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Studying the effect of bacterial infections on immune state of pneumonia patients at Babylon province

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Abstract: Pneumonia is a common disease that responsible for the mortality and morbidity rate among the world. Immunological parameters potently stimulates both innate and adaptive immune system. In Babylon province there is a little study dealing with the association between bacterial pneumonia and immune state at humoral and cellular levels. The present study aims to determine the bacterial causative agents of pneumonia in patients with measurement of some systemic humoral and cellular immune response aspect.

Methods : This study involved 118 patients suffering from pneumonia. Sputum specimens were collected aseptically, then stained with acid fast stain and gram stain to detect the bacterial isolates. The specimens were cultured on specific culture media. Antibiotic susceptibility test was done for bacterial isolates according to CLSI and we measured the growth inhibition zones in millimeters.

The patients were classified into nine groups according to the types of bacterial isolates, then blood samples were collected from them to measure the concentrations of IgG, IgA, IgM, C3 and C4 using single radial immunodiffusion assay with performed the Capillary Migration Inhibition test. In addition to assay the concentrations of IL-6 using ELISA kit.

Results : The results showed the sputum gram stain revealed the presence of neutrophils in 63 specimens from a total of 118 specimens (53.4%) while Ziehl-neelsen stain revealed *Mycobacterium tuberculosis* in 4 specimens with (3.4%). The common bacterial isolate were *Streptococcus pneumoniae* with rate (33.30 %), followed by *Staphylococcus aureus* with rate (14.30%), 8 cases (12.70%) for each one of *Klebsiella pneumonia* and *Burkholderi acepacia*, and 5 cases (7.90%) for each one of *Escherichia coli* and *Streptococcus pyogenes*, 3 cases (4.80%) for *Acinetobacter spp*. and 2 cases (3.20%) for each one of *Pseudomonas aeruginosa* and *Haemophillus influenzae*. The occurrence of pneumonia increased in patients aged between 47-62 years about 27 cases (42.9%), also it was increased in male rather than female 36 cases (57%). G^+ and G^- organisms showed susceptibility and resistant to different types of antibiotic that used in this study. The immunological parameters showed a significant increased ($p \leq 0.05$) in the concentrations of IgG, IgA, IgM, C3 and C4 with LIF and IL-6 at systemic humoral and cellular levels respectively compared with healthy control groups.

Conclusions: We can conclude that *Streptococcus pneumoniae* was the most dominant bacterial isolates in causing pneumonia and the best antibiotic that used for treating the infections were Imipenem and Trimethoprim-Sulfamethoxazole with significant increased ($p \leq 0.05$) in all immunological parameters that used in this study.

Key words : Pneumonia; Streptococcus pneumoniae; Antibiotic susceptibility; Immune state.

1. Introduction

Pneumonia is an infection of the lung occur through the invasive the pulmonary tissues by pathogens, it may be caused by a different types of microorganisms like: bacteria, virus, fungi, etc... .There are many conditions and risk factors that predispose to pneumonia include: smoking, alcoholism, dust, chemicals and gases, Immunodeficiency, pulmonary disease. Also, The use some drugs such as proton-pump inhibitors or H2 blockers[1;21&41].

The most common cause of pneumonia is bacterial infection especially (*S. pneumoniae, S. pyogenes, S. aureus, S. epidermidis*) as gram positive bacteria and (*H. influenzae, K. pneumoniae, P. aeruginosa, M. catarrhalis, N. meningitides, E. coli and Proteus spp.*) as gram negative bacteria. Respiratory tract is the port of entry of these bacteria followed by adherence to the epithelial cells then colonized and multiplicated in order to initiate the inflammation of lung tissues [2;3&4].

The bacterial pneumonia induced a host immune response at both systemic and mucosal levels. The humoral immune response represented by the production of antibodies especially IgG and the presence of this class indicated that the patient in chronic infections[5&6]. SIgA is the main immunoglobulin at mucosal surfaces and secretions, and it played an important role in protection against infections through interfering with the adherence of bacteria to the epithelial cells of the respiratory tract, and through enhancing surface phagocytosis[7].

