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Chlamydia pneumoniae seropositivity among public health and health informatics students in Ha'il University, Saudi Arabia

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ABSTRACT

Chlamydia pneumoniae is a common respiratory tract infectious bacterium and associated with chronic diseases such as cardiovascular diseases, atherosclerosis and Alzheimer's. The prevalence of *C. pneumoniae* infections in general communities and specific populations are mostly conducted in western countries. This work aims to investigate the seroprevalence of the immunoglobulin G (IgG) against *C. pneumoniae* among public health and health informatics students in Ha'il University, Saudi Arabia. Serum samples of sixty-six students were subjected for the microimmunofluorescence (MIF) test. Current/acute infections were estimated at IgG titres of 16-256, while past/chronic infections at a titre of 512. The seropositivity results were combined with the demographical, medical history and habits as evidenced by the surveyed students. The overall seroprevalence of IgG antibodies against *C. pneumoniae* was 48.48%, with a higher IgG detection rate in males (55%) than females (48.7%). The seropositivity in students had symptoms was 30.4% (symptomatic), while 58.1% of the students had no symptoms related to *C. pneumoniae* infection. The serological evidence of *C. pneumoniae* infection was found in association with asthma (3.03%), sore throat (1.51%), Cough (6.06%) and in smokers (4.55%). In conclusion, although our study relatively shows a moderate prevalence of *C. pneumoniae* IgG seropositivity, which is likely a leading cause of common respiratory tract infections among students, the results should be used for proper distribution of the internship students in the hospital departments to minimize the contact of *C. pneumoniae* infected students and subsequently reduces the transmission of *C. pneumoniae* to the high-risk groups patients.

Keywords: *Chlamydia pneumoniae*, Microimmunofluorescence, respiratory tract infections, asthma, epidemiology, Saudi Arabia.

1. INTRODUCTION

C. pneumoniae is a gram-negative obligate intracellular bacterium which is one of the leading causes of 6-20% of community-acquired pneumonia cases (Grayston et al., 1990; Kuo et al., 1995). Seroepidemiological studies estimated that 50-70% of adults have antibody to *C. pneumoniae* (Block et al., 1997; Emre et al., 1994; Gaydos et al., 1994; Gnarpe et al., 1991; Hyman et al., 1995). These studies have indicated that asymptomatic infections may be common. It is associated with several acute diseases of the respiratory system; it infects and damages the lining of the respiratory tract. Studies estimated that *C. Pneumoniae* infection is implicated in 10% of community-acquired pneumonia and 5% of pharyngitis, bronchitis, asthma and sinusitis (Kuo et al., 1995). The infection is spread among people via air droplets and direct contact (Cunha, 2006). Increased risk groups include crowded settings inhabitants e.g. schools and colleges, military barracks, nursing homes, hospitals and prisons (Fajardo et al., 2015; Schmidt et al., 2002). The incubation periods are relatively long and symptoms normally appear 3-4 weeks following exposure (Kuo et al., 1995). Remarkably, *C. pneumoniae* tends to persist in the body after acute infection and is likely involved in the development of chronic diseases like chronic obstructive pulmonary disease (COPD, the leading cause of morbidity and mortality worldwide), chronic asthma, coronary heart disease (CHD), atherosclerosis, Alzheimer's disease, strokes and transient cerebral ischemia (Balin et al., 1998; Cook et al., 1998a; Cook et al., 1998b; Hahn et al., 1991; Sriram et al., 2005; Von Hertzen et al., 1997). The mortality rate due to a *C. pneumoniae* infection is approximately 9%. However, co-infection with other bacteria or the presence of other severe diseases increases the mortality risk (Oba, 2007).

In the Kingdom of Saudi Arabia (KSA), as in most other developing countries in this region, there have been no or limited data on the incidence of *C. pneumoniae* among the populations and on the association of this pathogen with acute infections and several serious diseases, thought to be chronic like persistent cough and coronary heart diseases. One of the increased risk groups is the college students, in particular internship. These students attend training at different department in hospital and might be at high risk for infection. Vice versa, students also may infect other patient's in particular elderly people and chronic diseases patients and subsequently increases the risk of developing new critical diseases among these patients. Using the species-specific microimmunofluorescence (MIF) test, this study aims to investigate the seroprevalence of *C. pneumoniae* in internship students of the college of public health and health informatics, Ha'il University, KSA. Accordingly, the incidence of acute and chronic chlamydial infections in the subjects will be estimated. This study could contribute to the general understanding of asymptomatic respiratory infection with *C. pneumoniae* in young adults and importantly, it should serve as a basis for a proper distribution of students among departments to avoid direct contact with patients at risk factor. The study will also determine the distribution of asymptomatic infections according to sex and the association of the seroprevalence of *C. pneumonia* with other chronic diseases among internship students.

2. MATERIAL AND METHODS

Informed consent

Before being included in the study, participants had to give their consent to participate to the study. Written informed signed consent was obtained from participants. An information sheet was shared with the participants that obviously indicate the risks and the benefits associated with the participation to this study. A survey including demographic and medical history information of the students was also filled up by the participants. According to the local ethics committee of the University of Ha'il, all volunteers gave written consent for obtaining blood samples to be used for serological testing for the presence of *C. pneumoniae* and to fill in the survey. The informed consent, information sheet and the survey were previously approved by the Ethics Committee at the University of Ha'il (Nr. 40006).

