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Co-Infections Bacterial and Human Parainfluenza Virus of The Upper Respiratory Tract at Children in Kirkuk City

ABSTRACT

Background: Upper respiratory tract co-infection including (nose, mouth, pharynx and throat) originated from colonization bacteria, viruses and fungi epithelial layer lining the upper respiratory tract.

The aim: To determine the pathogens (bacteria and virus) common in the upper respiratory co-infection.

Materials & Method: the study covered children aged (5 months to 12 years) which has upper respiratory tract co-infection detection the pathogens causing infections through a series of diagnostic tests.

Using characteristics, microscope examination and biochemical test for diagnosis of different types of bacteria after doing sensitivity test for this bacteria used group of antibiotics. The Human parainfluenza virus (HPIV) was diagnosed using indirect enzyme linked immunosorbent assay by two kits including (IgM parainfluenza type 1,2,3 kit and IgG parainfluenza type 1,2,3 kit).

Result: A total of 150 patients of children, found 119 of children was infected with different types of bacteria including gram negative bacteria which formed percentage 8% and included (E.coli, Pseudomonas aeruginosa, Klebsella pneumoniae, Citrobacter freundii) and gram positive bacteria formed percentage 71.3% and included Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae, Viridans streptococci, Group B streptococci, Group C streptococci, Group G streptococci, Group D streptococci, Group F streptococci. These types of bacteria were resistant with different percentage for beta-lactam antibiotics which included cefepime, amoxicillin-clavulanic acid, ampicillin, penicillin G, tetracycline, ceftazidime, Gentamicin, ceftriaxone), as for antibiotics (vancomycin, erythromycin, clindamycin, chloramphenicol) some isolated bacterial species were sensitive to these antibiotics. Out of a total of sick children, 59% of them were infected with HPIV, 33% of them had IgM anti human parainfluenza virus and 26% had IgG anti human parainfluenza virus. Among the patients of children found, 41% ones had co-infection with bacteria and virus.

Conclusion All bacterial species (gram positive and gram negative) were resistant to beta-lactam antibiotics with varying percentage depending on the bacterial species -Participation of bacterial species (S.pyogenes, Staph.aureus and S.pneumoniae) with Human parainfluenza virus to cause Co-infection to each other in the respiratory system.

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Introduction

Upper respiratory tract co-infection (including nose, mouth, pharynx and throat) is a common disease originated from colonization bacteria ,viruses and fungi epithelial layer alining the upper respiratory tract (1,2). Cooperative relationships occur between these pathological causes namely bacteria include (Streptococcus spp, Staphylococcus spp, Klebsiella spp, Citrobacter spp, Pseudomonas spp and Escherichia coli) (3) and Viruses include (human parainfluenza virus, adeno virus, respiratory syncytial viruse, rhino virus, corona Virus (4, 5).From these relationships result agroup of disorders like (Pharyngitis, Tonsillitis, Bronchitis, Rhinitis and nasal pharyngitis may lead to pneumonia (6) . There were some factors affecting the comparative relationships and reduce colonization phenomenon by prevention pathogene adhesion in the epithelial cell and eliminating them like antibiotics and vaccines (7) . However, some bacterial species are resistant to the antibiotics because of their excessive use (8), therefor, this study deals with the knowledg of the pathogens (bacteria and virus) common in the co-infection of upper respiratory tract in addition to knowing the precentage resistance of bacterial species to antibiotics.

Material and Methods

Sample collection: Through a sectional study, samples were collected from 150 children(85 child of man and 65 of female) aged 5months to 12years . They referred to the hospitals of Kirkuk city during the period 27-2-2017 to 30 – 5- 2017 with sings and symptoms of respiratory tract infection . Two type of instruments were used for the purpose of sample collection . One sterile swab was used for collecting samples from throat , pharynx and nose after then transferred to the laboratory for diagnosis. Second sterile syringe(5ml) was used for blood that collected by Vacuum tube (clot activater) and centrifuged at 3000rpm for 3minutes and then separated the serum by micropipette and transferred to ependrof tube for storing it at - 20°C. Diagnosis Many bacteria were diagnosed in this study through using group diagnostic test including cultural characteristics and microscopic examination (9).Biochemical test includes (oxidase, catalase, indole, citrate utilization, motility, methyle red, kligers iron agar, voges- proskauer, eosine methylene blue, urease, muller Hinton agar, and xylose lysine deoxycholate agar) test for diagnosis gram negative bacteria (10-15) and used API Enterobactraceae (16). (mintol salt agar, cogulase , oxidase, catalase, gelatine, motility and Hemolysis) tests were used for diagnosis Staphlococcus spp (10, 11,

