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Investigate the Spread of *Haemophilus influenzae* in Patients Infected with Upper Respiratory Tract Infection in Samarra city Anemia

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ABSTRACT

The study included the collection of 155 specimens and divided to 64 specimens of people suffering of pharyngitis, 52 specimens of people suffering of tonsillitis, and 39 specimens of people suffering from sinusitis, and they were collected from Samarra General Hospital and some outpatient clinics for the period between 2/11/2021 to 27/ 4/2021 from all age groups, in order to isolate *Haemophilus influenzae*, specimens were culture on chocolate agar and blood agar. The specimen was diagnosed according to routine methods in terms of culture characteristics, microscopic diagnosis and biochemical tests, the culture and diagnosis results showed that the percentage of *Haemophilus influenzae* was about % 9 with 15 isolates, where 6 isolates were from children, 6 isolates from adults, 3 isolates from the elderly, and it was found that 9 isolates were of type B, while 6 isolates were of Non-type *Haemophilus influenzae*. The study included testing the sensitivity of isolates of *Haemophilus influenzae* to eight antibiotics belonging to different groups by way of spreading around the tablets, and these antibiotics are Ampicillin, Amoxillin, Erythromycin, Ceftriaxone, Levofloxacin, Rifampin, Tetracyclines, Trimethoprim / Sulphamethaxole, and these results showed resistance to multiple antibiotics, high resistance was recorded against Ceftriaxone and Tetracyclines at 100%, Ampicillin at 93,4%, Levofloxacin at 93,7 and Amoxillin at 80,1 %, and medium resistance against Erythromycin at 60 %, and Trimethoprim/Sulphamethaxole at 60.8%, for the antibiotic Rifampin, it was the most effective in isolates of *Haemophilus influenzae*, as it showed a sensitivity to this antibiotic with a rate of 73,3%.

INTRODUCTION

The respiratory tract infects humans with a large and diverse number of organisms, and the upper respiratory tract is considered a reservoir for a large number of negative and positive bacteria, aerobic and non-aerobic (Santacroce *et al.*, 2020), among these bacteria is *Haemophilus influenzae*, which is endemic to the upper respiratory tract in particular and infects infant, children, adult, and some types have capsule capsules, which are classified into six serotypes, namely, a, b, c, d, e, f serotype, and some do not have a capsule. Some studies have indicated that *Haemophilus influenzae* that possesses a capsule is the most dangerous (Crandall *et al.*, 2019), and type b is the most dangerous species for having a capsule, which is Polyribosyl ribitol phosphate (PRP), which helps the bacteria invade the respiratory tract and cause infections.

Including Pneumonia, Epiglottitis, Meningitis, and other infections in children and adults (Takeshita *et al.*, 2020), *Haemophilus influenzae* has a number of mechanisms that enable it to resist antibiotics, including the production of B-Lactamase enzymes that break down the B-Lactams ring of penicillin. Efflux systems are one of the resistance mechanisms in *Haemophilus influenzae* that pump antibiotics out of the bacterial cell.

The aim of our study is to isolate and diagnose *Haemophilus influenzae* from people visiting Samarra Hospital and private clinics in Samarra who are infected with upper respiratory tract, and check sensitivity of bacteria for different groups of antibiotics

MATERIALS AND METHODS

Specimen Collection:

155 specimens were collected from Samarra city hospitals, including Samarra General Hospital and Consultation Hospital, and from some outpatient clinics for the period between 2/11/2021 and 4/27/2022, by taking cotton swabs containing a carrier medium from patients diagnosed by a specialist doctor who suspects upper respiratory tract infection (64 specimens from pharyngitis, 52 specimens from tonsillitis, 39 specimens from sinusitis) from both genders and from all age groups. Respiratory infections, samples were cultured on chocolate medium and blood medium which contain antibiotics bacitracin tablets 10 micrograms after dissolving them in the medium for the purpose of purification and in the presence of two X.V factors, and were incubated in the incubator under anaerobic conditions for 48-24 hours at a temperature of 37 °C in the presence of an amount of CO₂ at 5% (anaerobic bags or Candle jar).

