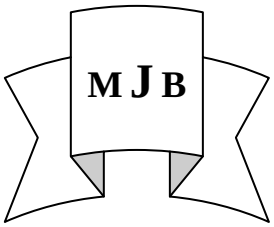
Bacteriological and Immunological Study on Infants Pneumonia at Babylon Governorate

Muhammad Sabri A. Razzaq Rajwa Hassan Al-Rubiee*
Jwan Ahmed Al-Hamawandi
College of Medicine ,University of Babylon, Hilla, Iraq.
*College of Science, University of Al-Mustansiriya, Baghdad, Iraq.



Abstract

Eighty-five blood samples were obtained from pneumonic infants below 6 months age admitted to Child and Maternal Hospital at Hilla City. The results revealed that 46:85[54.1%] gave positive blood culture whereas 39:85 [45.9%] gave no growth. Gram positive bacteria were the predominant types in comparison to gram-negative bacteria. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacteriam* accordingly were the predominant bacterial causative agent isolated during this work, while *Escherichia coli*, *Pseudomonas aeuginosa* were predominant Gram-negative bacterial isolates. E-rosette test showed a mean of subnormal value [13.3%, 20% and 12%] for pneumonic infants infected with Staphylococci, Corynebacteria, and Gram-negative bacteria respectively. IgG levels were higher [518.9 mg/dl] in pneumonic infants when compared to normal value, likewise the means of IgA [101.2 mg/dl] and IgM [99 mg/dl] were within the normal range.

الخلاصة

تم في هذه الدراسة اخذ خمسة وثمانون عينة دم من الرضع المصابين بذات الرئة وكانت اعمارهم اقل من ٦ اشهر من الراقدين في مستشفى بابل التعليمي للنسائية في الحلة وقد بينت النتائج بأن ٤٦:٨٥ [٥٤.١%] اعطت نتائج زرعيه موجبة بينما ٣٩:٨٥ [٤٥.٩%] لم تظهر اي نمو فيها. وكانت البكتريا الموجبة لصبغة الغرام اكثر سيادة بالمقارنة مع البكتريا السالبة لصبغة الغرام. اظهرت هذه الدراسة بأن البكتريا الموجبة الاكثر شيوعا" تمثلت بكل من المكورات العنقودية الذهبية ، والمكورات العنقودية الجلدية، والوتديات على التوالي، بينما اشريشيا القولون والزوائف الزنجارية مثلت النوع السائد من البكتريا السالبة لصبغة الكرام المعزولة. كانت قيم اختبار التشكيل الزهري التائي دون المستوى الطبيعي للأطفال الرضع المصابين بذات الرئة الناتج عن المكورات العنقودية، والوتديات، البكتريا السالبة لصبغة الغرام وكما يلي[١٣.٣%، ٢٠%، ١٢%] على التوالي، وكانت مستويات الكلوبيولين المناعية نوع IgG عالية [٥١٨.٩ ملغم/ ديسيليتر] و IgM [٩٩ ملغم/ ديسيليتر] ضمن المستوى الطبيعي.

Introduction

Acute respiratory infections [ARI] are one of the most important causes of morbidity and mortality in children throughout most of the world [1]. Pneumonia refers to inflammation of pulmonary tissue and is associated with consolidation of alveolar spaces [2]. Pneumonia may be caused by a variety of infectious agents (bacterial, viral, protozoal and fungal organisms) and inflammatory processes [systemic lupus erythymatosis [SLE], sarcoidosis, histocytosis] as well as some substance (hydrocarbons, smoke, dust, chemicals and gases) that are inhaled or aspirated [3]. The most common cause of pneumonia in children is viral infection; bacterial infections account for only (10-30%) of all pediatric pneumonia [4]. People with suspected pneumonia should undergo medical evaluation including a through physical exam may not always distinguish pneumonia from acute bronchitis or other respiratory infections depending on the severity of illness, additional studies such as complete blood count, blood cultures and sputum culture may be obtained [5].

Children with dysfunction of immune system, malnutrition, or defects in the normal mechanisms of the lung are very susceptible to pulmonary infection. The causative agents often are unusual, opportunistic organism, including gram-negative bacteria Cytomegalovirus [CMV], Varicella, Pneumocystis carinii or Fungi [6].

