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Comparison of virulence factors and capsular types of *Streptococcus agalactiae* isolated from human and bovine infections

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ABSTRACT

Streptococcus agalactiae is a leading cause of human and bovine infections. A total of 194 *S. agalactiae* isolates, 55 isolates from bovines and 139 from humans, were analyzed for capsular types, virulence genes (*scpB*, *hly*, *rib*, *bca* and *bac*) and mobile genetic elements (IS*1548* and GBSi1) using polymerase chain reaction (PCR) and multiplex PCR. Capsular type III was predominant (61%), followed by types V, II, Ib, and IV. The *scpB*, *hly*, *bca* and *bac* virulence genes were only found among human isolates. Twelve and 2 distinct virulence gene profiles were identified among human and bovine isolates respectively. The virulence gene profiles *scpB- hly-* IS*1548- rib-bca* (51%) and *scpB- hly-* IS*1548- bca* (19%) were only predominant among human isolates. The *rib* gene was the most common virulence gene in both human and bovine isolates. The study showed a high prevalence of virulence genes in *S. agalactiae* strains isolated from human infections, these result can support the idea that *S. agalactiae* isolated from humans and bovines are generally unrelated and probably belonged to separate populations.

1. Introduction

Streptococcus agalactiae (Group B Streptococci or GBS) is Gram-positive cocci, which emerged in the 1887 as a major cause of bovine mastitis, a disease of economic importance. In 1970; the organism emerged as a major cause of neonatal meningitis and sepsis [1]. Additionally, it is also important pathogen in immunocompromised adults and the elderly [2]. In 1990; the organism emerged as an important pathogen causing urinary tract infections in adults [3]. The ability of *S. agalactiae* to cause disease depends on the production of a numerous of virulence factors, including capsular polysaccharide, the alpha and beta antigens of the C protein, surface protein Rib, hyaluronate lyase and C5a peptidase that is encoded by the *cps*, *bca*, *bac*, *rib*, *hly*, and *scpB* genes respectively [4,5]. The capsular polysaccharide of *S. agalactiae* is the most important virulence factor and it is generally used for strain typing [6]. Ten distinct capsular types (Ia, Ib, II–IX) have been distinguished [4]. The α-C protein mediates adherence to epithelial cells, whereas the β-C protein participates in invasion, as well as resistance to phagocyte clearance [5,7]. Protein Rib (resistance to proteases) confers protective immunity and has been found in most strains of type III that caused invasive infections in neonates [5]. Hyaluronate lyase is a surface-exposed protein which cleaves hyaluronan, the host connective tissues, and facilitates the invasion and spreading of *S. agalactiae* through host tissues [7,8]. C5a peptidase, a surface-localized serine protease, cleaves human C5a, a component of the human complement system, thereby leading to reduced neutrophil chemotaxis and decreasing opsonophagocytic killing. Additionally, C5a peptidase mediates binding to fibronectin and contributes to the invasion into epithelial cells [9,10]. Prevalence studies for *S. agalactiae* in bovine and humans have been performed in various regions of Iran but data about distribution of capsular types and profiles of virulence gene in Iran, like as many countries, are scarce [4,6,11–13]. Therefore, the objectives of the present study were to characterize the capsular type and virulence factors among *S. agalactiae* isolated from human and bovine infections.

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2. Material and methods

A total of 194 *S. agalactiae* isolates, corresponding to 55 isolates from bovines and 139 from human source, were analyzed in the current study. The 55 bovine *S. agalactiae* isolates were obtained from cows with subclinical mastitis from 5 dairy farms situated in Tehran and 139 human isolates were obtained from urine source of patients attending to three hospitals in Tehran. The California Mastitis Test (CMT) was used to determine the presence of subclinical mastitis [14,15]. The isolates were identified to species level using standard biochemical methods including Gram staining, catalase, sulfamethoxazole − trimethoprim (SXT) and bacitracin susceptibility tests, CAMP and Hippurate Hydrolysis Tests [16]. To confirm the identity of isolate as *S. agalactiae*, the *dltS* gene was amplified by polymerase chain reaction (PCR) [17]. The study was approved by the Ethics Committee of Tehran University of Medical Sciences.

2.1. DNA extraction

The genomic DNA was extracted from *S. agalactiae* isolates using Exgene Cell SV Kit (Gene All, Seoul, Korea) according to the manufacturer's protocol and was stored at 4 °C until being processed.

2.2. Molecular capsular typing

The identification of capsular type (Ia, Ib, II-VIII) of all *S. agalactiae* isolates was carried out by multiplex PCR assay as described previously [17].

2.3. Detection of virulence genes

Detection of 5 different virulence genes (*scpB*, *hly*, *rib*, *bca* and *bac*) and mobile genetic elements (IS*1548*and GBSi1) were carried out by PCR using the oligonucleotide primers are listed in Table 1 [18–20].