The cellular systemic immune responses can be determined by the activation of Th1 and Th2 through the secretions of their mediators. Cytokines and chemokines are important in the development of both the innate and adaptive immune response, several types of them are produced during pneumococcal infection secreted by different types of immune cells when encountered to pathogens like: tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6) and interleukin-10 (IL-10), in addition to stimulate the production of acute-phase proteins and attract phagocytic cells such as Polymorphonuclear leukocytes (PMNLs) and macrophages to the site of infections[8&9]. Opsonization, which is followed by phagocytic uptake and killing of bacteria can be achieved by classical, alternative and pulmonary surfactant lectin pathways. The PMNLs and macrophages, which possess receptors for these complement factors[10]. IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine through its inhibitory effects on TNF-α and IL-1, it is secreted by T cells and macrophages in response to specific microbial infections[11&12].

Aim: The aim of this study is to determine the bacterial causative agents of pneumonia in patients and investigate the immune state of them at systemic humoral and cellular levels.

Material and Methods

A- Bacteriology Study

1- Specimens collection:

Sputum specimens were collected aseptically from 118 patients, with proof pneumonia as clinically diagnosed by physicians, who were admitted to Medical Marjan City and to General Al-Hashemyia Hospital in Babylon province during a period extended from January till December 2015. All specimens divided into two groups male (n=36) and female (n=27) each group subdivided into four groups according to the age [(15-30),(31-46),(47-62),(63 ≤)] male and female in sequence. The following data were recorded for each patient: age, sex, hospitalization. Patients who were treated with antibiotics before/on admission to hospitals were not included in our study. The specimens were collected by standard methods as mentioned in[13].

2- Sputum staining:

Each sputum specimens were stained with acid fast stain to detect *M. tuberculosis*, and with gram stain for investigating the presence of the polymorphonuclear neutrophils (PMNs) and the epithelial cells. Specimens containing less than 10 epithelial cells and more than 20 neutrophils per low-power field were considered appropriate for culturing on laboratory media [14&15].

3- Identification:

The specimens were cultured on specific culture media then incubated aerobically and anaerobically for primary isolation. Bacterial causes were identified by routine diagnostic tests including cultural, morphological and biochemical characteristics[14&15].

4- Antibiotic susceptibility test:

Antibiotic susceptibility patterns of bacterial isolates were determined by Bauer Kirby's disc diffusion method according to CLSI (Clinical and Laboratory Standard Institute)[16]. Mueller-Hinton plates were inoculated with a 0.5 McFarland standard suspension of organisms, and disks were placed, zones of growth inhibition were recorded in millimeters after overnight incubation.

B- Immunological study

This study was down by distributed the patient according to the bacterial isolates that cause pneumonia into 9 groups with one group considered to be control as following:

Group 1(G 1): patient infected with *S. pneumoniae*.
Group 2(G 2): patient infected with *S. aureus*.
Group 3(G 3): patient infected with *B. cepacia*.
Group 4(G 4): patient infected with *K. pneumoniae*.
Group 5(G 5): patient infected with *E. coli*.
Group 6(G 6): patient infected with *S. pyogenes*.
Group 7(G 7): patient infected with *Acinetobacter spp.*
Group 8(G 8): patient infected with *P. aeuginosa.*
Group 9(G 9): patient infected with *H. influenzae*.
Group 10(G 10):Control of healthy person.

Then we measured the mean concentrations of IgG, IgA, IgM, C3 and C4 using single radial immunodiffusion assay with performed the Capillary Migration Inhibition test as in[17&18]. In addition to assay the concentrations of IL-6 using ELISA kits (provided from Ray Bio, USA, Company).

