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RESEARCH ARTICLE

The Prevalence and Molecular Characterization of Some Virulence Genes in *S. agalactiae* and *S. dysgalactiae* Isolates from Milk, Milk Products, and Humans in Sharkia Governorate, Egypt.

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Abstract

In this study, *Streptococcus* species were identified in 68.75% of milk samples, 69.33% of kariesh cheese, 80% of hand swabs, 77.5% of pharyngeal swabs, and 45% of vaginal swabs. From milk samples, 37 *Streptococcus* species isolates were identified: *S. agalactiae* (37.83%), *S. dysgalactiae* (21.62%), *S. uberis* (13.51%), *S. pyogenes* (8.11%), and *S. pneumoniae* (2.7%). Analysis of hand swabs revealed *S. agalactiae* (40%), *S. dysgalactiae* (10%), *S. pneumoniae* (10%), and both *S. pyogenes* and *S. uberis* (15% each). For pharyngeal swabs, 19 isolates included *S. agalactiae* (15.79%), *S. dysgalactiae* (5.26%), *S. pneumoniae* (26.32%), and *S. pyogenes* (36.84%). Thirty- four *Streptococcus* species isolates were molecularly confirmed as: *S. agalactiae* (n = 20), *S. dysgalactiae* (n =11) and *S. pyogenes* (n = 3). Results indicated that virulence genes *scp*B, *rib*, and *lmb* were present in all *S. agalactiae* isolates (100%) from milk and cheese. However, *scp*B and rib were detected in 66.7% of S. agalactiae human isolates, and *lmb* was found in all isolates (100%). The *scp*B gene was identified in all *S. dysgalactiae* human isolates (100%) but was not detected in *S. dysgalactiae* isolates from milk and cheese. The rib gene was not found in *S. dysgalactiae* milk isolates but was detected in all *S. dysgalactiae* from cheese and human (100%). The lmb gene was found in all milk and cheese *S. dysgalactiae* isolates, but only detected in 50% of human isolates. In conclusion, *S. agalactiae* and *S. dysgalactiae* were frequently detected in dairy products and human swabs in Sharkia Governorate, Egypt, indicating potential zoonotic transmission. The virulence genes *scp*B, *rib*, and *lmb* were more common in *S. agalactiae* from milk and cheese than in those from humans, while these genes were more prevalent in *S. dysgalactiae* from humans than from dairy sources.

Keywords: *Streptococcus* spp., Prevalence, Zoonotic transmission, Virulotyping

Introduction

Mastitis is a widespread disease in dairy cattle causing considerable economic losses due to reduced milk yield and quality [1]. A major contributing factor is bacteria from the *Streptococcus* genus, which includes both contagious pathogens such as *Streptococcus agalactiae* and environmental pathogens such as *Streptococcus uberis* [2-4]

Group B *Streptococcus* (GBS), scientifically designated as *Streptococcus agalactiae*, represents a significant pathogen affecting various hosts, including humans, fish, and dairy cattle. In humans, it is a leading cause of neonatal infections and is increasingly

recognized as an invasive pathogen in adults [5]. Many adults carry this bacterium asymptomatically, primarily in the gastrointestinal and urogenital tracts, but also in the oropharynx and on the skin [6-8]. In dairy cattle, *S. agalactiae* is linked to intramammary infections leading to lower milk quality and quantity. It is found in other species, including dogs and cats, and in humans on dairy farms [7, 9, 10] and named as an "obligate intramammary pathogen" **[**11]. Studies have also detected *S. agalactiae* in bovine faeces and environmental samples [12], with evidence suggesting potential transmission between cattle and humans [13].