15, 17,18) and used API staph (19). Streptococcus spp was tested including (Bactriacin, Optochin, Sulphamethoxazo timethoprime and used Biochemical test include (bile salt tolerance, alkaline test , salinity tolerance , eschulin,motility,catalase test, oxidase test,gelatinase test) test for diagnosis Streptococcus spp and used (indole, mathyle red, citrate utilization, Catalase test, manitol salt agar test) for diagnosis Bacillus spp (14,15,17,20-23). Lancefield test used for differentiation Streptococcus grouping and used API Streptococcus (19) and confirmed the Bacilluse spp by API 50 CHB. The diagnosis virulence factor of Streptococcus pyogenes included (Capsule, Cysteine proteas and Hemolysin enzyme) factor (24, 22). After that the sensitivity test for was performed all bacterial species was done the know the sensitivity or resistance to

antibiotic including (amoxicillin-clavulanic acid, Gentamicin, chloraphenicol, erythro-mycin, cefepime, tetracyclin, penicillin-G, ceftzidime, clindamycin, vanco-mycin, Ceftriaxone (Bioanalyse, Turkey). ampicillin (Mastdisc, UK). For viruses diagnosis was done by using ELISA kit including (IgG Parainfluenza type 1,2,3 kit and IgM parainfluenza type 1,2,3 kit) (Demeditec, Germany).

Results

In this study of the 150 children who were sick ,119 were found have different types of pathogens include(Bacteria and virus), Two group of bacteria were isolated (one group negative for gram stain formed percentag 8% and second group positive for gram stain formed percentag 71%) . Each one of these groups included different species of bacteria that are show in in Table 1.

Table 1 percentages of bacterial species isolate of from URT of children age < 5 years.

Sequence	Gram negative bacteria	No.	%	Gram positive bacteria	No.	%
1	<i>E. coli</i>	1	8.3	<i>Bacillus spp</i>	2	1.86
2	<i>P.aeruginosa</i>	1	8.3	<i>Staph. aureus</i>	17	15.88
3	<i>K.pneumoniae</i>	4	33.3	<i>Staph. epidermidis</i>	13	12.14
4	<i>Citrobacter frundii</i>	6	50	<i>S. pneumoniae</i>	4	3.7
5	-			<i>S. pyogenes</i>	29	27.10
6	-			<i>Viridans s.</i>	8	7.47
7	-			<i>Group B streptococcus</i>	2	1.86
8	-			<i>Group C streptococcus</i>	9	8.41
9	-			<i>Group D streptococcus</i>	12	11.21
10	-			<i>Group F streptococcus</i>	6	5.60
11	-			<i>Group G streptococcus</i>	5	4.67
Total		12	100	Total	107	100
Note that the total number of samples that did not give bacteria growth is 31 specimens						

N=Number ,Staph=Staphylococcus, S=Streptococcus, P=Pseudomonas K=Klebsiella, E=Escherichia

in this study, it was found all that types of bacteria isolated from the patient were resistant to beta-lactem antibiotics in different percentage depending on the bacterial species (Figure 1) either resistance for antibiotics macrolides, glycopeptides, miscellaneous antibacterial antibiotics that found not all bacteria species resistant to it because some species have sensitivity to this antibiotic (Figure 2,3,4) adopted in measuring the inhibition diameters on the spelling (25).