Aims of the Study

To determine the bacterial causative agents of pneumonia in infants below 6 months age and measurement of some humoral immune response aspect by assessment serum immunoglobulin.

Patients and Methods

A total of eighty infects patients below six months which have pneumonia , clinically diagnosed by pediatrition in Babylon province at a period from November 2003 to May 2004.

Specimens: the blood samples are divided into three part:

1. Half ml was immediately inoculated into sterilized blood cultures bottles for bacteriological study.
2. Two ml were put in sterilized plane tube and allowed to clot at $37^{O}C$ for [30-45] minutes, then serum was separated by centrifugation for 15 minutes at 3000rpm. Hemolysed sample was avoided.
3. Two ml were put in EDTA anticoagulant tubes for E-rosette technique.

Bacterial diagnosis . It was done according to [7-9].

Singles Radial Immuno Diffusion Test [SRID]: this Kit was used for the quantitative determination of many human serum proteins [such as IgG, IgM, IgA].

Erythrocyte-Rosette formation test [E-rosette test]: It was done according to [10].

Results and Discussion

Blood culture: The samples had positive results in 46:85 [54.1%]. The determination of bacterial causative agents of pneumonia in infants was based on the blood culture techniques, this is due to the fact that sputum cannot be used at this age range [below 6 months] because of difficulties in the utilization sample from infants [11]. Besides, it has been widely appreciated for many years among physicians and microbiologists that blood cultures are among most important laboratory tests performed in the diagnosis of serious infections in infants [12]. Meantime, blood cultures should be performed in patients with suspected bacterial pneumonia and do appear to have low sensitivity but are still worthwhile to identify the causative pathogens and corresponding susceptibilities to antimicrobial agents [13]. On the some manner, blood cultures appear to be the sole specimens used for the diagnosis of bacterial causative agents of infant infections, hence bacterial isolates from blood is diagnostic of pneumonic infection in infants [6].

Gram positive cocci:

The type of gram positive cocci isolated from blood of pneumonic infants expressed in table [1] that *Staphylococcus aureus* was the major Gram positive cocci 13:30 [43.3%], followed by *Staphylococcus epidermidis* 9:30 [30.0%], Macrococcus sp. 4:30 [13:3%], and *Streptococcus pyogenes* 4:30 [13.3%]. These results are agreed with [14] who showed that *Staphylococcus aureus* and *Staphylococcus epidermidis* are the predominated species among the Gram positive bacteria isolated from infants infected with bacterial pneumonia. [15] showed that *Staphylococcus aureus* is main Gram positive bacteria associated with pneumonia in infants. Likewise, *Staphylococcus aureus* are reported to be the most frequent bacterial pathogens in certain third-world communities [1]. *Staphylococcus aureus* is leading cause of pneumonia in infants [16]. Whereas, [17] pointed that *Staphylococcus aureus* is cause of sever community-acquired and nosocomial pneumonia. However, *Staphylococcus epidermidis* strains are considered as pathogen in cases of pneumonia [18]. *Staphylococcus epidermidis* is the causative agents isolated

from blood stream of the infants under five months infected by pneumonia, and is the leading causes of infants intensive care units acquired infection [19]. Besides, 4 [13.3%] isolates were diagnosed as *Streptococcus pyogenes*. This result was not identical to those obtained by [20] who pointed that *Streptococcus* species is one of the commonest Gram positive cocci that cause pneumonia in infants, and is responsible for [38%] of bacterial pneumonia in sputum obtained from adult.

Table 1 Gram positive cocci bacterial isolated from pneumonic infants

Species of bacteria	Pneumonic patients	
	Total No.	%
Staphylococcus aureus	13	43.3
Staphylococcus epidermidis	9	30.0
Macrococcus sp.	4	13.3
Streptococcus pyogenes	4	13.3
Total	30	100%

Gram positive rods: results on Table [2] revealed the frequency of Gram-positive rods as causative agents of pneumonia in infants, *Corynebacteriam* spp. Have been isolated from 5:46 [5.7%] cases. *Corynebacteriam* causes respiratory tract infections mainly in children [21]. Diphtheroids are normal inhabitants of the birth canal but may cause infected pneumonia [22]. Also, *Arcanobacterium* which was isolated from only one cases 1:46 [2.2%]. These results were show in Table [3-2]. This study confirmed the presence of this bacteria in pneumonic patients, so, it is the first study to isolate this bacteria from pneumonic cases. *Arcanobacterium* spp. Was recovered from only 0.5% of patients with respiratory tract infection in adults but this highly rate increased in younger children and may reach 10% [23].