Streptococcus dysgalactiae has two subspecies: *S. dysgalactiae subspecies equisimilis* (SDSE), associated with human pharyngitis, skin infections as well as severe diseases in vulnerable individuals [14]. Whereas *S. dysgalactiae subspecies dysgalactiae* (SDSD), is recognized as a major animal pathogen responsible for mastitis in cattle, infective arthritis in sheep, and neonatal mortality in puppies [15-17]

The pathogenesis and severity of *S. agalactiae* and *S. dysgalactiae* infections are linked to various virulence factors that aid in host colonization, bacterial dissemination, immune evasion, and internalization in mammary gland cells [18]. Over 15 virulence genes, such as *scp*, *rib*, and *lmb*, have been identified, influencing the pathogen's invasive abilities. The *bac* gene is associated with immune evasion [19]. Other significant virulence factors include fibrinogen-binding protein (*fnb*), laminin-binding protein (*lmb*), fibronectin-binding protein (*pavA*), β-C protein (*cba*), capsule, C5a peptidase (*scp*), hyaluronate lyase, α-C protein, β-hemolysin/cytolysin, and CAMP factor (*cfb*) [20]. Additionally, the biofilm-forming ability of *S. agalactiae* is considered a major virulence factor affecting its survival and persistence in the environment and host [21].

In diagnostic laboratories, bacterial identification often depends on labour-intensive biochemical tests and serological grouping, which can yield unsatisfactory results [22-23]. Currently, the molecular methods based on Polymerase Chain Reaction (PCR) and sequencing have been developed to identify *Streptococcus* spp. by amplification of species-specific sequences of the *tuf* (encoding the elongation factor Tu) and *16S rRNA* (encoding the 16 S ribosomal RNA) genes. These molecular assays are preferred for their robustness, reproducibility, and accuracy in bacterial identification compared to traditional phenotypic testing [24-25]. This study focuses on the phenotypic and genotypic identification of *Streptococcus* species, especially *S. agalactiae* and *S. dysgalactiae* in milk, kariesh cheese from dairy cows as well as in humans' hand, pharyngeal and vaginal swabs. Additionally, the virulence profiles of the identified strains were analyzed.

Materials and methods

Ethical approval

The current study was reviewed and approved by Zagazig University Institutional Animal Care & Use Committee (approval number ZU-IACUC/2/428/2023). Medical ethics were also followed according to the declaration of Helsinki. Animals' samples were collected after the owners' agreement to participate in the study as well as human samples were collected after getting verbal and / or written consent of participation (Regarding the children, the consent was obtained from their parents).

Samples' collection and preparation

Milk samples

A total of 160 milk samples were collected from four dairy cattle farms (n = 120) and dairy outlets (n = 40) at Sharkia Governorate. Milk from dairy farms were collected from 78 cattle with mastitis (The udder showed swelling, hardness, hotness, redness, or pain and the milk was watery with presence of clots, flakes, or pus) and 42 apparently healthy cattle. Milk samples were collected according to the National Mastitis Council [26].

Kariesh cheese samples

A total of 75 cheese samples were collected from farmers' houses in Sharkia Province and were processed according to APHA [27]. In a blender, eleven grams of each cheese sample were added to 99 mL sterile, freshly prepared aqueous solution of 2% sodium citrate at 40-45°C, mixed well for 2 min till complete emulsification, and then decimal dilutions were prepared using a sterile buffered peptone water (BPW; Thermo Fisher Scientific, Oxoid Ltd., UK).

Hand swabs from milk handlers

A total of 40 hand swabs were collected from dairy farm workers and workers in retail outlets (n = 20, each). During the milking process, a moistened sterile swab was rolled over the hands, fingertips, nails, and area between fingers [28]. The swab was then inserted into tubes containing BPW and then directly transferred to the laboratory for bacteriological analysis.

Vaginal swabs

Twenty vaginal swabs from pregnant women were collected from three laboratories at Sharkia Governorate. The investigated women were either apparently healthy (n = 4) or suffered from vaginitis (n = 16). Swabs were collected by specialized health care personnel by gently passing sterile cotton swabs several times across the vaginal surface, including the lesion [29]. Each vaginal swab was immediately immersed into a sterile tube containing BPW and then directly transferred to the laboratory for bacteriological analysis.

Pharyngeal (Throat) swabs

A total of 40 pharyngeal swabs were collected from children attending private clinics (n = 30) and Zagazig University Pediatric outpatient clinics (n = 10). The pediatrician or the laboratory personnel asked the child to open his/her mouth as wide as possible. The oral cavity was checked for any signs of inflammation and for the presence of any exudates or pus on tonsils using a tongue depressor. The throat swab was then collected by rubbing a sterile cotton swab over the tonsillar area avoiding touching the tongue or lips to reduce contamination by oral microbiota [30]. The collected swabs were then placed in a sterile tube containing BPW, labeled, and then immediately transferred to the laboratory for bacteriological analysis.