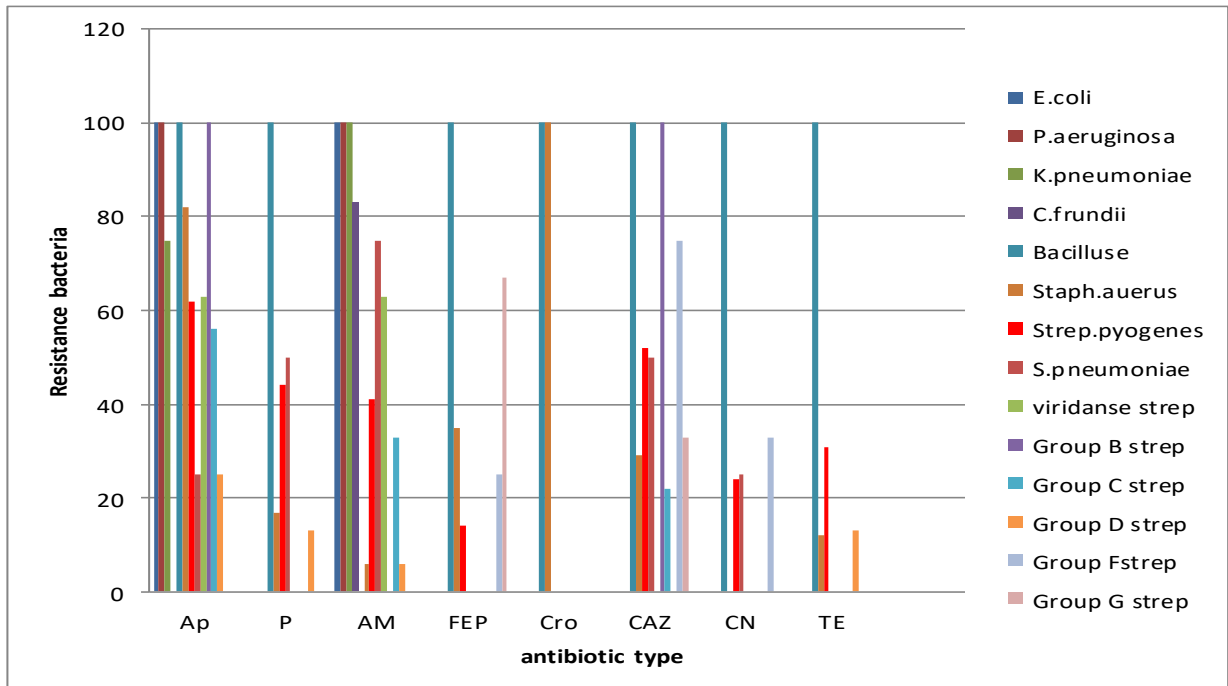


Figure (1) the percentage of resistant bacteria isolates from URT of children to beta-lactem antibiotic. AP=Ampicillin, TE=Tetracyclin, CN=Gentamicin, P=penicillin G, AM=Amoxacillin, FEP=Cefepime, CRO=Ceftriaxone, CAZ=Ceftazidime