Table 2 Distribution of Gram-positive rods of bacterial isolated from pneumonic infants

Species of bacteria	Pneumonic patients	
	Total No.	%
Corynebacteriam spp.	5:46	8.7
Arcanobacterium spp.	1:46	2.2

Gram-negative rods: the results in Table [3] showed the rate of infection with Gram negative rods, *Escherichia coli* was the predominant type among other Gram negative bacteria 4:46 [8.7%], while other isolate were distributed as *Pseudomonas aeuginosa* 3:46 [6.5%]. *Acinetobacter* 2:46 [4.3%], *Klebsiella pneumoniae* 1:10 [2.2%]. *Escherichia coli* is one of the most common pathogens isolated from culture of pneumonic patients. These finding agree with the results of [24]. *Pseudomonas aeuginosa* is one of the top ten cause of bacteremias in 2002 [25]. Moreover, it was represents one of the major causes of nosocomial pneumonia in newborns [26].

Table 3 The Gram-negative rods of bacteria isolated from pneumonic patients

Species of bacteria	Pneumonic patients	
	Total No.	%
Escherichia coli	4:46*	8.7
Pseudomonas aeuginosa	3:46	6.5
Acinetobacter spp.	2:46	4.3
Klebsiella pneumoniae	1:46	2.2

* Total positive culture of Gram positive and Gram negative

E-rosette test: The results of E-rosette test were showed in Table [4], it that the mean value of E^{+}-rosette-T-lymphocytes in infants infected with G^{+ve} streptococci was 13.28%, which less than the normal value which ranging from [21-33%] [10], or [25%] [27]. The E-rosette value of patient infected with *Corynebacteriam* was 20%, which tended to be within the minimal normal value, whereas, patients infected with G^{-ve} rods, showed results less than normal value [12%]. E-rosette technique is widely used as a tool for T-cell count and to differentiate between T and B cells, the majority of rosette forming cells vary during the immune response and there is a linear relationship between the number of rosette-forming cells and the total number of lymphocyte [28].

Table 4 E-rosette tests of patients infected with bacterial isolated

Total No.	Bacterial groups	E-rosette %
7	G^{+ve} cocci [Staphylococci]	13.3%
1	G^{+ve} rods [Corynebacteria]	20.0%
2	G^{-ve} rods [*E. coli* and *Acinetobacter*]	12.0%

Normal value is [21-33%, 25%]

Humeral immunological assays: the mean concentration of immunoglobulins [IgA, IgG and IgM] was demonstrated in Table [5], the mean concentration of IgM was 99.0 which was slightly above the limits of normal value [up to 92 mg/dl]. Only one case was with 250.7 mg/dl which indicate that patient was still in the early stages of immune responses [acute state]. Mean of IgG concentration was within the normal value as [190-1400] mg/dl and, whereas IGA was slightly upper the normal value [5-88] mg/dl.

Table 5 Concentration of IgG, IgA and IgM immunoglobulins [mg/dl] in pneumonic infants

Immunoglobulins type			
	IgA	**IgG**	**IgM**
Concentration mg/dl	0.02	466.8	22.8
	139.8	296.8	63.9
	357.7	768	68.6
	104.8	619.9	88.0
	50.4	513.2	78.1
	43.3	610.4	193.9
	72.6	203.2	88.0
	23.2	489.6	98.3
	80.4	610.4	250.7
Mean	101.2	518.9	99.0
S.D*	32.0	52.7	22.2