***Isolation and identification of Streptococcus* spp.**

A loopful from each processed milk and different swab samples was streaked on Edward's medium (Biolife, Turkey) and incubated at 37°C for 24-48 h. The suspected colonies showed slight blue dew drop like appearance were purified and identified morphologically by Gram's stains. Biochemical identification of the isolates was carried out by standard biochemical tests using media and reagents provided by Thermo Fisher Scientific, Oxoid Ltd., UK [31]. Serotyping of biochemically confirmed *Streptococcus* isolates was carried out by latex agglutination test using

Streptococcal Grouping Test Kit DR0585 (Thermo Scientific Oxoid, Basingstoke, Hampshire, England) as described by the manufacturer`s instructions. The serologically confirmed *S. agalactiae*, *S. dysagalactiae* and *S. pyogenes* isolates were preserved on brain heart infusion broth (Thermo Fisher Scientific, Oxoid Ltd., UK) with 30% glycerol at -20°C until used for further molecular techniques.

Molecular identification of Streptococcus spp.

The DNA was extracted from overnight broth cultures of serologically confirmed *S. agalactiae*, *S. dysagalactiae* and *S. pyogenes* by QIAamp DNA mini kit (Qiagen) with modifications from the manufacturer's recommendations. A uniplex PCR assay was conducted targeting *Streptococcus* spp. *tuf* &*16S rRNA* genes, *S. agalactiae 16S rRNA*, *S. dysgalactiae 16S rRNA* and *S. pyogenes 16S rRNA* genes using oligonucleotide primers supplied by Metabion, Germany (Table 1). The PCR reaction volume (25 µL) consisted of a mixture of 12.5 µL of 2x premix Emerald Amp GT PCR mastermix (Takara, Japan), 1 µL of each primer (20 pmol), 5 µL of DNA template and 5.5 µL of PCR grade water. The amplification was conducted in a T3 thermal cycler (Biometra) and the cycling conditions were provided in table 1. The amplified PCR products, 100 bp DNA ladder (Fermentas), positive and negative controls were loaded to 1.5% agarose gel stained with ethidium bromide and run for 30 min at 1-5 volts / cm. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software.

Molecular identification of virulence genes in S. agalactiae and S. dysagalactiae isolates from different examined sources.

The extracted DNA of each *16S* rRNA positive *S. agalactiae* and *S. dysagalactiae* strain was screened using a uniplex PCR assay for the presence of s*cpB, lmb,* and rib virulence genes coding for Streptococcal C5a peptidase adhesion, laminin-binding surface proteins and Surface protein Rib., respectively. The primers used were supplied by Metabion, Germany (Table 1). The PCR reaction volume (25 µL) consisted of a mixture of 12.5 µL of 2x premix Emerald Amp GT PCR mastermix (Takara, Japan), 1 µL of each primer (20 pmol), 5 µL of DNA template and 5.5 µL of PCR grade water. The amplification was conducted in a T3 thermal cycler (Biometra), and the cycling conditions were provided in Table 1. The amplified PCR products, 100 bp DNA ladder (Fermentas), positive and negative controls were loaded to 1.5% agarose gel stained with ethidium bromide and run for 30 min at 1-5 volts / cm. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software.

Statistical analysis

The data were edited using Microsoft Excel version 16 (Microsoft Corporation, Redmond, WA, USA). A Chi-square test (PROC Freq; SAS Institute Inc., Madison, WI, USA, 2012) was conducted to investigate significant differences in the prevalence of the isolated *Streptococcus* spp. as well as the distribution of *scpB, rib, lmb* virulence genes in representative isolates of *S. agalactiae* and *S. dysgalactiae* from milk, cheese, and human samples. *P* values less than 0.05 were considered statistically significant. Figures were generated using GraphPad Prism 9 software.

