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RESEARCH ARTICLE

GRAM POSITIVE AND GRAM NEGATIVE BACTERIA FROM SPUTUM OF CLINICALLY TUBERCULOSIS SUSPECTED PATIENTS

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ABSTRACT

Objective: To determine associated bacteria from sputum of tuberculosis suspected patients in Basra governorate.
Methods: 150 TB suspected patients sputum clinical specimens were collected at The Advisory Clinic for Chest Diseases and Respiratory (ACCDR) in Basra Governorate, Iraq. They were subjected to cultural, microscopical and biochemical tests.
Results: Out of 150 sputum samples thirty seven samples (24.6%) revealed associated bacteria, of which 13 (35.1%) was *Pseudomonas* spp., 11 (29.7%) *Bacillus* spp., 5 (13.5%) *Vibrio* spp., 4 (10.8%) *Staphylococcus* spp. and 4 (10.8%) as *Klebsiella* spp.
Conclusion: The association rate of gram-negative bacteria were significantly higher than that of gram-positive bacteria. They belong to five genera, three gram negative and two gram positive bacteria, which potentially may cause complications to TB patients.

INTRODUCTION

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis* and it is a major cause of morbidity and mortality, particularly in developing countries (Cegielski *et al*., 2002; Corbett *et al*., 2003; Tufariello *et al*., 2003). Many Infections caused by opportunistic bacteria are of the most important complications in patients with pulmonary tuberculosis. TB patients become susceptible to secondary bacterial infection as a result of the inhibition of the human defense system during the course of active tuberculosis (Naz and Tariq, 2005). Presence of *M. tuberculosis* causes an immune response in which many types of white blood cells are recruited to sites where the bacteria are growing. The bacteria within the tubercle can survive for decades, and conditions leading to a weakened immune response can allow the bacteria to break out of the lesion and reactivate to develop into active TB (Salyers and Whitt, 1994). This weakens the immune system, and secondary infections can be caused by opportunistic bacteria. There are several pathogenic species which can survive in lungs beside normal flora of respiratory tract and may produce lesions beside tuberculosis (Southwick, 2007). Various pathogenic bacteria and fungi have been found in tuberculous sputum e.g. Streptococci, Staphylococci, Pneumococci, *Heamophilus influenzae*, *Moraxella catarrlis*, *Klebsiella pneumonae*, *Pseudomonas aeroginosa*, *Actinomyces* and Diptheria like bacilli (Kumar *et al*., 2007). About 90% of those infections with *M. tuberculosis* are asymptomatic (Niederweis *et al*., 2010). In this study attention focused on opportunistic pathogenic bacteria, regarding their identification of patients with suspected tuberculosis.

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MATERIALS AND METHODS

Sample Collection

This study includes 150 samples from 150 patients admitted to the ACCDR, during a one year period (March 2013 February 2014). Samples of sputum were collected in sterile, screw-cap containers. The expectorated sputum was taken by asking the patient to cough deeply into the container, followed by immediate screwing off the cap. Samples were transported to the laboratory within two hours and processed immediately or refrigerated at 4ºC as soon as possible.

Microbiologic Examination

TB and associated bacteria samples were inoculated on nutrient agar and incubated aerobically at 37°C and read within 24 h and 48 h.

Characterization of Organisms

All the pure cultures were characterized to genus level using different tests conforming to required standard diagnostic criteria according to Brenner *et al.* (2005). The criteria included morphological, cultural and biochemical tests (Gram stain, oxidase, catalase, spore formation, mannitol fermentation, glucose oxidation/ fermentation, growth on MacConkey agar and growth on TCBSmedium).

RESULTS

Of one hundred fifty patients with suspected tuberculosis attended the ACCDR who were suffering from upper respiratory tract infections, thirty seven samples (24.6%) showed associated bacteria, of which 13 (35.1%) were *Pseudomonas* spp., 11 (29.7%) *Bacillus* spp., 5 (13.5%) *Vibrio* spp., 4 (10.8%) *Staphylococcu*s spp. and 4(10.8%) as *Klebsiella* spp. (Table 1 and 2).

Pseudomonad infections, specially in immune suppressant persons (Kielhofner *et al*., 1992), such as patients whose suffering from tuberculosis. These findings confirm a previous study where *Pseudomonas* has been reported as a major pathogen causing secondary infections in hospitalized tuberculous patients (Shishido *et al*., 1990; Naz and Tariq., 2005). In addition to that 11 (29.7%) was *Bacillus* spp., and 5 (13.5%) *Vibrio* spp. These genera are opportunistic bacteria can be transmitted from one host to another without having to cause disease.

The prevalence of 4 (10.8%) found for *Staphylococcus* spp. was higher compared with many previous studies like Nabeetha *et al.* (2005) who found 6.5% of *Staphylococcus* in sputum samplesin Trinidad and 3.8% found by Moine *et al*. (1994) in France. On the other hand, a lower prevalence (0.4%) was found in the USA (Martson *et al*., 1997). Also the results showed the presence of 4 (10.8%) as *Klebsiella* spp. and this agrees with Mayaud *et al*. (2002), who reported that, the *Klebsiella* (13%) organisms frequently associated with bacterial infections of the respiratory tract infection.

Table 1. Biochemical characteristics of bacteria isolates from samples of 37 clinically TB suspected patients

Genera	Identification criteria							
	Gram stain	oxidase	Catalase	spore formation	mannitol fermentation	glucose oxidation fermentation	growth on MacConkey agar	growth on TCBS medium
Bacillus	+	+	+	+	N/A	-	-	N/A
Staphylococcus	+	+	+	-	+	N/A	N/A	N/A
Pseudomonas	-	+	+	-	N/A	-	N/A	N/A
Vibrio	-	+		N/A	N/A	+	N/A	+
Klebsiella	-	-	+	N/A	N/A	N/A	Pink colonies	N/A

- (No growth), + (Growth), N/A (Not applicable)

Table 2. Number and percentage of gram positive and gram negative bacteria from samples of 37 clinically TB suspected patients of various age groups

Bact. Genera	No. and % of isolates	Sex		Age range
		M (21)	F (16)	
Bacillus	11(29.7)	5	6	28-70
Staphylococcus	4(10.8)	2	2	30-47
Pseudomonas	13(35.1)	7	6	25-60
Vibrio	5(13.5)	3	2	40-87
Klebsiella	4(10.8)	4	-	40-35

DISCUSSION

Although the prevalence, by gender of patients, of each of the bacteria did not differ significantly, over all the prevalence of infection was higher in male than in female patients. They were found in 21 male patients (56.75%) and 16 female patients (43.24%). The higher percentage of secondary bacterial infections in the malesis more than females (Table 1). This may be due to the male working in various fields, to non sanitary, and crowded area especially in cases of poor ones. Thus male is more exposed to infection (WHO, 2009). The results showed that gram negative bacteria dominance versus gram positive bacteria and this agrees with previous study which confirmed the infection rate of gram negative bacteria in respiratory tract infection was significantly higher than that of gram positive bacteria (He *et al*., 2014). It is also shown that the highest bacterial appearance was *Pseudomonas* sp., which represented 35.1%. *Pseudomonas* considered opportunistic pathogens that rarely cause disease in healthy persons, but several well-described conditions that render patients susceptible to

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