analyses of type b and non-type-able *H.influenzae,* which shows that this protein is stable and conserved [3]. Other OMP is P2 which is a major protein comprises more than 50% of the OMPs. This protein exists on the outer membrane as a trimer and act as a prion, so, it is mostly located on the outer membrane of non-type-able *H. influenzae* [4].

H. influenzae is also variable for the presence of polysaccharide capsule and is classified on the basis of production of polysaccharide capsule, strain types a through f produce antigenically distinct capsules and non-type-able strains produce no capsule [5]. Others classify *H. influenzae* into 3 main categories: nonencapsulated strains, encapsulated type b strains, and capsulated non-type b strains (types a and c-f) where type b is the most virulent form [6]. Several studies have demonstrated molecular capsule typing methods to be more sensitive and specific than other methods [7].

Encapsulated *H. influenzae* isolates contain genes for the production of their respective polysaccharides capsules at the cap locus which is composed of three distinct regions, designated region I to III. The genes contained within regions I and III, designated bex DCBA and hcs AB, respectively, are highly conserved across all six capsular types and are required for transport of capsule constituents across the outer membrane [8,9]. Yet, region II gene encodes capsule type a-through f-specific proteins and thus varies by serotype.

The organization and genetics of the cap locus are complex, duplications, partial loss, and complete loss of the cap locus can occur and give different results [10]. Early studies revealed that all isolates of *H. influenzae* are different in terms of pathogenic potential. It is very important to mention that most systemic isolates express the type b capsule, whereas most respiratory tract isolates contain unencapsulated, referred to as non-type-able [11]. These are more commonly part of the normal flora, less invasive and frequently involved in opportunistic respiratory tract infections. Many classification approaches showed that most non-type-able *H. influenzae* (NTH1) were genetically quite distinct from type b (Hib) strains and more heterogenous, yet, ribotyping and ERIC (Enterobacterial Repetitive Intergenic Consensus), PCR have been used to relate strains. The horizontal exchange of *H. influenzae* genetic loci between strains due to natural DNA transformation make the classification complicated and may explain the differences seen using different methods [12].

The objectives of this study was to isolate *H. influenzae* from different clinical samples and differentiate both capsulated (type-able) and non-capsulated (non-type-able) one by molecular detection method and to make a comparison between the two types by cultural, molecular, and clinical aspects.

Methods

Samples and Bacterial culture

A total of 220 clinical samples were taken from different clinical samples from patients attending the three main hospitals (Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital and Merjan Medical City) during the period from February 2012-June 2012. The samples were transported using specific transport media and processed on blood, chocolate agar and tryptic Soya agar sublimated with X, V disc and subjected to standard bacteriological method and incubated in 5% CO_2 at 37^{o}C for 24 hrs., biochemical tests like catalase, oxidase, urease, indole, nitrate reduction, carbohydrate fermentation was done according to MacFaddin [13].

DNA extraction from Gram-negative bacteria

This method was performed according to the genomic DNA purification kit supplemented by a manufacturing company (Promega, USA).

Molecular method used in detection of type-able and non-type-able *H.influenzae*

H. influenzae was detected by PCR, using 20 µl PCR reaction mixture as in table 1. Primer used and thermal cycle condition were illustrated in table 2. The amplification product was separated on (1-1.5%) agarose gel containing ethidium bromide for 45 min. at 70 V. The size of the