Table 1. Oligonucleotide primers' sequences and PCR cycling conditions used in this study

Target gene	Primers' sequences 5\-3\	Amplified product (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Streptococcus* spp. *tuf	GTACAGTTGCTTCAGGACGTATC	197	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[32]
	GCTTCGATTTCATCACGTTG							
Streptococcus* spp. *16S rRNA	CGGGGGATAACTATTGGAAACGATA	912	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	[33]
	ACCTGTCACCCGATGTACCGAAGTA							
S. agalactiae* *16S rRNA	CGCTGAGGTTTGGTGTTTACA	405	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[34]
	CACTCCTACCAACGTTCTTC							
S. dysgalactiae* *16S rRNA	GGGAGTGGAAAATCCACCAT	572	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[20]
	AAGGGAAAGCCTATCTCTAGACC							
S. pyogenes* *16S rRNA	CTA CTT GGA TCA AGA CGG GT	419	95˚C 5 min.	95˚C 1 min.	55˚C 1 min.	72˚C 1 min.	72˚C 5 min	[35]
	TTA GGG TTT CCA GTC CAT CC							
Lmb	AGTCAGCAAACCCCAAACAG	397	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[36]
	GCTTCCTCACCAGCTAAAACG							
ScpB	ACAACGGAAGGCGCTACTGTTC	255	94˚C 5 min.	94˚C 30 sec.	47˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	[37]
	ACCTGGTGTTTGACCTGAACTA							
Rib	CAGGAAGTGCTGTTACGTTAAAC	369	94˚C 5 min.	94˚C 30 sec.	51˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
	CGTCCCATTTAGGGTTCTTCC							

ScpB virulence gene codes for Streptococcal C5a peptidase adhesion (Prevents neutrophils, promotes adherence)
Lmb virulence gene codes for laminin-binding surface proteins (Promote adherence to host laminin)
Rib virulence gene codes for Surface protein Rib. (Resistance to proteases)

Results and discussion

Occurrence of Streptococcus species in milk and cheese sample

In the present study, *Streptococcus* spp. was bacteriologically and biochemically identified in 68.75% of the 160 milk samples examined (Table 2). This finding was lower compared to 74.49% recorded by Farzana *et al.* [38] in Bangladesh, yet it is higher than the percentages reported in numerous prior studies: 12.5% in Algeria [39], 16.47% in China [14], 4% in Egypt [40], 12% in Egypt [41], 16.28% in Egypt [19], 4.5% in Argentina [18], 11.08% in China [42], 39.7% in Egypt [43], and 18.6% in China [44]. The variation in the prevalence of *Streptococcus* spp. may be linked to the extent of contamination of bulk milk samples by bacteria originating from the environment and milking equipment, as well as pathogens present in the milk from affected quarters. Therefore, prompt and accurate identification of mastitis in cattle can prevent the condition or at least reduce the costs associated with its effects, which can worsen with delays [45].

Among the 37 representative *Streptococcus* species isolates from milk subjected to serological identification, the distribution was as follows: *S. agalactiae* constituted 37.83%, *S. dysgalactiae* 21.62%, *S. uberis* 13.51%, *S. pyogenes* 8.11%, and *S. pneumoniae* 2.7% that respectively corresponded to 8.75%, 5%, 3.13%,1.8%, and 0.63% of the total examined milk samples (Table 2). In China, similar isolation rates of *S. agalactiae* were respectively reported at 34.38% and 33.6% by Tian *et al.* [46] and Han *et al.* [47] who calculated the prevalence of *S. agalactiae* in relation to the bacteriologically positive samples in the same manner as the current study. However, lower percentages of 5%, 29%, 1.4%, 24.6%, 16%, 17.2%, 19.3%, 14.7%, 13.3%, 20.1%, and 13.7% were documented respectively by Gianneechini *et al.* [48] in Uruguay, Tenhagen *et al.* [49] in Germany, Abdel Hameed *et al.* [50] in Poland, Amosun *et al.* [51] in Nigeria, El-Mossalami and Hamed [52] in Egypt, Ebrahimi *et al.* [53] in Iran, El-Jakee *et al.* [54], El-Desouky *et al.* [55], Markos *et al.* [56] in Ethiopia, Saed and Ibrahim [57] in Egypt, and Ismail *et al.* [43] in Egypt. In these studies, the prevalence of *Streptococcus* spp. was calculated in relation to the total examined milk samples.