Study population and samples

103 students from the college of public health and health informatics (University of Ha'il, KSA) routinely undergo for hepatitis C and B test before attending the internship program at the local hospitals. These students were asked to volunteer to this study. Samples were collected (May. 17th-25th 2020) from the student 2 weeks before starting their internship program. A blood sample of 5 ml was obtained from each student by a certified laboratory technician at the Ha'il university clinics. Sera samples for the routinely hepatitis test were prepared by allowing the blood to stand for 20 to 30 min at room temperature and subsequently were aspirated. A total of 100 µl of each serum was then transferred to sterile Eppindorf tubes and frozen at −20 °C until used for *C. pneumoniae* test. All samples were sequentially numbered.

Serology

Sera collected from students were tested for the presence of specific IgG antibodies against *C. pneumoniae* purified elementary bodies (EBs) using the MIF test, an established serological standard for determining *C. pneumoniae* infection (Dowell et al., 2001). As shown in Figure 1, sera were serially diluted with phosphate buffered saline solution (PBS) pH 7.4 to 1:16-1:512. Ten microliters of serum dilutions as well as a drop of positive and negative control sera were pipetted to MIF slide wells dotted with inactivated *C. pneumoniae* elementary bodies. Slides were then incubated for 30 min in a moist chamber. After being washed with PBS (pH 7.2) for 10 min, slides were then rinsed with distilled water and dried. Next, 10 µl of commercially available FITC-labeled anti-human IgG was added to each well, and the slides were again incubated in a moist chamber for 30 min. Finally, specimens were washed, dried, mounted with a mounting medium, and then covered by proper cover slips. The slides were examined under fluorescence microscope. Specimens displaying a bright uniform fluorescence associated with bacterial particles were considered positive. A titer of <1:16 will be considered negative, whereas a titer of ≥1:16 are positive. A titer of ≥1:512 were considered acute (recent or current) infection, as recommended by others (Al-Younes, 2014; Lin et al., 2004; Miyashita, 2006).

3. RESULTS

Characteristics of the study population

The prevalence of *C. pneumoniae* antibodies among public health and health informatics students preparing to attend the internship program at the local hospital was evaluated. A total of 66 students (20 males and 46 females) out of 102 (40 male, 62 female) responded to answering the questionnaire and volunteer with their sera to be tested for *C. pneumoniae* infection (response rate 64.7%: female 69.7% and male 30.3%). The range of the student age was 22-26 years (mean: 22.1, SD ± 0.8). Three students are married (4.6 %). We also analyzed the demographic, characteristics and typical risk factors of the study population. Among the volunteered students, 5 students (7.6%, 4 female and 1 male) reported that they suffer from chronic respiratory diseases. This includes, asthma (2 female and 1 male), sinusitis (1 female) and one non declared case by a female student. Only one male student reported to suffer from acute infection (sore throat) during the last 3 weeks. None of the students had a history with bronchitis. Five students (7.6%, 4 female and 1 male) declared they had antibiotic treatments in the last 3 weeks. All students confirmed that they had a successful treatment of respiratory diseases after using antibiotics. Fifteen volunteer students (11 female and 4 male) reported that they had cough at least 1-2 times a year, while one female student had regular cough along the year. Hypertension was only declared by one female student. Most of the study population were nonsmokers as only 4 (6.1%) had the smoking habit. Among the students, only one female student declared that she had diabetes mellitus type II. None of the volunteered students reported to have diagnosed cardiovascular diseases, atherosclerosis, cerebral infections or other chronic diseases.

Serological status

Using sera collected from the volunteered students, the presence of IgG antibodies against *C. pneumoniae* was assessed using the MIF test (Figure 1). The status of the students from both genders to *C. pneumoniae* infection is shown in Figure 2. Overall, 32 out of 66 (48.48 %) students' sera showed seropositivity against *C. pneumoniae* antigens. The results revealed that male students had higher prevalence (11/20, 55%) of the IgG antibodies against *C. pneumoniae* than female students (21/46, 45.7%), however, this variation was statistically not significant. Subsequently, the reactivity of sera samples were tested at several titers ranging from 16 to 512. Sera samples showed reactivity with *C. pneumoniae* antigens only at titers of 16-256 was considered as an evidence of past/chronic infection, while samples reacted against *C. pneumoniae* antigens at titer of 512 (in addition to positive reactivity at titer of 16-256) was considered as an evidence of current/acute infection. Our analysis showed that 40.9% (27/66) sera samples had serological evidence of past/chronic infection. On the other side, only 7.58% (5/66) of the sera samples showed an evidence of current/acute infection. Remarkably, past/chronic was higher in male than in female students (male: 10/20 (50%) and female: 17/46 (40%). In contrast, current/acute infection was likely higher in female than in male students (4/46 (8.7%) female/1/20 male (5%). Nevertheless, the variations between both past/chronic or current/acute infection and gender were statistically not significant.