Results and Discussion:

Pneumonia considered to be one of the most important disease that leading to the morbidity and mortality among the worldwide. Sputum gram strain is reliable indicator and useful guide to direct initial antibiotic therapy in community-acquired bacterial pneumonia[19]. The sputum gram stain **(Table 1)** showed the presence of neutrophils in 63 specimens from a total of 118 specimens (53.4%).The presence of a large number of neutrophils and few of squamous epithelial cells indicates the infection in acute stage and the sputum specimen positive for pneumonia infections[14]. Ziehl-neelsen stain revealed the presence of *M. tuberculosis* in 4 specimens with (3.4%).

Table (1) Staining profile for sputum specimens

Staining type		Numbers of patients	%
Direct Gram stain	Neutrophil	63	53.4%
	Other cell	51	43.2%
Ziehl-neelsen stain		4	3.4%
Total		118	100%

The results of cultural, morphological and biochemical characteristics for 63 sputum specimens explained that neutrophil cells domination, revealed that the most frequent bacterial isolates was *S. pneumoniae* (21 cases) with rate (33.30%), followed by *S. aureus* (9 cases) with rate (14.30%), 8 cases (12.70%) for each one of *K. pneumonia* and *B. cepacia*, and 5 cases (7.90%) for each one of *E. coli* and *S. pyogenes*, 3 cases (4.80%) for *Acinetobacter spp*. and 2 cases (3.20%) for each one of *P. aeruginosa* and *H. influenzae* (**Figure 1**).

The domination *of S. pneumoniae* followed by *S. aureus* isolated organism from pneumonic patients are nearly in agreement with the study[20] conducted in Ethiopia on patients with community acquired pneumonia and reported that *S. aureus* constituted (10.5%) of isolated bacterial pathogens, while disagree with the study[22] which found that *S. aureus* and *S. pyogenes* were constituted (2.8%) and (1.4%) respectively.

Among gram negative bacteria isolated in this study we found *K. pneumonia* and *B. cepacia* were predominant types, while other isolates were distributed as *E.* coli, *Acinetobacter spp*., *P. aeruginosa* and *H. influenza*.

B. cepacia and *Acinetobacter spp.* have been emerged as nosocomial pathogen and recently isolated from cases with community acquired pneumonia[23&24]. The current results were compatible with study[25] that isolated *P. aeruginosa, K. pneumoniae* and *E. coli* from lower respiratory tract infections, However in contrast to our results, the same study reported *P. aeruginosa* as predominant gram negative isolate.