While *S. dysgalactiae* was initially considered to be less virulent than *S. agalactiae*, recent findings have demonstrated its capacity to induce severe infections of the mammary gland. Although some researchers classify *S. dysgalactiae* as an environmental pathogen, it has been shown to persist within the mammary gland, utilizing this site as a reservoir. As a result, it is also classified as a contagious pathogen [58]. The isolation rate of *S. dysgalactiae* from milk in the present study was recorded at 21.62% out of the 37 serologically screened samples that respectively corresponded to 5% of the total examined milk samples. These results were similar to 21.88% recorded by Tian *et al.* [46], and lower than 46.7%, and 23% reported by Markos *et al.* [56] and Saed and Ibrahim [57], respectively. Conversely, lower isolation rates of 4.7%, 12.3%, 7.5%, 7.2%, 6.8%, and 3.12% were documented respectively by Abdel Hameed *et al.* [50], Amosun *et al.* [51], Zhang *et al.* [59], Shen *et al.* [60], Ismail *et al.* [43], and Parasana *et al.* [61].

S. uberis was isolated from milk samples at a percentage of 13.51% out of the 37 serologically screened samples that respectively corresponded to 3.13% of the total examined milk samples (Table 2). This finding is significantly lower than the higher rates of 80%, 55.4%, 45.31%, and 40% reported by Zadoks *et al.* [62], Amosun *et al.* [51], Tian *et al.* [46], and Markos *et al.* [56], respectively. In contrast, this percentage is higher than the 10.6% and 5% reported respectively by Ismail *et al.* [43] and Parasana *et al.* [61].

S. pyogenes is not only associated with the human upper respiratory tract and other soft tissues infection but it has the potential to be transmitted to animals, resulting in sub-acute mastitis, and may subsequently be re-secreted in milk. This reverse zoonosis mechanism could facilitate the spread of infection to other human hosts [63]. *S. pyogenes* is isolated from 8.11% and 7.41% of the examined milk and kariesh cheese samples, respectively (Table 2) compared to 2% and 17% in milk samples examined respectively in Kafr El-Sheikh [64] and Sohage [65].

Kariesh cheese is a traditional soft cheese commonly produced in rural Egyptian farmhouses. This acid dairy product is derived from defatted milk. The traditional production process involves allowing raw milk to coagulate through the action of indigenous microbial flora, followed by the removal of the fat layer. The microbial communities associated with traditional fermented dairy products are complex and have not been thoroughly characterized. Microbial diversity plays a crucial role in determining the sensory properties of traditional cheeses; however, certain members of these intricate communities may present potential health risks [66].

Among the 27 representative *Streptococcus* isolates from kariesh cheese subjected to serological identification, the distribution was as follows: *S. agalactiae* constituted 33.33%, *S. dysgalactiae* 18.52%, *S. uberis* 25.93%, *S. pyogenes* 7.41% (Table 2). *S. agalactiae* were isolated from kariesh cheese in previous studies in Egypt: 16% in Beni Suef [67], 12% in Alexandria [52], and 12% in Menoufia [68]. From the direct comparisons between these studies, there are challengesdue to variations in study design, management systems, streptococcus detection methodologies, and potential breed differences, in addition to the influence of geographical and climatic factors.

Table 2. Results of serological identification of 107 representative bacteriologically and biochemically streptococcus positive samples from different sources

Source	Total examined	Bacteriologically & biochemically +ve Samples [No. (%)] [a]	No. of serologically screened isolates	Serologically identified species [No. (%)] [b]					
				S. agalactiae	*S. dysgalactiae*	*S. pyogenes*	*S. uberis*	*S. pneumonae*	Mixed culture
Milk	160	110 (68.75%)	37	14 (37.83%)	8 (21.62%)	3 (8.11%)	5 (13.51%)	1 (2.7%)	6 (16.22%)
Kariesh cheese	75	52 (69.33%)	27	9 (33.33%)	5 (18.52%)	2 (7.41%)	7 (25.93%)	0 (0%)	4 (14.81%)
Hand swab	40	32 (80%)	20	8 (40%)	2 (10%)	3 (15%)	3 (15%)	2 (10%)	2 (10%)
Pharyngeal swab	40	31 (77.5%)	19	3 (15.79%)	1 (5.26%)	7 (36.84%)	0 (0%)	5 (26.32%)	3 (15.79%)
Vaginal swab	20	9 (45%)	4	4 (100%)	0	0	0	0	0
Total	335	234 (69.85%)	107	38 (35.5%)	16 (14.95%)	15 (14.02%)	15 (14.02%)	8 (7.48%)	15 (14.02%)

[a]: The percentage is calculated in relation to the total examined samples in each source.
[b]: The percentage is calculated in relation to the total serologically screened isolates in each source.