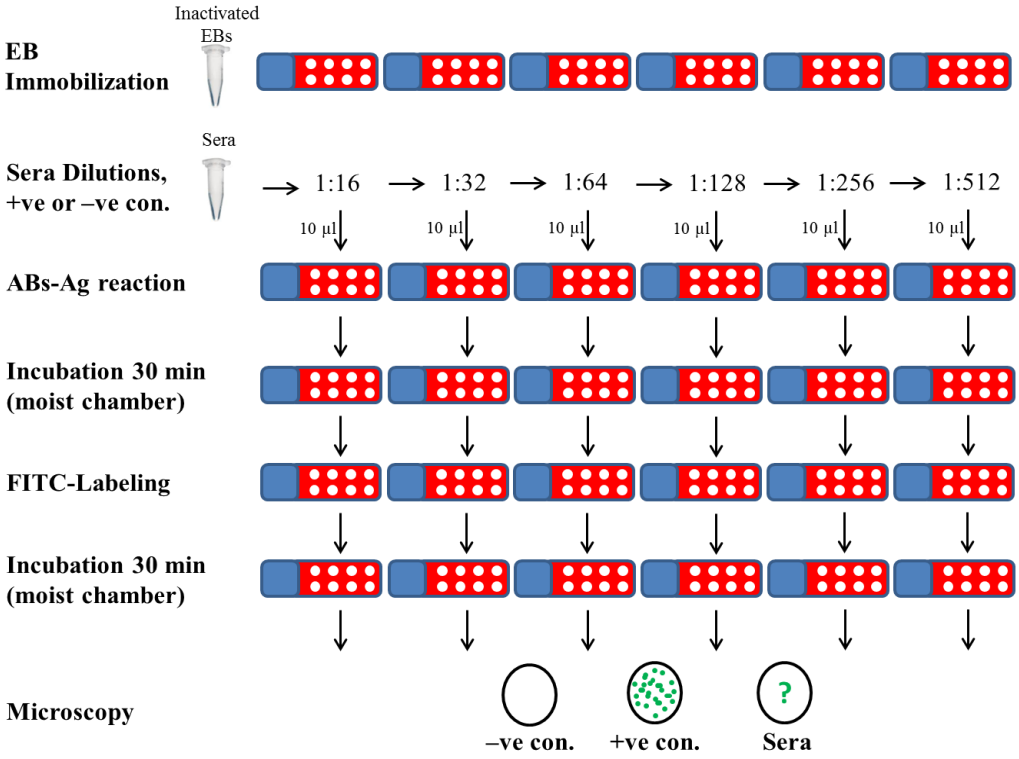
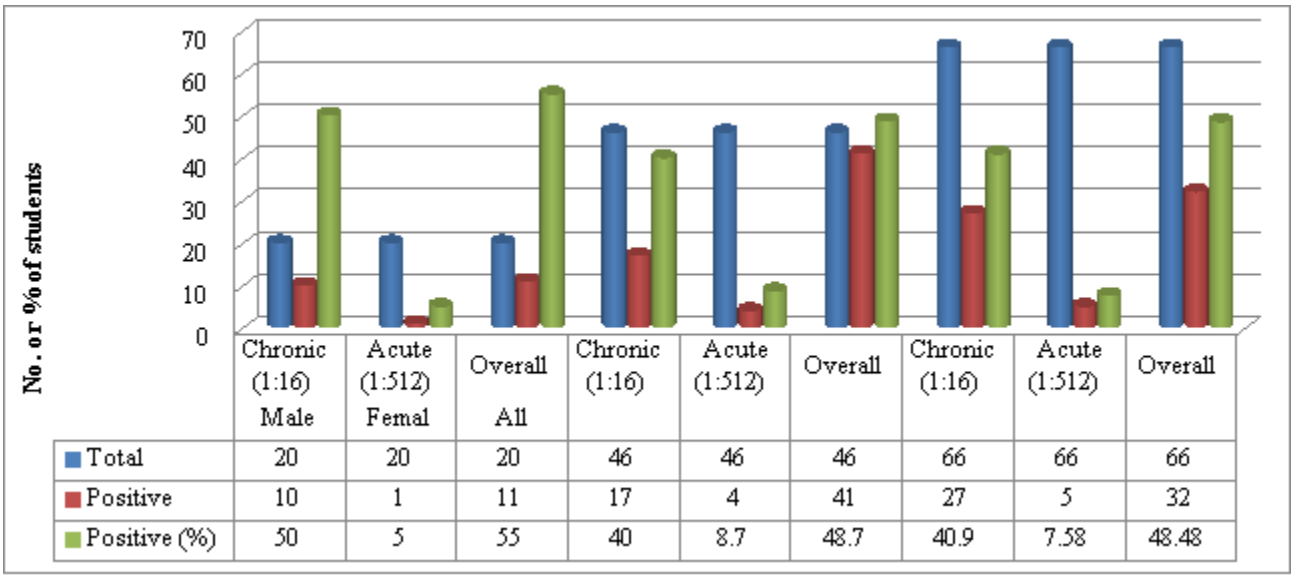


