patient into this study. Due consideration was given to the ethical issues with regard to human subject research and this study was conducted with the consent of the respective hospitals and clinics.

Statical analysis

All data were analyzed using SPSS software (V.28 Inc., Chicago, USA) and Microsoft Excel 2019 to estimate frequencies and percentages .

3. RESULTS AND DISCUSSION

127 samples from sputum and throat swab were collected from patients who presented symptoms of respiratory tract infection. positive bacterial growth was 120 of the samples and the 7 samples were exclude from this study. The majority of the bacterial pathogen were obtained from male 71 (59.2%) whereas 49 (40.8%) from female.

3.1 Results

Table (1) shows the largest proportion (30%) of the children who took part belonged to the age group of 10-14 years, with 27.5% falling within the 1-4 years category, 21.7% within the 5-9 years group, and 20.8% under the age of 1 year and shows lower respiratory tract infections (LRTI) is more prevalence in investigated children than upper respiratory tract infections (URTIs),where the LRTI was 71.7%, and URTI was 28.3%.This result was agreement with those results that obtained by [11-14] who reported that LRIs were the leading infectious cause of death and the fifth-leading cause of death overall.

Table (1): Sociodemographic Characteristics of Children Studied in Current Study

Variables	Frequency	Percent %
Age:		
<1 yrs	25	20.8
1-4 yrs	33	27.5
5-9 yrs	26	21.7
10-14 yrs	36	30
Sex:		
Male	71	59.2
Female	49	40.8
Respiratory tract infections:		
URTI	34	28.3
LRTI	86	71.7
Severity of disease:		
Mild	43	35.8
Moderate	40	33.3
Severe	37	30.8

Table (2): Clinical Profile of Respiratory Tract Infections in Paediatrics

Clinical Profile	Frequency	Percent %
Cough	99	82.50
Fever	102	85.00
Nasal Discharge	13	10.83
Difficulty Breathing	44	36.67
Sore Throat	12	10.00

Table (2) shows shows that the fever and cough are the most symptoms of pneumonia revealed among the infected children with percent proportions of 85 % and 82.5 %, respectively. The results of study agreed with [15-17] who revealed in their study that fever and cough were common symptoms of respiratory infections in pediatrics.

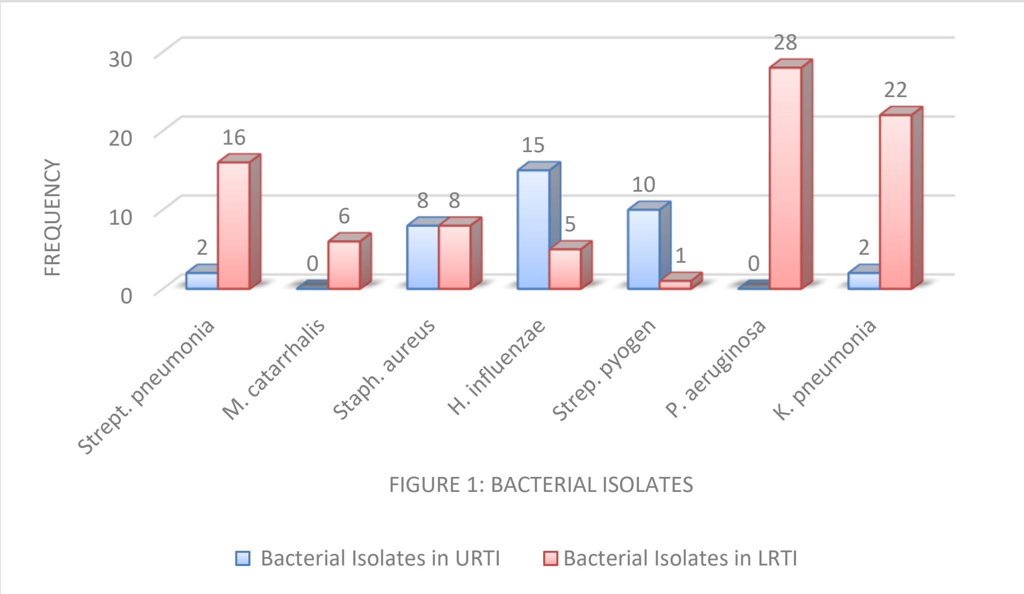

Figure 1: frequency of bacterial isolated from patients with respiratory infection.

In this study observed P.*aeruginosa*, *Strep.pneumonia*, *K.pneumonia* and *Staph. aureus* were the common and prevalent bacteria in lower respiratory tract infections (LRTIs). This study result was in line with other studies conducted by [18-20]. While, the most predominant bacteria in upper respiratory tract infections (URTIs) were *H. influenzae*, *Strep. pyogen*, *Staph. aureus* and *Strep. pneumonia*, this result was supported by different studies such as [21]and [22].

In this study gram negative bacteria highest isolates were 76 (63.3%) whereas gram positive bacteria isolates were 44(36.6%). These results similar to other study conducted by [23]. Bacteria that were isolated from upper respiratory tract

These bacterial isolates include *P.aeruginosia* 26(21.6%), *K.pneumonia* 24(20%), *Strep.pneumonia 18(15%), H.influenzae* 20(16.6%), *S.aureus* 16(13.3%), *Strep.pneumonia* 10(8.3%) and *M.catarrhalis* 6(5%) 120 samples, 86 bacterial isolates were from sputum samples while 34 samples isolates from throat swab. *P.aeruginosia* is the most common isolate from sputum followed by *K.pneumonia* ,*Strep.pneumonia* and *M.catarrhalis* .*H. infiuenzae* most common bacteria isolates followed by *Strep.pyogenstrep.*

