

where type-specific serum agglutination had classically been used to confirm the presence and specificity of *H. influenzae* capsule. However, a strain may fail to react with typing sera and thus be classified as non-type-able for several reasons <sup>(24)</sup>.

**First:** inaccuracy in performing and interpreting slide agglutination tests had been well documented.

**Second:** strain with one copy of the *cap* region in which *bexA* is partially deleted is referred to as capsule deficient variants because they contain a majority of the *cap* locus.

**Third:** a previously serotypeable strain could have a deletion of the entire capsule locus, as apparently occurred with strain Rd (non-type-able variant of type d strain).

**Fourth and finally:** a strain may lack the entire *cap* locus as consequences of long-past evolutionary events, i.e., may be a true NTHi strain <sup>(25)</sup>. Davis <sup>(21)</sup> found that *bexB* which is located adjacent to *bexA* in region I of the capsule locus and encodes another protein important in capsule exportation, is a more reliable marker of the capsule locus because it can be detected in *H. influenzae* strains that possess a single *cap* locus and a *bexA* mutation in that locus. Another study done by Mojgani et al <sup>(16)</sup> isolated *H. influenzae* at a rate different from the rate of this study where NTHi isolation rate was (38.6%). While type-able one was (61%) by using *bexA* primer and found that most of NTHi isolates were from nasopharyngeal secretion while type-able *H. influenzae* were mostly from CSF. The results of this study are in contrast with the results of other studies where they identified and isolated NTHi at a rate of about (33%) while type-able one was (66.6%) but most of their isolates were from invasive *H. influenzae* disease <sup>(26)</sup>. Many studies proposed that the ancestor of *H. influenzae* was encapsulated and the non-type-able clones arose by convergent evolutionary loss of the ability to synthesize or extracellularly express a polysaccharide capsule. However, the wide heterogeneity of non-type-able strains, the more clonal features of type-

able strains and the evidence that most type b specific genetic regions are flanked by repeat sequences and thus may represent acquisition of foreign genetic elements, make it more likely that an unencapsulated ancestral *H. influenzae* strains acquired these elements and became more virulent <sup>(27)</sup>.

While the detection of non-type-able *H. influenzae* at 1<sup>st</sup> by absence of capsule gene and by P2 gene that is conserved for them. In this study it was used to differentiate between both type-able and NTHi and since it expresses on NTHi so it is possible to confirm that the remaining 4 isolates that were non-capsulated by using Bex primers (A, B) were non-type-able because some isolates that have mutation or deletion or single copy of *cap* locus sometimes fail to express capsule and can be regarded as non-capsulated, but these isolates are not true NTHi.

The emerging role of invasive disease because NTHi is intriguing because this organism had traditionally been considered relatively non-invasive bacteria predominantly associated with community-acquired pneumonia, chronic obstructive pulmonary disease exacerbations and otitis media <sup>(28)</sup>. Regarding the Detection of type b *H. influenzae*, the study revealed that two isolates were of capsulated (type-able) *H. influenzae* and further primers were used to detect the serotypes of these two isolates and specifically type b (Hib) since these two isolates were from CSF of patient with meningitis and so type b could be the causative agent among these isolates. However, the results showed that only one isolate was type b (Hib) while the other one was capsulated non type b *H. influenzae*.

In this study two primers were used, one called bex to detect Hib from CSF of patient with meningitis specifically and to exclude *N. meningitis* and *S. pneumoniae* while the other one called Hib which confirmed the results and both gave the same results. Center for Disease Control and Prevention <sup>(29)</sup> revealed that infection with Hib can cause meningitis in 50% of cases of adults and children and this result is similar to the results of this study and they also