Diagnosis of Bacterial Isolate:

After culture bacteria on chocolate medium and blood medium, the diagnosis was made as following:

Form Colonies on chocolate and blood medium, form colonies under a light microscope using Gram stain, perform the biochemical test as oxidase test, catalase test,

mannose fermentation test, triple sugar test, capsule test, satellite test, bacterial need test for factor v and x, agglutination test.

Antibiotics Sensitivity Test:

The sensitivity of bacteria to eight antibiotics was tested using the Disk diffusion method, the bacterial suspension was prepared by transferring a bacterial colony to tubes containing 5 ml of physiological saline solution and the density was compared with a standard turbidity constant solution which is equivalent to 1.5×10^8 cells/ml, then a sterile cotton swab was inserted into the tube containing the bacterial suspension, and then spread on the center of Mueller-Hinton which contain factors v, x by diffusion method for a gland several times to be distributed evenly on all directions of the dish at an angle of 45 in order to ensure the good spread of bacteria, then, the antibiotic tablets were placed on the surface of the culture medium at the appropriate dimensions of the dish so that the inhibition zones do not overlap between them, and then incubated anaerobic for 24 hours at a temperature of 37° C and with a presence of 5% CO₂, After completing the incubation, the results were read using a ruler, then the results were compared with standard tables according to whether they are sensitive or resistant.

RESULTS AND DISCUSSION

Bacterial Isolate:

155 specimens were collected from people with upper respiratory tract infection, distributed as 64 samples from pharyngitis, 52 from tonsillitis, 9 from 3 from sinusitis from people who visited Samarra city hospital and some outpatient clinics, for the purpose of isolating and diagnosing *Haemophilus influenzae*, where the specimen were culture on the Chocolate medium, which promotes the growth of fastidious bacteria such as *Haemophilus* and *Neisseria* that need certain nutrients for their growth, including two factors (Hemin X) and V (Nad).

The results of the isolation resulted in 138 bacterial growth and 17 fungi growth. The bacterial isolates were distributed into 73 isolates (47%) belonging to the genus

Staphylococcus, 25 isolates (16.1) belonging to the genus *Streptococcus*, 19 isolates (12.2%) belonging to the genus *Haemophilus*, 21 isolates (7.7) belonging to *Klebsiella pneumoniae*, 3 isolates (1.9) belonged to

Escherichia coli, 3 isolates (1.9%) belonged to *Moraxella catarrhalis*, 3 isolates (1.9) belonged to *Pseudomonas aeruginosa* as shown in Figure 1.

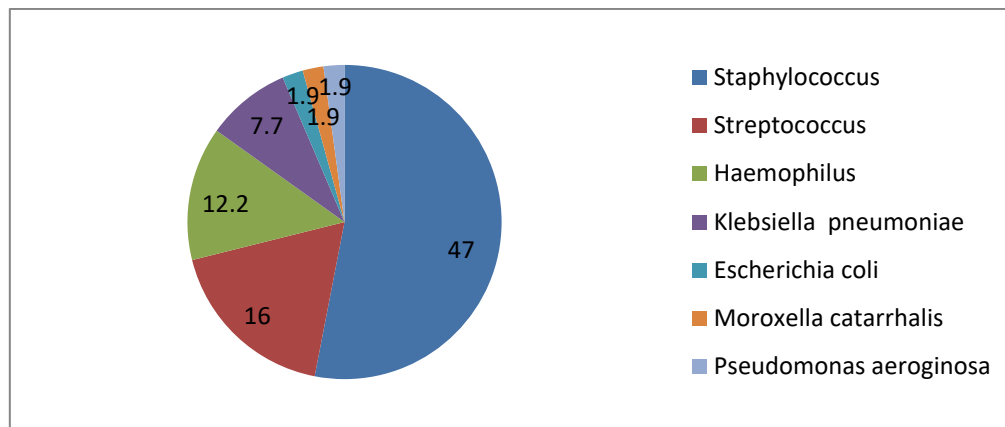


Fig.1: shows the percentage of bacteria isolated from the upper respiratory tract.

It was found through the results of the isolation that the species belonging to the genus *Haemophilus* are not the dominant of the isolates obtained, but that it comes in the third place in terms of the number of bacterial species that appeared at the initial isolation,

and the species that were isolated are *Haemophilus influenzae*, with a number of 15 isolates, where 6 isolates were from children, 6 isolates from adults, 3 isolates from the elderly, as shown in Table 1 and *Haemophilus parainfluenzae* with 4 isolates from adults.