*Standard deviation

References

1. Teixera, L.M. [2004]. Characteristics of bacteria as etiological agents of acute respiratory infections in children: consideration for diagnosis. PP: 105-115.
2. Krecsmar, C.M. [2002]. The respiratory system. In Behrman, R.E. Nelson Essentials of pediatrics, 3rd ed. W.B. Saunders Company, London. Pp.459-497.
3. Chapel, H., Hacney, M. and Misbah, S. [1999]. Essential of clinical immunology. 4th ed. Black well Science.
4. Franklin, M. [2004]. Pneumonia. N. Engle. J. Med. 350:1904-1930.
5. Ben-Ami, R., Navon, V and Carmeli, Y. [2003]. Infection of ventriculoatial shunt with phenotypically variable *Staphylococcus epidermidis* Masquerading as polymicrobial bacteria due to various coagulase-negative Streptococci and Kocuria varians. J. C. Microbiology. 41[6]:2444-2447.
6. Prober, C.G. [2002]. Pneumonic in Behrman, R. E. Textbook of Pediatric, 18th ed. W.B. Saunders Company, London.
7. Macfaddin, J.K. [2000]. Biochemical tests for identification of medical bacteria. Lippincott. Williams and Wilkins. New york. Pp: 214-289.
8. Baron, C., Howard, M. and Turner, L. [1994]. Medical Microbiology, 2nd ed. Wiley liss, New-York.
9. Holt, J., Krieg, Sneath, P. and Wiliams, S. [1994]. Berges's manual of determinative bacteriology, 9th ed. Williams and Wilkins, Baltimore, USA.
10. Burrell, R. [1979]. Experimental immunology, 5th ed. Burges publishing company, Minnesota. pp:23-24.
11. Gotoff, S.P. [2000]. Infections of the neonatal infant. In Behrman, R. E.

Kliegman, R. M, and Jenson, H. Nelson textbook of pediaties. 16th ed. W.B Sanders. London.pp:

12. Washington, J. A. [1975]. Blood culture: principle and techniques Mayo. Clin. Proc.50:91-97.

13. Alberta Medical Association [2002]. Guideline for the diagnosis and management of community acquired pneumonia: Pediatric Avenue N.W.

14. Luna, G.M. Gherardi, C. and Vay, C. [2001]. Bacterial resistance and antimicrobial therapy in respiratory medicine and intensive care. Medicine [B-Aires]. 6[15]:603-613.

15. Rozaidi, S.W., Sukro, J. and Dan, A. [2001]. The incidence of nosocomial infection in the intensive care unit, Hospital University Kebangsaan Malaysia: ICU-acquired nosocomial infection. Med. J. Malysia. 56[2]: 207-222.

16. Cordero, L., Sananes, M. and Ayers, L. [2001]. A comparison of two airway suctioning frequencies in mechanically ventilated, very-low birth weight infants. Respire. Care. 46[8]: 783-788.

17. Fakih, G. and Saravolatz, L. [2003]. Treatment of *Staphylococcus aureus* pneumonia. Current treatment options in infectious diseases. 5:136-142.

18. Martin, M., Pfaller, M. and Wenzal, R. [1989]. Coagulase negative staphylococcal bacteremia: Mortality and Hospital stay. Ann. Intern. Med. 110:9-16.

19. Villari, P. Sarnatoro, C. and Lacuzio, L. [2000]. Molecular epidemiology of *Staphylococcus epidermidis* in neonatal invasive care unit over a 3-year period. J. of clin. Microbiol. 38[5]: 1095-1137.

20. Luo, B, Huang, Y and Wa-Es. [1998]. A prospective study [5]: 453-457.

21. Brooks, G., Butel, J. and Morse S. [2004]. Medical Microbiology, 21th ed. A lange Medical Book, Middle East edition. pp:279-289.

22. Alessandro, M. D. [2004]. Pneumonia neonatal [group-B *Streptococcus*]. Paedia paedia: Neonatal chest diseases.

23. Funke, G. and Bernard, K. [2003]. Coryneform Gram positive rods. In Murray, P, R, Baron, E. Manual of Clinical Microbiology, 8th ed. pp: 472-501.

24. Furner, D. and Dagon, R. [2001]. The sensitivity of common bacteria to antibiotics in children in southern Israel. Hare fuah. 140[10]:923-929.

25. CDR Weekly. [2003]. Bacteremia: *Pseudomonas* spp. And stenotro phomonas maltophilia bacteremia: England, Wales and Northern Ireland: 2002. CDR weekly. 13[34].

26. Fadeeva, G., Sterkhova, G. and Volodin, N. [2001]. Etiology and microbiological diagnosis of nosocomial pneumonia in newborns. Antibiot. Khimioter. 46[5]:16-23.

27. Al-Sa'adi, M.A.K. [2004]. An evaluation study of cellular immunological functions in anergic tuberculous patients. Ph.D. Thesis. College of Science. Babylon University.

28. Kotton. C. [2004]. Encylopedia: T [thymus derived] lymphocyte count. University of Pennsylvania Health system.