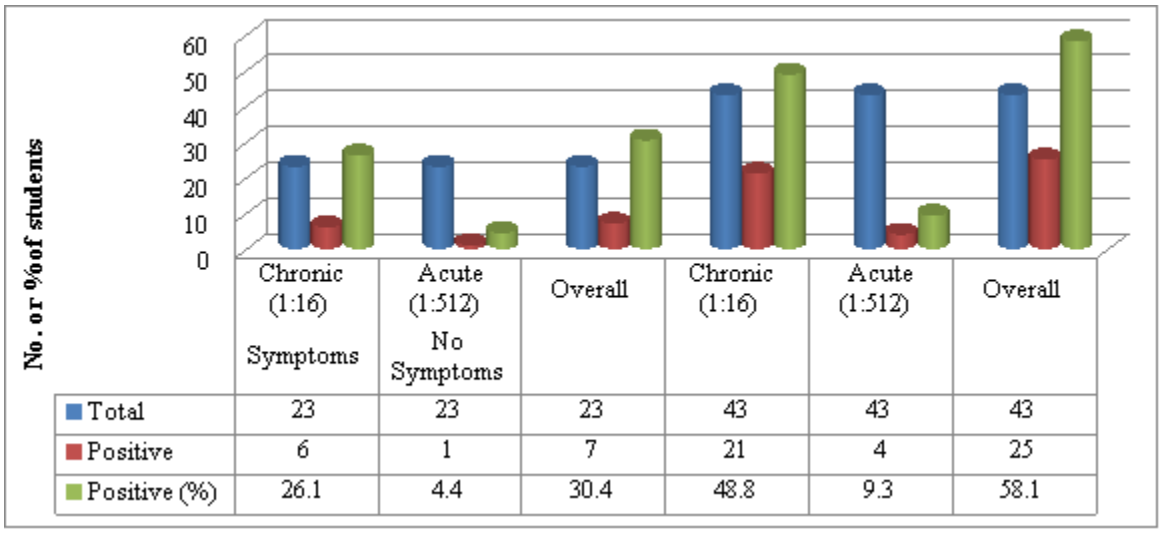
Figure 1 Schematic presentation of the MIF method.
Abs: Antibodies, Ag: Antigens, –ve con: Negative control, +ve con: Positive control.

We also strive to assess the association of the seropositivity expressed either as past or current infection and the appearance of symptoms documented by the student survey. As shown in Figure 3, 23 students reported to have different symptoms that might be correlated to *C. pneumoniae* infection, while 43 were not. Among the 23 students showed symptoms, 6 (26.1%) and 1 (4.4%) students had seroevidence for past/chronic and current/acute infections, respectively. On the other hand, 21 (48.8%) of the students who had no symptoms related to *C. pneumoniae* infections showed seropositivity for past/chronic infections, while 4 (9.3) of them were seropositive at the current/acute titer. Collectively, 30.4% of the seropositive *C. pneumoniae* students had symptoms related to *C. pneumoniae*, while 58.1% of the students who did not show symptoms to *C. pneumoniae* were seropositive to a *C. pneumoniae* infection.



	Chronic (1:16) Male	Acute (1:512) Femal	Overall All	Chronic (1:16)	Acute (1:512)	Overall	Chronic (1:16)	Acute (1:512)	Overall
Total	20	20	20	46	46	46	66	66	66
Positive	10	1	11	17	4	41	27	5	32
Positive (%)	50	5	55	40	8.7	48.7	40.9	7.58	48.48

Figure 2 *C. pneumoniae* seropositivity among students.
Dilution of 1:16 indicates past or chronic infection of *C. pneumoniae*. Subjects were positive at dilution of 1:512 were excluded and considered as current or acute only. Dilution of 1:512 indicates current or acute infection of *C. pneumoniae*.



	Chronic (1:16) Symptoms	Acute (1:512) No Symptoms	Overall	Chronic (1:16)	Acute (1:512)	Overall
Total	23	23	23	43	43	43
Positive	6	1	7	21	4	25
Positive (%)	26.1	4.4	30.4	48.8	9.3	58.1

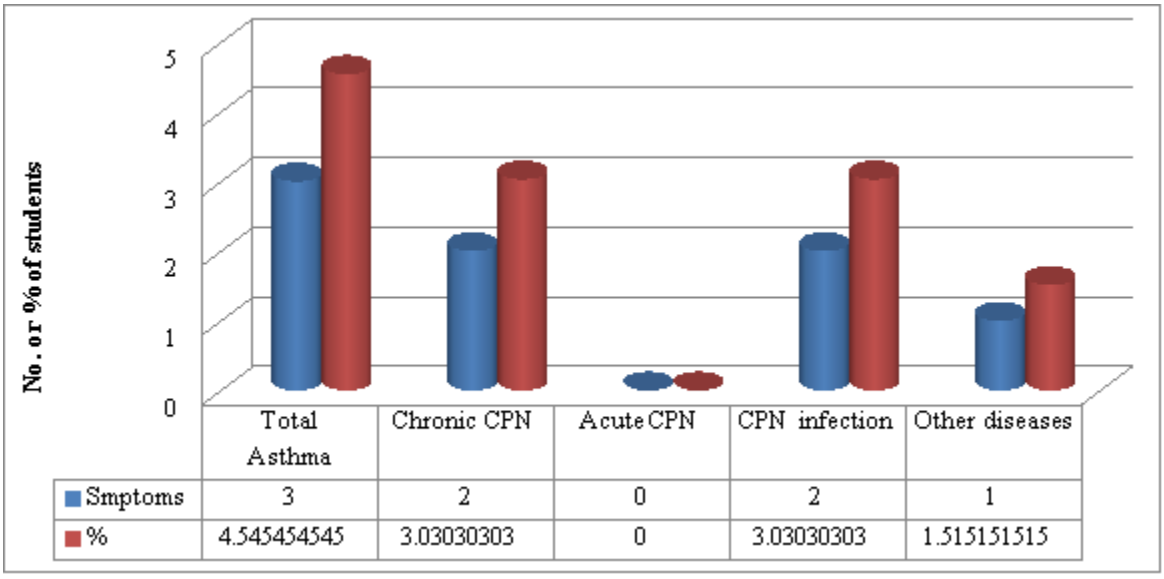
Figure 3 *C. pneumoniae* seropositivity among students associated with symptoms.
Students suffers from symptoms related to either chronic or acute *C. pneumoniae* infection was figured out. The prevalence of *C. pneumoniae* antibodies among students showing symptoms or no symptoms is shown.