Table 1:- Isolate sources, number and percentages of *Haemophilus influenzae* isolates.

Source isolate	Number sample	Number Isolate	Children	Adults	Elderly
pharynx	64	6	2	3	1
Tonsil	52	6	3	2	1
Nose	39	3	1	1	1

Our results were almost identical to what was obtained by the researcher Farajzadeh *et al.*, (2004), who collected 170 samples and was able to isolate *Haemophilus influenzae* from 14 patients, while it did not match what was obtained by the researcher Shooraj *et al.*, (2019), where the dominant bacteria were from 108 isolates out of 328 isolates in Iran.

The isolates were diagnosed by microscopic examination and the isolates showed a spherical rod negative for the gram stain and the biochemical examination showed that they were positive for the oxidase

and catalase test and fermented for glucose sugar and non-fermented for lactose, sucrose and mannose, and also proved the need of bacteria for VX agents, and the growth of *Staphylococcus aureus* in a satellite method.

Epidemic of *Haemophilus influenzae* in Iraq:

From our results, we can show that the proportion of *Haemophilus influenzae* in patients with a respiratory infection is about 12% with 15 isolates and included the isolation of 6 of the pharynx out of 64 isolation, 6 of the tonsils out of 52 isolation and 3 of the nose out of 39 isolates that the

proportion of males in people infected with *Haemophilus influenzae* is higher than that of females, where the proportion of males was 60% and the percentage of females 40%, Table 2 shows that 6 isolates of the tonsils included 4 males and 2 females and were 5

isolates of type b and 1 Nontypeale, while 6 isolates of the pharynx included 4 males and 2 females and were 3 isolates of type b and 3 Nontypeale, while 3 isolates of the nose included 1 male and 2 females and was one isolation of type b and 2 of Nontypeale.

Table 2: Number of isolates of *Haemophilus influenzae* Nontype and type b in males and females.

Area Isolates	<i>Haemophilus influenzae</i> type b	Nontype <i>Haemophilus influenzae</i>	Male	Female
Pharynx	3	3	4	2
Tonsils	5	1	4	2
Nose	1	2	1	2

The results were compared with other results in which bacteria were isolated from different cities in Iraq, and the bacteria were isolated years ago when the researchers Al-Taie and Al-Rawi (2001) were able to isolate the bacteria from people with upper respiratory tract inflammation by 27%, where the number of isolates was 387 out of 1420 and it turned out that type b is the most common among the isolated species if they number 153 isolation, and in 2005 the researcher Nader was able to isolate bacteria from patients with upper respiratory inflammation, with 17 out of 219 isolations in Mosul, Also, the bacteria were isolated from children with Meningitis by researcher Almelan (2015) with 18 isolations out of 250 isolations and all isolation was type b, and as the researcher Jreemich (2015) was able to isolate bacteria from people with tonsilitis in Diwaniya with 12 isolations out of 131 isolations and found that type b is the most common type, As the researcher H Ali (2016) isolated bacteria from patients with tonsilitis by 12% with 18 isolates and found that type b is the most common with 15 isolates. The researcher Rasol (2019) isolated 8 isolates out of 191 isolates from *Haemophilus influenzae* type b taken from children with meningitis in Najaf, and in the city of Hilla the researcher (Bunyan 2019) was able to isolate bacteria

from patients with sore throat with 8 isolates out of 204 isolation, In addition, the bacteria were isolated from adults with pneumonia by researcher Alubaidi *et al* (2020), where the number of isolates was 26 out of 100 and all isolates were type b. In people infected with the Covid-19 virus, the bacteria were isolated in 15 out of 170 isolates by researcher Amin (2022). It turns out that type b is the most common of people infected with *Haemophilus influenzae* in Iraq in terms of infection sites and age groups.

***Haemophilus influenzae* Satiability to Antibiotics:**

The sensitivity of *Haemophilus influenzae* isolates under study was tested against 8 antibiotics distributed among the Beta-lactams group antibiotics for penicillans and spectrum media, which included Ampicillin (AM) and Amoxillin (AMC), Macorlides group Erythromycin (E), Cephalosporin group Ceftriaxone (CXT), Fluroquinolones group Levofloxacin (LEV), Rifampin group Rifampin (RIF), Tetracyclines group Tetracycline (TE), group Sulfonamides Sulphamethaxole /Trimethoprim (SXT), The Kirby-Bauer disc method was used for all isolates by measuring the diameter of the inhibition zone around the tablet used and the results were compared to the CLSI. (2021).