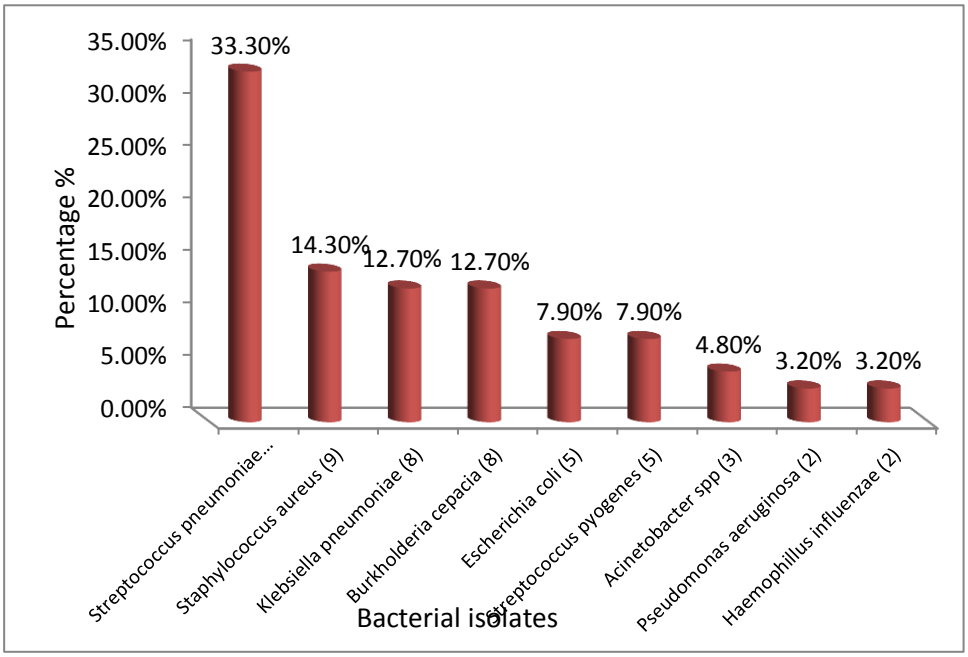

Figure (1) Frequency of bacterial isolates from pneumonic patients

Incidence of mixed bacterial infection in this study was (3.2%) as shown in **(Table 2)**. Most studies have revealed that more than one causative microorganism could be detected in a considerable amount of pneumonic cases. However, the recorded rates for mixed infection differ in the range of 2.5–38% [25&26].

Table (2) Bacterial growth types in primary isolation

Bacterial growth type	Number	%
Single growth	61	96.8%
Mixed growth	2	3.2%
Total	63	100%

According to age, the occurrence of pneumonia increased in patients aged range between 47-62 years. Decreasing pulmonary functions, due to their age related to the physiological and immunological changes and other comorbidities, as aging process advances may explain this fact[26]. In respect to sex, our results **(Table 3)** showed consistency with study[27] that recorded occurrence of pneumonia in the males and females were (60.4%) and (39.6%) respectively, which may probably due to cigarette smoking was more common among male[28].

Table (3) Occurrence of bacterial pathogens in relation to age and sex

Age-group (years)	Male (%)	Female (%)	Total (%)
15-30	8	7	15 (23.8%)
31-46	8	6	14 (22.2%)
47-62	18	9	27 (42.9%)
63 ≤	2	5	7(11.1%)
total	36 (57%)	27 (43%)	63 (100%)

Ten different antibiotics representing different antibacterial families were tested on most common bacterial isolates for studying there antimicrobial sensitivity pattern **(Table 4).** In present study G^+ organisms showed 100% susceptibility to imipenem. Imipenems were broad-spectrum carbapenems antibiotics, Beta-lactam rings of these antibiotics are resistant to hydrolysis by most β-lactamases[16]. *S. pneumoniae*, which was the most common isolate showed 57% sensitivity to oxacaillin, that was comparable with studies conducted in Iran[23&29], where 43-70% of the isolates were resistant. All S. *pneumoniae* isolates from this study were susceptible to trimethoprim‑sulfamethoxazole which is comparable with a study conducted in Ethiopia[20]. In contrast to this, studies conducted in Nigeria[30] explained that all isolates were indicated as resistant to trimethoprim‑sulfamethoxazole. On the other hand, more than 90% of tested *S. pneumoniae* isolates were susceptible to erythromycin, chloramphenicol, tetracycline and ciprofloxacin which is comparable with[31] and a study conducted in Kenya where >97% isolates were resistant to erythromycin and chloramphenicol[32].

In this study, *S. aureus* showed (88.9%) susceptibility to chloramphenicol, (77.8%) for each gentamycin and vancomycin, (66.7%) for each oxacaillin, erythromycin, where's show low sensitivity (44.4%) to tetracycline and trimethoprim‑sulfamethoxazole, (22.2%) to ciprofloxacin. These results were nearly agreeable with results obtained by Al-Hassnawi [33] who clarified that *S. aureus* isolates exhibited high sensitivity (93.2%) to imipenem and chloramphenicol, moderate sensitivity (86.4%) to gentamycin, (75%) to erythromycin and (70.5%) to oxacaillin, however the same study recorded resistance rate (59%) to tetracycline, but in contrast to our study also recorded high sensitivity (86.4%) to trimethoprim‑sulfamethoxazole.

In our study, most G^- bacilli isolates showed high resistance to oxacillin, where's sensitive to imipenem except *E. coli* showed resistance (60%). Among G^- bacilli, *K. pneumonia* and *E. coli* showed (50%), (60%) sensitivity to gentamycin and (62.5%), (60%) sensitivity to chloramphenicol. However, *K. pneumonia* and, *E. coli* showed low sensitivity to tetracycline, ciprofloxacin, trimethoprim‑sulfamethoxazole and ceftriaxone. *B. cepacia* showed 100% resistance to gentamycin. A lack of binding sites on the lipopolysaccharide of *B. cepacia* leads to intrinsic resistance to the cationic antimicrobials, polymyxins and aminoglycosides. Overall resistance to third generation cephalosporins (ceftriaxone) was high on account of the production of extended spectrum β-lactamases (ESBLs) by the most G^- bacilli isolates. The resistance may also be due to the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated[34].