Occurrence of Streptococcus Species in hand, pharyngeal, and vaginal swabs

Among 20 representative *Streptococcus* isolates from 32 bacteriologically and biochemically positive hand swabs, *S. agalactiae* was detected at 40%, while *S. dysgalactiae* and *S. pneumoniae* were found at 10% each, and *S. pyogenes* and *S. uberis* were each identified at 15% (Table 2). El-Gedawy *et al.* [40] reported *S. agalactiae* isolation from 2% of hand swabs of workers examined. Additionally, Ndiaye *et al.* [69] isolated *S. pyogenes* and *S. pneumoniae* from the palm of hands of populations in rural Senegal at 33.6% and 1.95%, respectively, compared to 1.88% and 31.81% recorded by Ndiaye *et al.* [70].

Furthermore, four (44.44%) serologically screened *Streptococcus* isolates from 9 bacteriologically and biochemically positive vaginal swabs were identified as *S. agalactiae* (Table 2). Previous studies documented *S. agalactiae* isolation rates of 7.1% , 13.6% ,18.75% , 13.6% , 15.5% ,20% and 13.7% [71-77] respectively.

Moreover, of the 31 bacteriologically and biochemically positive pharyngeal swabs examined in this study, 19 isolates were serologically screened, revealing the presence of *S. agalactiae* (15.79%), *S. dysgalactiae* (5.26%), *S. pneumoniae* (26.32%), and *S. pyogenes* (36.84%) (Table 2). *S. pyogenes* has previously been isolated from pharyngeal swabs at

rates of 24% ,7.31% , 2.4% and 36% [78-81].

The serological analysis results revealed significant discrepancies among various sources (p<0.05 or p<0.001; Table 2). No bacterial presence was noted in vaginal swab samples except for the full prevalence of *S. agalactiae*. The isolates exhibited significant predominance in milk, cheese, and hand swab specimens. Conversely, *S. pyogenes* isolates were predominantly found in pharyngeal swabs. Significant variations in the prevalence of the examined microbes were observed across all studied samples (p<0.05 or p<0.001; Figure 1). Regarding the bacteriologically and biochemically positive samples [No. (%)], notable differences were identified among the samples (p = 0.0062), with the highest percentages occurring in pharyngeal swabs, cheese, and milk at 77.5%, 69.33%, and 68.75%, respectively, based on the total samples examined in each source (Table 2).

From the results of this study and previous ones, significant disparities between the prevalences of different *Streptococcus* species in different sources were found to depend mainly on the method for identification either phenotypic, serologic and /or genotypic.