Table 3: Antibiotic Sensitivity Test Results.

Antibiotics	Number of resistant isolates	Percentage of resistant Isolates	Number of Sensitive isolates	Percentage of Sensitive isolates
AM	13	93.4	2	6.6
AMC	12	80.1	3	19.9
E	9	60	6	40
CTR	15	100	0	0.0
LEV	14	93.7	1	6.3
RIF	11	73.3	4	26.7
TE	15	100	0	00
SXT	9	60.8	6	39.2

Table 3 shows that there is high resistance to the Ampicillin and 93.4% by the isolates under study. and the results were also close to what the researcher concluded Zhou *et al.*, (2021) that the ratio of the resistance of bacteria to the antibiotic is 96%, and the results showed the resistance of isolates to the Amoxillin by a percentage of 80% and this result came identical to what the researcher Amin (2022) presented, which was the percentage of resistance of bacteria to this antidote 80.1%, As the results showed the resistance of isolates to the Ceftriaxone by 100% and these results were consistent with the findings of Amin (2021) that the resistance of bacteria to the antibodies Ampicillin, Amoxillin and Ceftriaxone is due to its ability to produce Beta-lactamase enzymes that break down the beta-lactamase ring, which works to analyze penicillins and cephaloporins in particular, whose genes are carried either on the plasmid or chromosome in many nuclei of bacteria. The antibiotic becomes ineffective as a result of the loss of the target site of the antidote, and the antibody becomes ineffective by changing the binding sites of the Penicillin Binding Protein (PBP), in addition to the interference in the treatment leads to antibiotic-resistant mutations and thus makes the bacteria resistant and a producer of the enzyme beta-lactamase. (Thegerström, 2018; Li *et al.*, 2020; Heinz, 2018).

In addition to the presence of Pump efflux or tat gene systems that work effectively

in resistance against this group (Chopra and Roberts, 2001), and the results also showed high resistance of bacteria to Levofloxacin by 93.7% and the results were almost identical to the findings of the researcher Zhang *et al* (2018) which had a resistance ratio of bacteria to the antibiotic of 92% and the resistance of bacteria to the is due to mutations in genes encoded to produce DNA enzymes (Gyrase, (Topoisomerase –IV) Chen *et al.*, (2018) The average resistance of the bacteria was against the antibiotics Trimethoprim/Sulphamethaxole and Erythromycin where the ratio of the resistance of the bacteria to the Trimethoprim / Sulphamethaxole %60.8 and this result is similar to obtained by the researcher Su *et al.*, (2020), which was 58% resistance and one of the reasons for the resistance of bacteria to the antibiotic is the occurrence of mutations in the gene Fola and thus contributes to the ineffectiveness of the antigen (Sierra *et al.*, 2019), and for the Erythromycin the resistance ratio was 60% and this result is similar to what the researcher Daoud *et al.*, (2006) stated where the resistance rate was 55% and that the low permeability of the cell membrane and the occurrence of mutations in rRNA S23 or ribosomal protein are causes of resistance (Peric *et al.*, 2003).

The bacteria showed sensitivity to the Rifampin where it was 73.3% and the isolates of *Haemophilus influenzae* type b were the most sensitive, and a study showed the effectiveness of the antibiotic in inhibiting

the growth of bacteria by disrupting the enzyme RNA-dependent RNA polymerase (RNAP) that bacteria use to manufacture essential proteins (Hardie and Fenn, 2022).

Conclusions

Our current study showed that *Haemophilus* recorded the third highest rate of isolation in specimens taken from people with organ infection Upper respiratory in the city of Samarra, the study showed that *Haemophilus influenzae* type b is predominant among isolation and that the prevalence of *Haemophilus influenzae* among children and adults is higher than in the elderly, and the percentage of males is higher than the females. *Haemophilus influenzae* showed high resistance to Ampicillin, Amoxicillin, Ceftriaxone, Tetracyclines, and Levofloxacin, moderate resistance to Erythromycin, Trimethoprim/ Sulphamethaxol, and sensitivity to Rifampin.

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