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad QuickCalcs software available online http://www.graphpad.com/quickcalcs/contingency1/. Achi-square test was used to evaluate the differences in distributions of genes between *S. agalactiae* isolated from human and bovine infections. A P value of ≤ 0.05 was considered significant. All figures results were rounded down if they were less than 0.5, and if they were 0.5 or more rounded up.

3. Results

Forty-eight (87%) of the bovine and 136 (98%) of the human *S. agalactiae* isolates were capsular typed by the multiplex PCR. Only ten isolates were non-typeable. The capsular type distributions of 194 isolates are given in Table 2. Five different capsular types (Ib, II, III, IV, V) were identified. Capsular type III and II were detected in bovine and human *S. agalactiae* isolates, whereas capsular types Ib, IV and V were only found in human *S. agalactiae* isolates. Among the identified types, capsular type III was the most frequent, representing 61% of all isolates. No strains of capsular type Ia, VI–VIII were found. The virulence gene profiles we observed are summarized in Table 3. Generally, human strains demonstrated a greater heterogeneity of virulence profiles than did bovine strains. Among human strains, 12 distinct virulence gene profiles were observed but 98 strains (71%) belonged to either the *scpB*- *hly*- IS*1548*- *rib*- *bca* 71 (51%) profile or the *scpB*- *hly*- IS*1548*- *bca* 27 (19%) profile. The bovine strains exhibited 2 different profiles which did not occur in human strains. The most common virulence gene was *rib* detected in 89% of bovine isolates, whereas four virulence genes (*scpB*, *hly*, *bca* and *bac*) were only distributed in human *S. agalactiae* isolates. In addition, the high prevalence of the IS*1548* was found among human isolates than bovine isolates. The genes *scpB*, *hly*, *bca* and IS*1548* were significantly highly detected in strains isolated from humans compared to those from bovine ($P < 0.0001$). In contrast, the *rib* gene was significantly detected in strains isolated from bovine compared to those from humans ($P < 0.0001$). The GBSi1 was not detected in any of the isolates. Four isolates; one from a human and three from bovine were negative for all virulence marker genes.

4. Discussion

In the current study, the capsular type of 95% isolates was identified. In Turkey, Ekin et al. was determined the capsular type of 56% of isolates and in United States, Dogan et al. was determined the capsular type of 82% of isolates [21,22]. This discrepancy in determination of capsular types may be explained by use of various methods such as PCR-based capsular gene typing methods or an agglutination method, such as the latex test. In this study, capsular type III was the predominant type in both human and bovine. Similar findings have been observed in United States where capsular type III was shown to be common in human and bovine [22]. However, capsular type V and IV were found to be the most frequent in Norway and United Arab Emirates, respectively [23,24]. The diversity in capsular type distribution might be related to the time and source of the isolation, ethnic origin, geographic area and diagnostic techniques [4,25]. It has been reported that capsular type III is the most common cause of invasive disease in human especially in newborns less than 7 days, but its significance for bovine isolates is not proven yet [2,5]. In this study PCR assay for detection of virulence genes revealed that high percentage of the human isolates were positive for the *hly*, *scpB* and *bca* genes, while none of bovine isolates had these genes. The high prevalence of human *S. agalactiae* strains carrying these genes has been reported previously [4,26,27]. In contrast to the findings in our study, Duarte et al. observed that 66% and 79% isolates from bovine harbored the *scpB* and *bca* gene respectively [28]. Duarte et al. also observed that 97% and 77% isolates from human harbored the *scpB* and *bca* gene respectively [28]. Among the 194 *S. agalactiae* isolates tested, 69% were *rib* positive and this gene was detected more frequently among bovine than in human strains (95% vs. 58%). The high frequency of *rib* gene may indicate that this gene is essential for the development of bovine mastitis. However, Jain et al. show that 26% of bovine isolates harbored the *rib* gene [29]. Manning et al. showed that the *rib* gene was present in 28% and 20% of colonizing and invasive strains respectively [20]. In the current study the *bac* gene prevalence was similar to those study in which the low frequency of the *bac* gene has been detected among bovine and human isolates [26,28]. However compare to our study, the higher rate of the *bac* gene has been reported from the United States and Poland [20,30]. In this study, the IS*1548* was found more frequently among human than in bovine (77% vs. 5%). In a study from India low prevalence of IS*1548* (8.9%) has been reported in bovine isolates [31]. However, discrepancy in the frequency of virulence genes was observed among different studies, which can be explained by the difference in the origin of the isolates as well as other factors. The comparison of human and bovine isolates demonstrated that virulence genes were less prevalent among bovine isolates than among human isolates, which is in accordance with the report of Duarte et al. [28]. In the current study the majority of the *S. agalactiae*