In present study, it has been observed that most G^- bacilli isolates showed low resistance to older drugs like chloramphenicol. It indicates that routine exposure of bacteria only to newly developed antibiotics eliminated resistance against older out of use antibiotics and present bacterial strains have grown sensitive to these outdated agents[16].

Table (4) Sensitivity patterns of bacterial isolates

Antibiotics	Bacterial spp. (N)					
	S. pneumonia **(21)**	***S. aureus*** **(9)**	***S. pyogenes*** **(5)**	***K. pneumonia*** **(8)**	***B. cepacia*** **(8)**	***E. coli*** **(5)**
Oxacillin	12 (57%)	6 (66.7%)	1 (20%)	2 (25%)	0 (0)	1 (20%)
Imipenem	21 (100%)	9 (100%)	5 (100%)	7 (75%)	7 (87.5%)	2 (40%)
Gentamycin	15 (71.4%)	7 (77.8%)	3 (60%)	4 (50%)	0 (0)	3 (60%)
Erythromycin	19 (90.5%)	6 (66.7%)	5 (100%)	NT	7 (87.5%)	NT
Chloramphenicol	20 (95%)	8 (88.9%)	5 (100%)	5 (62.5%)	7 (87.5%)	3 (60%)
Tetracycline	19 (90.5%)	4 (44.4%)	4 (80%)	2 (25%)	5 (62.5%)	3 (60%)
Ciprofloxacin	19 (90.5%)	2 (22.2%)	4 (80%)	3 (37.5%)	5 (62.5%)	1 (20%)
Trimethoprim – Sulfamethoxazole	21 (100%)	4 (44.4%)	NT	3 (37.5%)	7 (87.5%)	1 (20%)
Ceftriaxone	NT	NT	NT	2 (25%)	5 (62.5%)	1 (20%)
Vancomycin	18 (85.7%)	7 (77.8%)	5 (100%)	NT	NT	NT

NT: not tested

Immunological study

The host defense mechanisms against pneumonic infection are either non-immunological nor immunological mechanisms, the first one includes normal cough reflex, intact mucosal surface and clearance mechanisms of the upper respiratory tract. In this study we saw an increased in the concentrations of IgA antibody as in **(Table 5)** in the groups ranges from 1 to 6 compared with control group this may be according to the variable systemic immune responses in each analyzed patients or to the little numbers of samples of the rest groups. Also, the pneumonic immune system functions dependently not only from systemic apparatus, but also from lower intermucosal immune system[35&40]. In addition to higher increased in the concentrations of IgG antibody in the same groups with decreased in the concentrations of IgM antibody except in first group which indicated that patients were in the late stages of immune responses [chronic state]. We can conclude that these infections may be correlated with the increased in the number of stimulating B cells responsible for antibodies production in addition to their correlation with the induction of cytokines from stimulating T cells[36].

Table (5) Concentrations of immunoglobulins types in pneumonic patients

Patients group	Mean Concentrations (mg/dl)		
	IgA	**IgG**	**IgM**
(G 1)	80.2	665.8	93.8
(G 2)	79.5	623.5	91.5
(G 3)	67.2	593.3	90.6
(G 4)	64.8	518.9	90.3
(G 5)	50.4	512.2	90.1
(G 6)	49.0	500.4	90.9
(G 7)	42.6	423.2	89.0
(G 8)	23.2	389.6	78.3
(G9)	20.4	310.4	79.7
(G 10)	48	493	93

Additionally we found a significant increased ($p \leq 0.05$) in C3 & C4 in the complement compartments in comparison with control group as in **(Table 6)**. These results were usual because antigen represented by different bacterial groups or antigen antibody complexes stimulated the complement pathway to avoid infections[37].