Furthermore, we directly analyzed if there is any correlation between the seropositivity of *C. pneumoniae* IgG antibodies and its possible role in the development of association with other symptoms/diseases/habits obtained in the submitted surveys. Table 1 shows the correlation between the seropositivity against *C. pneumoniae* and the history of asthma, sinusitis, sore throat, cough, hypertension, diabetes mellitus type II and smoking habit as obtained by the survey. We observed seropositivity of past/chronic infection in 3.03% of asthma (Figure 4A), 1.51% of sore throat-suffering students (Figure 4B), 6.06% of students suffering cough at least 1-2 times/year (Figure 4C), 1.51% of students suffering cough along the year (Figure 4D) and 4.55% of smoker students (Figure 4E).

Table 1 Association of sero-evidenced of current and past *C. pneumoniae* infection with potential chronic diseases and habits.

		Males (20 students)			Females (46 students)			Both genders (66 students)		
Variable	**Infection**	**OVERALL Total**	**Positive**	**% positive**	**Total**	**Positive**	**% positive**	**Total**	**Positive**	**% positive**
Asthma	Acute	1	0	0	2	0	0	3	0	0
	Chronic		1	5		1	2.17		2	3.03
Sinusitis	Acute	1	0	0	0	0	0	1	0	0
	Chronic		0	0		0	0		0	0
Sore throat	Acute	1	1	5	0	0	0	1	1	1.51
	Chronic		0	0		0	0		0	0
Cough at least 1-2 times/year	Acute	4	0	0	11	0	0	15	0	0
	Chronic		2	10		2	4.35		4	22.72
Cough along the year	Acute	0	0	0	1	0	0	1	0	0
	Chronic		0	0		1	2.17		1	1.51
Hypertension	Acute	0	0	0	1	0	0	1	0	0
	Chronic		0	0		0	0		0	0
Diabetes mellitus type II	Acute	0	0	0	1	0	0	1	0	0
	Chronic		0	0		0	0		0	0
Smoking habit	Acute	4	1	5	0	0	0	4	1	1.51
	Chronic		2	10		0	0		2	3.03