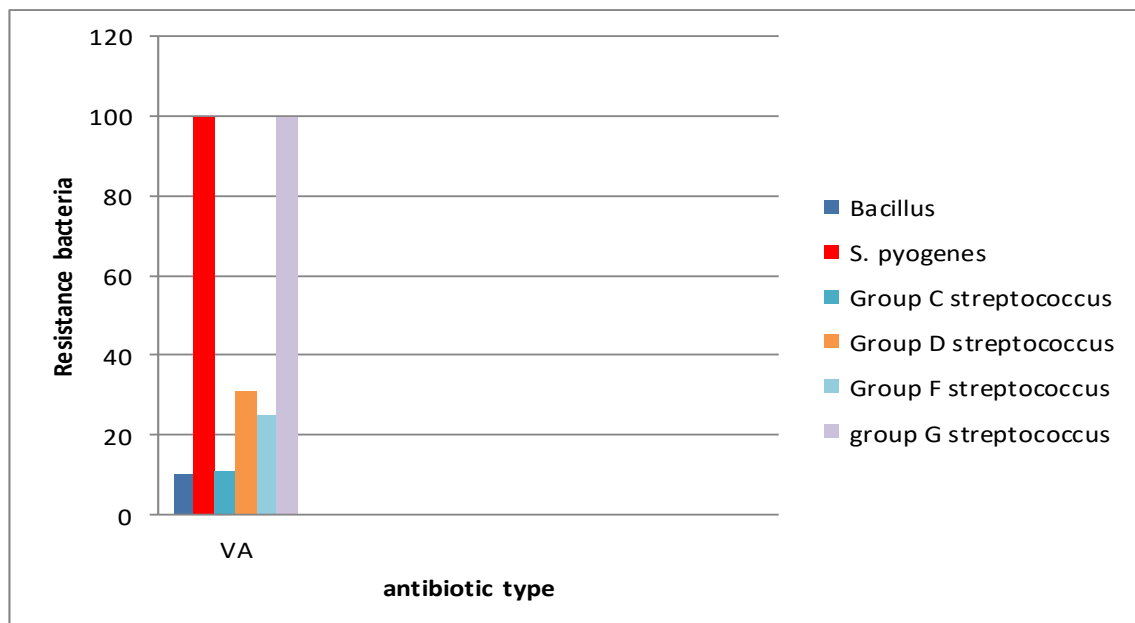
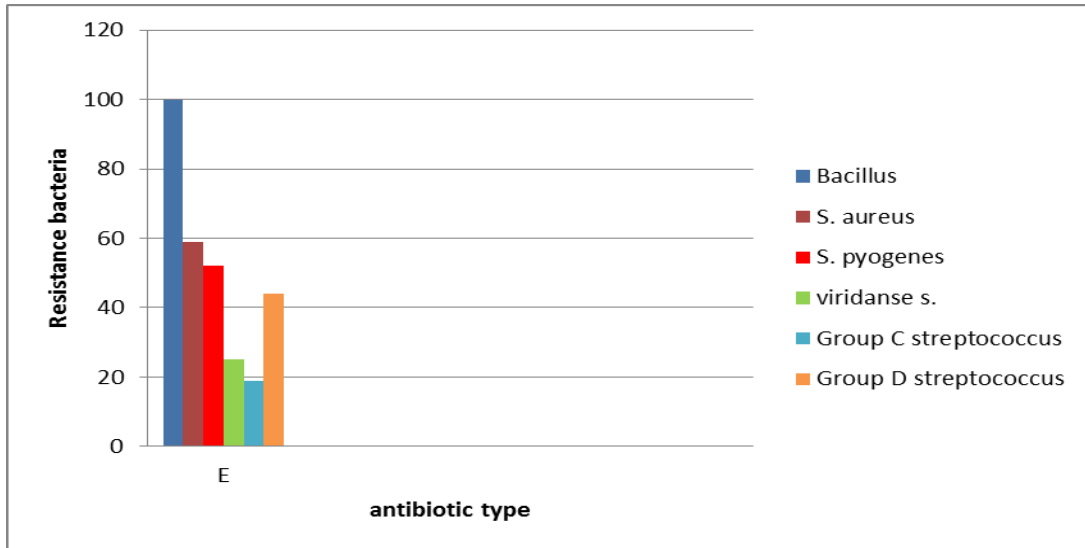


Figure (2) The percentage of resistant bacteria isolates from URT of children to glycopeptides (VA=Vancomycin) .



Figure(3) The percentage of resistant bacteria isolates from URT of children to macrolides (E=Erythromycin).

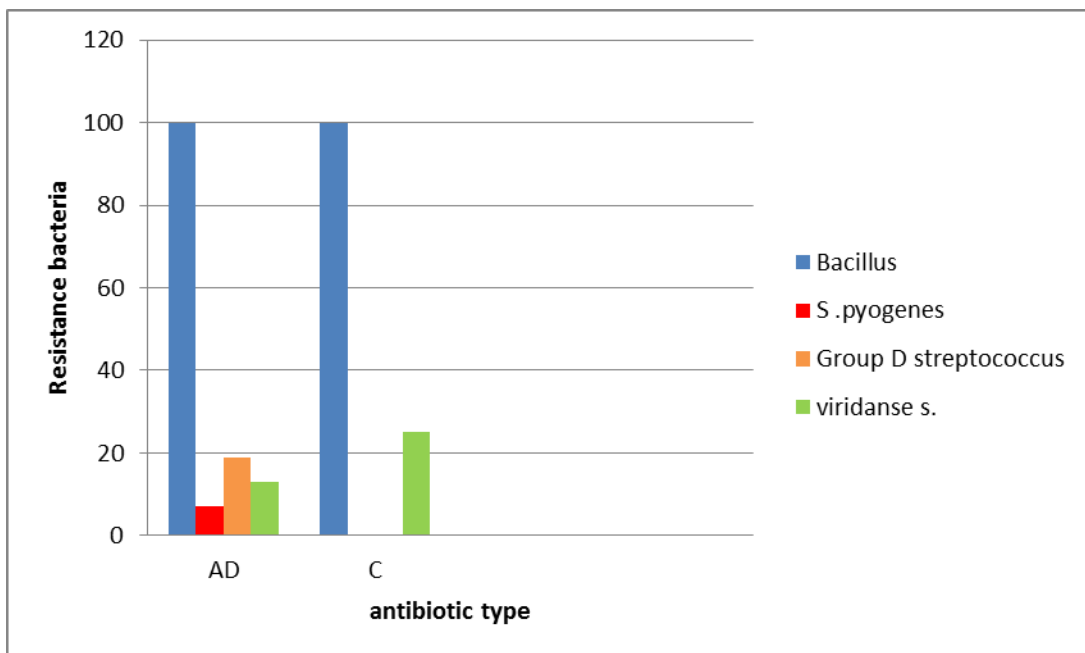


Figure (4) The percentage of resistant bacteria isolates from URT of children to miscellaneous antibacterial antibiotics. C=Clindmycin. AD= Chloromphenicol