Table (3) Relationship Between Bacterial Isolates and the Severity of Respiratory Tract Diseases in Pediatrics

Bacterial Isolates	No. of Bacterial Isolates in:		
	Mild Cases	Moderate Cases	Severe Cases
Strep.pneumonia	0	8	10
M.catarrhalis	4	2	0
Staph.aureus	3	8	5
H. influenza	5	10	5
Strep.Pyogen	9	1	0
P. aeruginosa	0	6	20
K. pneumonia	2	4	18

Table 3 it appears that numerous bacteria could cause severe respiratory diseases, such as *P. aeruginosa* and *K. pneumonia*, where they were the most frequent bacteria in severe cases with frequencies of 20 and 18. This result was verified by several studies such as [24] and [25] who reported those bacteria were the most significant in severe respiratory diseases. Whereas, *H. Influenzae*, *Staph. aureus* and *Strept.pneumonia* were more repeated bacteria associated with moderate respiratory diseases, with counts of 10, 8 and 8. Our finding was in agreement with studies performed by [26] and [27]. However, in mild cases, *Staph. pyogen* is the most prevalent bacterium, with 9 counts.[28] and [29] proved the same result in their work.

Table (4) Antibiotic resistance pattern in bacterial isolates from patients with respiratory tract

Antibiotics	Percentage of bacterial isolates resistant to antibiotic						
	P.aurginosa =26	*K.pneumonia* n=24	*Strep.pneumonian*=18	*Staph.aureus* n=16	*Strep.pyogen* n=10	*H.inflenzae* n=20	*M.catarrhalis* n=6
Gentamycin	11.5 %	0.0%	0.0%	0.0%	0.0%	-	-
Cefuroxime	7.6%	8.3%	11.11%	0.0%	0.0%	-	-
Amikacin	0.0%	4.1%	5.5%	0.0%	0.0%	-	-
Cephalexin	100.0%	87.5%	88.8%	-	70.0%	-	-
Ceftazidime	80.0%	95.8%	61.1%	62.5%	80.0%	-	-
Amoxicillin	100.0%	100.0%	100.0%	100.0%	90.0%	-	0.0%
Ampicillin	100.0%	100.0%	100.0%	100.0%	100.0%	5.0%	-
Piperacillin	100.0%	79.1%	100.0%	68.75%	-	-	-
Ciprofloxacin	92.0%	91.6%	-	81.25%	100.0%	-	-
Azithromycin	-	-	-	-	-	10.0%	16.0%
Levofloxacin	-	-	100.0%	-	-	0.0%	16.0%
Cefotaxime	-	-	-	-	-	0.0%	0.0%
Erythromycin	-	-	-	-	-	5.0%	16.0%

*n= the number of bacterial isolates
*- = non do

The antimicrobial effects ampicillin and amoxicillin showed the highest degree of resistance. Additionally, Ceftazidime, piperacillin-tazobactam, and cephalexin were found to have high levels of resistance. Table (3) shows highest resistance of *p.aeruginosa* was observed against to most of the antibiotics used, cephalexin, piperacillin and amoxicillin with (100.0%) fllowed by 92% was resistance to ciprofloxacin and ceftazidime (80.0%). on the other hand, the highest sensitivity observed to amikacin followed by cefuroxime (7.6%) and gentamycin (11.5%). These results similar to other study conducted by [30] showed that 100% of isolate *p.aeruginosa* were resistance to cephalexin.

In other study conducted by [31] showed that *p.aeruginosa* was highest resistance to followed by 96% cephalexine. *K.pneumonia* was highest sensitivity to gentamicin (0.0%) followed by amikacin (4.1%) and cefuroxime (8.3%). This study show *M.catarrhalis* was highly sensitive to amoxicillin, cefotaxime followed by azithromycin, erythromycin and levofloxacin. Whereas *H.inflenzae* was more sensitive to levofloxacin and cefotaxime fllowed by erythromycin (5.0%) and azithromycin (10.0%). Results are similar to other previous study by [32-33].

Table (5) multidrug resistance pattern in bacterial isolate from respiratory tract infections.

Type of bacterial isolates	Total	Multidrug resistance
P. aeruginosa	26	15(57.6%)
K. pneumonia	24	9(37.5%)
Strep. Pneumonia	18	7(38.8%)
Staph. aureus	16	10(37.5%)
H. Influenzae	20	12(60.0%)
Strep. Pyogen	10	0(0.0)
M. catarrhalis	6	0(0.0)
Total	120	53(44.1%)

All bacterial isolates were screened for antimicrobial resistance, and multidrug resistance was identified 53(44.1%) of the tested bacterial isolate**.** (Table 5) *P. aeruginosa* exhibited the highest amount of multidrug resistance, at 57.6%**.** *M. catarrhalis* and *Strep. Pyogen isolates* did not exhibit any multidrug resistance.

CONCLUSION

Through this study, we conclude that children under the age of five years are more susceptible to respiratory infections. lower respiratory tract infections (LRTI) was more prevalent in investigated children than upper lower respiratory tract infections (URTI). Fever and cough were common symptoms of respiratory infections in pediatrics *P. aeruginosa* and *K. pneumonia* were the most common types of bactreia isolated followed by *H. influenzae*, *Strep. pneumonia*, *Staph. aureus*, *Strep.pyogen* and *M. catarrhalis*.
However, these organisms have become resistant to at least two routinely used antibiotics, such as cephalexin, amoxicillin, ampicillin, ciprofloxacin, ceftazidime, and piperacillin-tazobactam. The high degree of antibiotic resistance found in the research area necessitates the use of susceptibility tests and cultures in order to properly manage respiratory infections.

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