A



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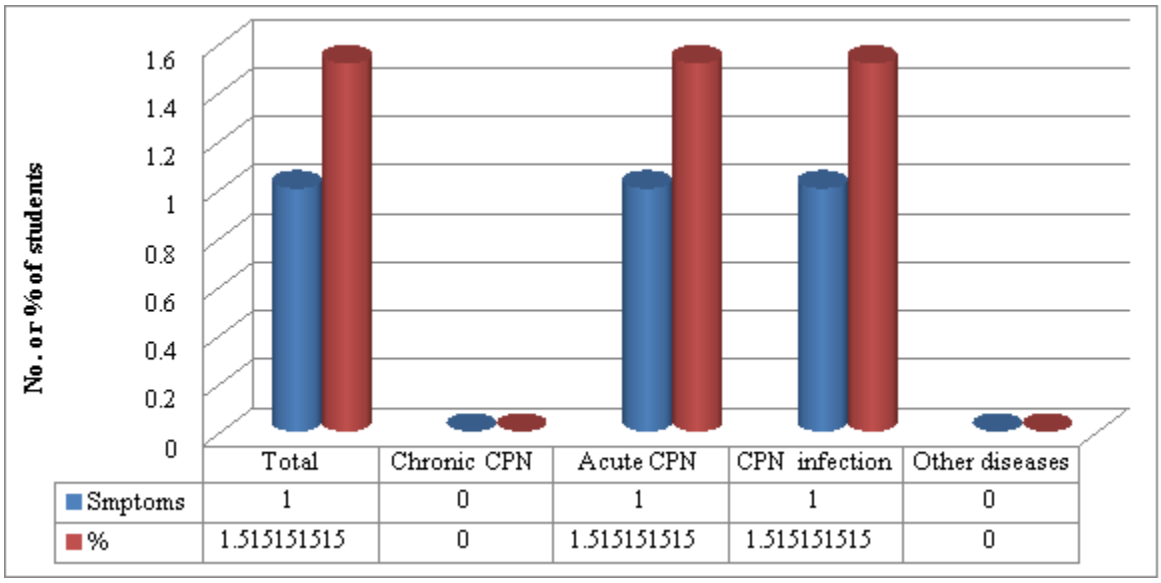
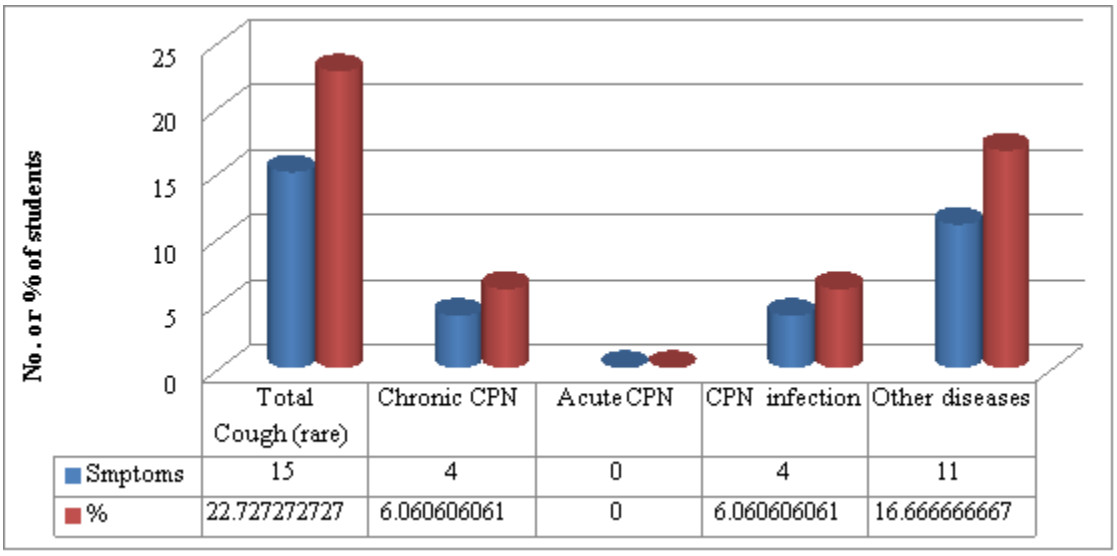


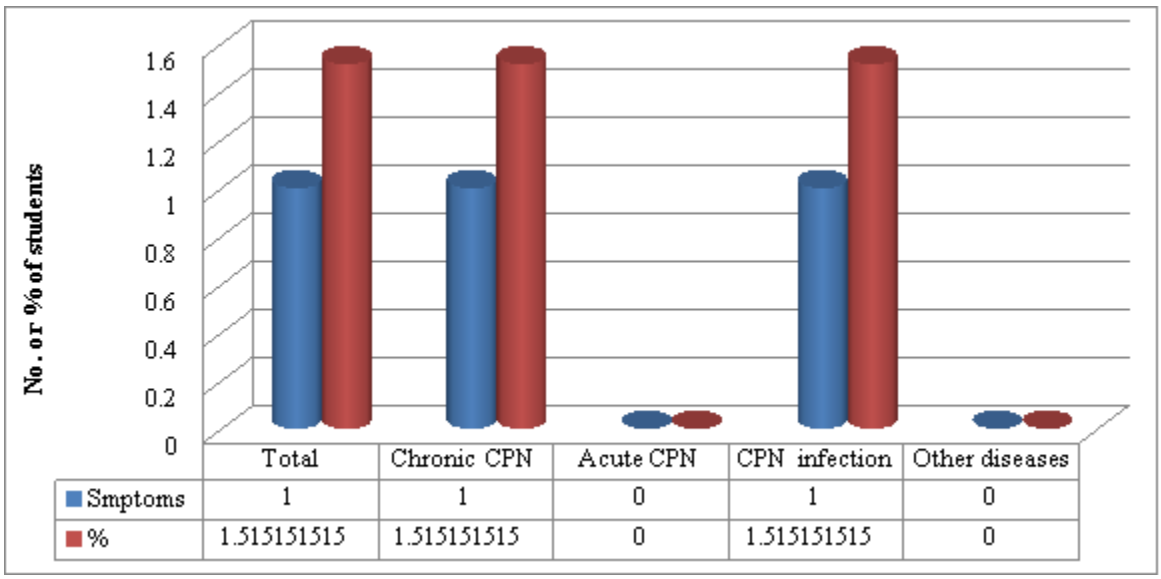
	Total	Chronic CPN	Acute CPN	CPN infection	Other diseases
Smptoms	1	0	1	1	0
%	1.515151515	0	1.515151515	1.515151515	0

C



	Total Cough (rare)	Chronic CPN	Acute CPN	CPN infection	Other diseases
Smptoms	15	4	0	4	11
%	22.727272727	6.060606061	0	6.060606061	16.666666667

D



	Total	Chronic CPN	Acute CPN	CPN infection	Other diseases
Smptoms	1	1	0	1	0
%	1.515151515	1.515151515	0	1.515151515	0