In this study, Human parainfluenza viruse type (1,2,3) was diagnosed in 59 % from 88 samples taken from sick children (male and female) with anti human parainfluenza viruse (IgM and IgG) with percentage (33% and 26%) respectively (Table 2).

Table 2 the Percentage of anti human parainfluenza virus (IgM and IgG) detection in serum of children with URT infection.

Sex	Anti- human parainfluenza virus							
	IgM				IgG			
	Positive result	%	Negative result	%	Positive result	%	Negative result	%
Males	10	19	42	81	8	15	44	85
Females	5	14	31	86	4	11	32	89

Human parainfluenza virus (HPIV) participation with bacteria leading co-infection in upper respiratory tract is confirmed by the current study where it was found that some children (male and female) infected with bacteria are infected with viruses at the same time where their percentage is 41% (Table3).

Table 3 co-infection percentage of HPIV and some type of bacteria isolated from URT of children.

Sex	Children infected with co-infection			Children without co-infection	
		No.	%	No.	%
Males (52)	HPIV+<i>Strep.pyogenes</i>	13	27	38	37
	HPIV+<i>Staph.aureus</i>	1			
Femals (36)	HPIV+<i>Strep.pyogenes</i>	1	14	31	86
	HPIV+<i>Strep.pneumoniae</i>	4			

Discussion:

Samples were taken by swabs from the upper respiratory tract areas (Tonsils, Pharynx and nose) were tonsils are the most were exposed to infection ,As it is constantly exposed to different types of bacteria,They filter bacteria entering through the respiratory system and prevent it to entry in to the body (26). Many scientific studies carried out by researchers on the upper respiratory tract infection obtained similar results of the current study . The researcher

(27) obtained in his study 35% of Streptococcus pneumoniae, 32.12% of Klebsiella pneumoniae, 22.97% Pseudomonas aeruginosa. (28) obtained in his study 47.976% of Staphylococcus spp, 18.497 of streptococcus spp, 42.4% of Bacilluse spp and 2.312% of Klepsiella spp, 0.578% of Pseudomonas spp and (29) obtained 16.3% of Streptococcus pyogenes, 18.75% of group G Streptococcus and 9.25% of group C Streptococcus. The difference in the

percentages of bacterial type recur to some reasons including (geographical location for patient, age, and indiscriminate use of antibiotic). In this study also found the percentage of parainfluenza virus was higher than that obtained by the researcher (30) regarding detection of parainfluenza virus by the indirect ELISA from children under the age of one year. In the study, it is found that males are more likely to develop respiratory diseases than females because of several reasons, including high level of immunoglobulin IgM more than males this makes them inhibitory to many of the foreign antigens entering the body such as viruses reduce the proportion of infection with different bacterial species (31,32). This was confirmed by a study by the researcher (33), which found many children with respiratory diseases die after carrying out a number of laboratory tests and ensuring that they did not suffer from other diseases, a number of tests were also carried out that revealed different types of viruses, the result was positive testing for the HPIV virus which was the main cause of child death and the reason for the involvement of bacteria in the cause of diseases is the presence of viruses that provide suitable invasion conditions in addition to the vulnerability of the body of the child

Conclusion

Gram negative and Bacillus spp showed a low percentage in the role of bacteria in the upper respiratory diseases.

2- Gram positive bacteria especially (*Staphylococcus* and *Streptococcus*) showed high percentage in the role of bacteria in the upper respiratory diseases.

- All bacterial species (gram positive and gram negative) were resistant to beta-lactam antibiotics with varying percentage depending on the bacterial species.

- Participation of bacterial species (*S.pyogenes*, *Staph.aureuse* and *S.pneumoniae*) with Human parainfluenza virus to cause coinfection to each other in the respiratory system.

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