

Discussion

The results indicate that isolation rate differs according to site of isolation and method applied and this can also be attributed to different virulence factors expressed by *H. influenzae* in different sites of human body as the natural host for it and can be correlated also with the severity and invasiveness of the disease. The results of this study agreed with the results obtained in another study⁽¹⁴⁾ where they detected *H. influenzae* by cultural method and by using X, V discs and specific culture media at a rate of about (18.05%). Also another study found that the isolation of *H. influenzae* is *increased* by using selective media and X, V factors discs and isolated it in a rate of (27.16%)⁽¹⁵⁾. The same result was described by Mojgani⁽¹⁶⁾ where he isolated *H. influenzae* from CSF of patient with meningitis, eye, mucous from patient with conjunctivitis and nasopharyngeal and ear swabs from patient with otitis media and throat infection, they isolated it at about (31.4%), based on their morphology and growth requirement for X and V factors.

In contrast to the results of this study, other study found 80 isolates of *H. influenzae* on the basis of their growth requirement and serotype distribution⁽¹⁷⁾. Generally, different isolation rate could also explained by different factors like age, season, the size of the facility, antibiotic treatment, morbidity from acute URIs, the sampling technique, and the methods that give more accurate sensitivity and specificity. While molecular detection method confirmed the isolation of *H. influenzae* and focused on role of P6 outer membrane protein OMP P6 gene in this molecular detection method and it detect about 6 isolates (60%) of *H. influenzae* by PCR. Although, *H. influenzae* have many OMPs like P5, P2, but unlike p2 protein, p6 protein show a very high homology (97%) in the amino acid analyses of type b and non-type-able *H. influenzae* strains, which shows that this protein is stable and conserved⁽³⁾.

Many studies concentrated and focused on this genetic primer and found that *H. influenzae* detection could be achieved with varying degree

of success with primers specific for rRNA-encoding genes⁽¹⁸⁾, yet, the rRNA sequences of *H. influenzae* and *H. parainfluenzae* show approximately 95% homology. So, these genes are not ideal targets for the unequivocal identification of *H. influenzae*⁽¹⁹⁾. Previous studies had shown that NTHi OMP P6 had 100% homology among human respiratory isolates from adults and children⁽²⁰⁾. Regarding the determination of capsular type ability by molecular methods detection, the result revealed that 2 isolates out of 6 (33.3%) were type-able (i.e., capsulated) while 4 isolates out of 6 (66.6%) were non-type-able.

The results also showed that these 2 capsulated isolates of *H. influenzae* were from CSF of patient with meningitis and this is accepted regarding the presence of capsule that make it resistant to macrophage, complement and human defense and provides the ability to be invasive and so cause severe disease and even make it resistant to many antibiotics. The highly conserved *bexA* and *bexB* genes, which are required by type-able strain for the transport of capsule components across the outer membrane, were assayed by PCR following the protocol of Davis⁽²¹⁾. The advantage of this method over traditional slides agglutination techniques using type-specific antisera or methods detecting *bexA* alone is that *bexB* PCR will detect rare strains that are *bexA* negative but *bexB* positive, which render them phenotypically non-type-able but genetically far closer to type-able strains⁽²²⁾.

Another studies used PCR reaction to detect *bexA* gene and found that its absence or presence determined whether an isolate was encapsulated or non capsulated, their study revealed a rate of NTHi to be about 93.5% while type-able one was (6.45%) distributed into different serotypes from a-f⁽²³⁾. So our results differed from this study and this may be attributed to dependence of their study on *bexA* alone and also their isolates were mostly nasopharyngeal isolates. Many studies found that differentiating type-able from non-type-able *H. influenzae* strains can be challenging