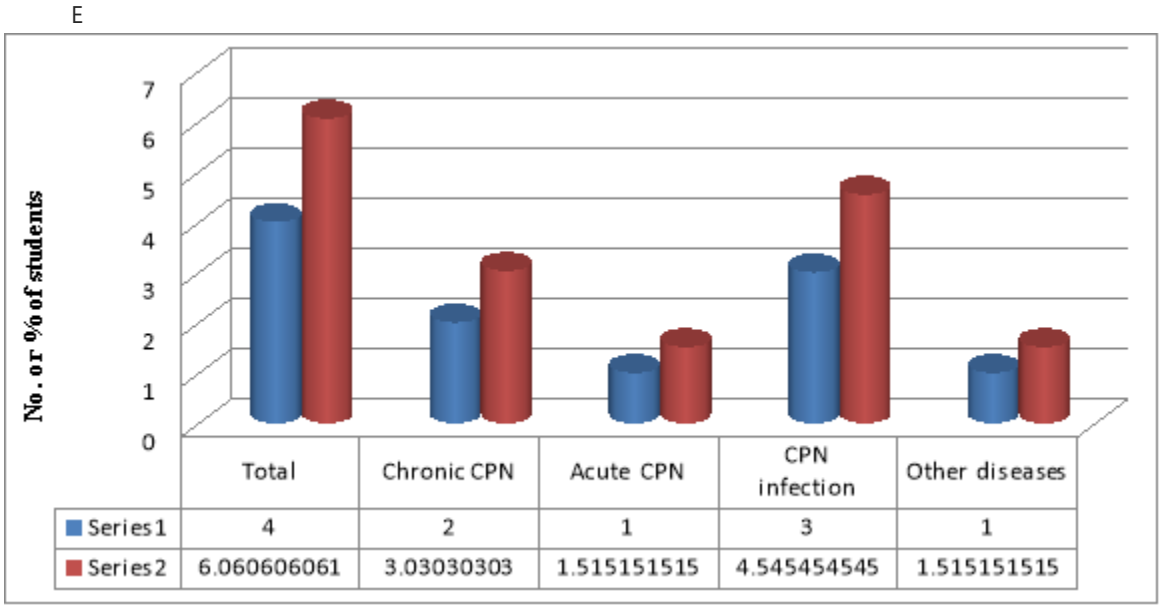


Figure 4 Association of specific *C. pneumonia*-related diseases with the presence of *C. pneumoniae* antibodies among students. The specific *C. pneumoniae*-related diseases shown are: asthma (A), sore throat (B), cough at least 1-2 times/year (C), cough several times a year (D) and the smoking habits (E). The number of students showing the symptoms to each disease (as informed by the survey) is indicated (total). The number and percentages of the students showing the presence of chronic, acute and total *C. pneumoniae infections* is indicated as Chronic CPN, Acute CPN and CPN infection, respectively. The number of students showing *C. pneumoniae*-related symptoms without evidence for *C. pneumoniae* infection were also indicated (other diseases).

4. DISCUSSION

C. pneumoniae infection is one of the leading causes of COPD, which has been categorized among the top 10 deadly disease that is responsible for 3.0 million deaths worldwide. *C. pneumoniae* is also associated with other deadly diseases that are categorized by the world health organization (WHO) within the top 10 deadly diseases worldwide. This includes heart diseases and Alzheimer's disease, which accounting for more than 9.5 and 2.5 million deaths in 2016, respectively (WHO: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Last visited on June 5th2020). The mortality rate due to a *C. pneumoniae* infection is approximately 9%. Remarkably, co-infection with other bacteria or the presence of other severe diseases increases the mortality risk (Oba, 2007). Therefore, transmission of *C. pneumoniae* should be avoided in particular to patients suffering from other infectious respiratory diseases. However, investigation of the presence of *C. pneumoniae* infection is relatively neglected. This might be because *C. pneumoniae* infection is mostly asymptomatic. This leads to less intervention strategies for preventing the spread of *C. pneumoniae* infection in particular among the high risk groups (e.g. schools and colleges, military barracks, nursing homes, hospitals and prisons). Herein, we wanted to intend a strategy in which we strive to minimize the interaction between internship students and patients suffering from infectious respiratory diseases and other chronic diseases associated with *C. pneumoniae* (e.g. heart diseases and Alzheimer') by distributing the students across hospital departments according to their seropositivity for *C. pneumoniae*. Thus, we assessed the prevalence of *C. pneumoniae* antibodies among 66 students from the college of public health and health informatics who are preparing to join the internship program at local hospitals. Serological tests of *C. pneumoniae* are the golden tool for demonstrating the current and past infection, in particular the MIF test which is the serological method of choice (Dowell et al., 2001). Although, criteria to define current/acute infection of *C. pneumoniae* using IgG antibodies in MIF appear to be controversial, in particular when single serum is used, most of the studies defined the IgG titer of 512 as indicative for current/acute infection of *C. pneumoniae* (Choi et al., 1998; Freidank & Brauer, 1993; Gaydos et al., 1994; Grayston et al., 1990; Halme et al., 1998; Hyman et al., 1995; Kern et al., 1993; Miyashita et al., 2001).