Table (6) C_3 and C_4 concentrations in pneumonic patients

Patients group	Complement compartments Concentrations (mg\dI)			
	C_3 concentrations		C_4 concentrations	
	M±S.D.	P- value	M±S.D.	P- value
(G 1)	243.200±3.121	0.041^a	63.420±2.440	0.101^a
(G 2)	198.400±2.576	0.020^b	52.830±1.310	0.100^b
(G 3)	126.340±2.543	0.020^c	49.150±1.205	0.020^c
(G 4)	124.561±2.432	0.010^d	47.500±1.904	0.001^d
(G 5)	116.341±2.101	0.002^e	44.860±1.065	0.000^e
(G 6)	114.168±2.001	0.001^f	41.364±1.098	0.000^f
(G 7)	110.004±1.800	0.000^g	36.500±0.089	0.000^g
(G 8)	108.034±1.800	0.000^g	35.570±0.683	0.000^g
(G9)	107.314±0.461	0.000^g	34.200±0.310	0.000^g
(G 10)	108.500±1.203	0.000^g	35.100±0.000	0.000^g

The cellular immune response represented by significant increased ($p \leq 0.05$) in leukocytes migration inhibition factor and IL-6 in comparison with control group as in **(Table 7 & 8).** LIF played an important role in specific and non specific immune response. It was produced from different types of immune cells after exposure to different bacterial antigens and toxins. The presence of different bacterial antigens as cell sensitizers inhibit the migration of leucocytes among patients and this may be lead to suggest that an epitope activating Th1 which in turn initiate Tdh subsets of T cells that involved of hypersensitivity reactions[38].

Table (7) LIF in pneumonic patients

Patients group	Percentage of LIF(%)	
	M±S.D.	P- value
(G 1)	124.485±0.195	0.101^a
(G 2)	114.283±0.320	0.002^b
(G 3)	107.843±0.261	0.012^c
(G 4)	101.432±0.034	0.010^c
(G 5)	99.702±0.061	0.001^d
(G 6)	97.970±0.073	0.001^d
(G 7)	86.21±0.014	0.000^e
(G 8)	86.53±0.176	0.000^e
(G9)	76.05±0.085	0.000^f
(G 10)	96.30±0.037	0.000^d

The innate immune response to severe bacterial infections was orchestrated by the proinflammatory cytokines TNF, IL-1, IL-6, and IL-8. IL- 6 was a cytokine of low molecular weight regulatory proteins secreted by an array of cells such as lymphoctes, monocytes, macrophages, fibroblasts ,neutrophil, endothelial cells and mast cells. It was derived from activated T lymphocytes that had many functions including induction of B-cell growth factors, differentiation and antibody productions[39].

Our results indicating a significant ($p \leq 0.05$) high concentrations of IL- 6 in serum of patients in all tested groups except in last group compared with control group, this suggest that during clinical pneumonia cytokines were produced at the site of the infection .

Table (8) Concentrations of IL-6 in pneumonic patients

Patients group	IL-6 concentration (pg\ml)	
	M±S.D.	P- value
(G 1)	260.59±4.951	0.031[a]
(G 2)	192.50 ±3.169	0.012[b]
(G 3)	159.51 ±2.647	0.012[c]
(G 4)	157.80 ±2.962	0.010[c]
(G 5)	123.25±2.432	0.001[d]
(G 6)	110.59±1.432	0.001[d]
(G 7)	99.53±1. 425	0.000[e]
(G 8)	93.51 ± 1.464	0.000[e]
(G9)	92.58±1.263	0.000[e]
(G 10)	92.62±0.971	0.000[e]

Conclusions

We can conclude that *S. pneumoniae* was the most dominant bacterial isolates in causing pneumonia and the best antibiotic that used for treating the infections were Imipenem with Trimethoprim-Sulfamethoxazole and we found a significant increased ($p \leq 0.05$) in all immunological parameters that used in this study.

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