The seroprevalence of *C. pneumoniae* antibodies among the volunteered students was 48.48% (32/66). In general, the seroprevalance of *C. pneumoniae* among communities varied from 40-70% (Aldous et al., 1992; Choi et al., 1998; Einarsson et al., 1994; Gnarpe & Gnarpe, 1993; Kanamoto et al., 1991; Karvonen et al., 1992; Kese et al., 1994). However, most of the studies, which investigated the prevalence of *C. pneumoniae* infections among university students, showed the prevalence higher than 60%. The

seroprevalence of *C. pneumoniae* in healthy Belgian students was estimated to 61% (Van den Abeele et al., 1992). Freidank and Brauer reported that 64.9% of 353 German medical students had antibodies to *C. pneumonia* (Freidank & Brauer, 1993). Surprisingly, we observed *C. pneumoniae* IgG antibodies in only 48.48% (32/66) of the students. Lower incidence of *C. pneumoniae* infection was also reported among university students in northern California (Katzman et al., 1991). The authors suggest that there is may be geographic and temporal differences or fluctuations among populations.

C. pneumoniae infection is more common in males than females. In a German study, the prevalence rates of IgG antibodies against *C. pneumoniae* in medical students were reported to be higher in males (69.4%) than in females (57.3%). Similarly, Katzman and colleagues investigated the prevalence of *C. pneumoniae* infection among university students in northern California and found that the prevalence in males is 10% higher than that in females (Katzman et al., 1991). Our results are consistent with these studies. Male students (55%) were more susceptible for *C. pneumoniae* infection than female students (45.7%). Although most of the studies highlight this variation between males and females, there was no convincing explanation presented for this phenomenon. Thus, investigating the underlying factor(s) responsible for the variation between both genders should be further addressed in future studies. Nevertheless, the higher prevalence rates among males observed here is fully consistent with other previous reports(Choi et al., 1998; Freidank & Brauer, 1993; Gencay et al., 1998; Gnarpe & Gnarpe, 1993; Gnarpe et al., 1999; Hjelm et al., 2001; Kese et al., 1994; Koh et al., 2002; Lin et al., 2004; Miyashita et al., 2001). Surprisingly, these variations are limited or even mirrored when current/acute infection is considered (Females: 8.7%, Males: 5%). This has also been evidenced by other studies (Al-Younes, 2014; Katzman et al., 1991). An explanation could be assumed that the immune system response in female are susceptible to alteration due to the menstrual cycle, which in turn impacts the immune response on one hand and increases current infection of *C. pneumoniae* as well as other infectious agents on the other hand (Berbic & Fraser, 2013). Indeed, this might leads to fluctuation and subsequently a controversial estimating of the level of the immune response against *C. pneumoniae* and other infectious microorganisms in females. In this context, this might lead for statistically insignificant variations as obtained in our study.

We also observed that asymptomatic infection of *C. pneumoniae* (58.1%) is approximately one-fold higher than symptomatic infection (30.4%). Seroepidemiological studies have demonstrated that symptomatic infections with *C. pneumoniae* are uncommon and asymptomatic infections are more common (Block et al., 1997; Emre et al., 1994; Gaydos et al., 1994; Gnarpe et al., 1991; Hyman et al., 1995). Seropositivity to *C. pneumoniae* infection was correlated with the risk of several diseases. In our study population, we failed to demonstrate the role of *C. pneumoniae* infection in developing of/association with bronchitis, cardiovascular diseases, atherosclerosis, hypertension, diabetes, sinusitis and other chronic diseases. These atypical results are likely due to the relatively low numbers of our study population. Moreover, these chronic diseases are dominantly found in elderly individuals and it is rarely found in young individuals (Mean age: 22.1 years). However, we could demonstrate the association of *C. pneumoniae* infection with asthma (3.03%), sore throat (1.51%), Cough (6.06%) and in smokers (4.55%). These results are in fully agreements with other studies. It has been reported that *C. pneumoniae* infection is implicated in 4.3%-5.7% of asthma and sinusitis (Cook et al., 1998a). *C. pneumoniae* were also demonstrated to present (52%) with a sore throat (Thom et al., 1990). Furthermore, Birkebaek and colleagues confirmed serological evidence for the presence of *C. pneumoniae* infection in 1-4% acute coughing and in 40%-46% of chronic coughing (Birkebaek et al., 2000). Moreover, an association between increased *C. pneumoniae* IgG antibodies and current smoking habit has been also observed (Hahn, 1992). Ultimately, the necessity to control and minimize the mortality and morbidity due to respiratory infectious pathogens, particularly by internship students is a crucial issue.

5. CONCLUSION

The incidence of *C. pneumoniae* documented here in signifies the urgent need for added surveillance in the student internship program at the local hospitals. Frequent diagnosis of *C. pneumoniae* as well as other infectious diseases aids health care providers to implement high infection prevention and control protocols. In our internship program, we used the results of this study to properly minimize the contact of *C. pneumoniae* infected students with high-risk groups of patients in the hospitals. Diagnosis of other infectious diseases before attending internship program at hospitals is also highly recommended.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Ethical approval

The study was approved by the Medical Ethics Committee of Ha'il University (ethical approval code: 40006).

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Data and materials availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

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