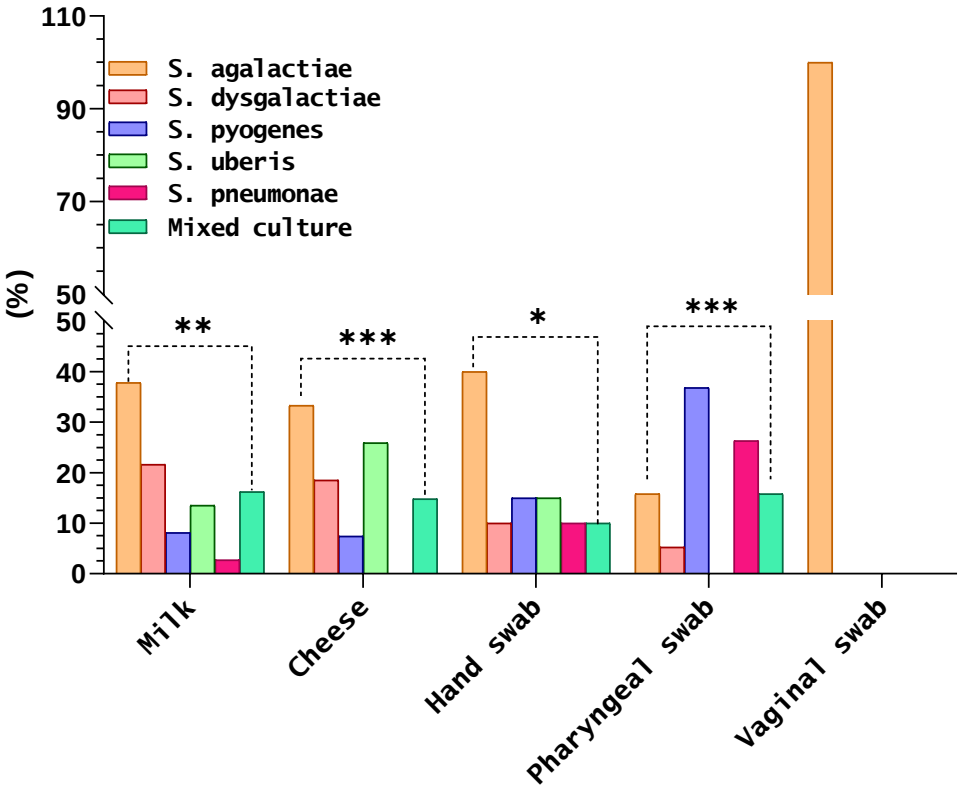

Figure 1. Prevalence of the examined *Streptococcus* species across all studied samples. *p<0.05. **p<0.01 ***p<0.001

Molecular identification of thirty-four representative serologically identified Streptococcus isolates from different sources

The results in Table 3 revealed that thirty-four serologically identified *Streptococcus* isolates from different sources were molecularly identified as (1 *S. agalactiae* and 3 *S. dysgalactiae*) from 4 cheese samples, (7 *S. agalactiae* and 5 *S. dysgalactiae*) from 12 milk samples, (5 *S. agalactiae* and 2 *S. dysgalactiae*) from 7 hand swabs, (3 *S. agalactiae*, 1 *S. dysgalactiae*, and 3 *S. pyogenes*) from 7 pharyngeal swabs, and 4 *S. agalactiae* from 4 vaginal swabs.

In previous studies for molecular confirmation of *Streptococcus* species isolates, Zhang *et al.* [59] confirmed 88 suspected *S. dysgalactiae* isolates from dairy cows with clinical mastitis in China through the application of *16S rRNA* gene sequencing. Kosecka-Strojek *et al.* [24] identified 21 *Streptococcus* species in Poland utilizing Sanger sequencing, achieving identification rates of 90% with *16S rRNA*, 86% with *rpoB*, 62% with *tuf*, and 57% with *sodA*. Abd El-Razik *et al.* [19] reported that 22 out of 28 *S. agalactiae* isolates from milk samples in Egypt were confirmed via PCR targeting the *sklA3* gene. Moreover, Hernandez *et al.* [18] performed species-specific PCR on 68 *S. agalactiae* isolates in Argentina, confirming the presence of the *dltR* gene in 56 strains. Xu *et al.* [82] confirmed all 51 *S. dysgalactiae* subspecies *dysgalactiae* isolates from mastitis cases in China using *16S rRNA* sequencing. Meanwhile, Farzana *et al.* [38] confirmed all 55 *Streptococcus* isolates (comprising 29 *S. agalactiae* and 26 *S. dysgalactiae*) from dairy farms in Bangladesh through species-specific *16S rRNA* analysis. Lastly, Dhital *et al.* [83] identified 73 *Streptococcus* isolates from a total of 984 raw milk samples in Taiwan, detecting species such as *S. uberis* (41%), *S. lutetiensis* (17.8%), and *S. agalactiae* (6.8%).

Table 3. Molecular identification of 34 serologically identified *Streptococcus* species isolates from milk, cheese, and human samples.

Sample code	Source	*Tuf* gene +ve samples	*16S rRNA* positive samples specific for		
			S. agalactiae	*S. dysgalactiae*	*S. pyogenes*
137	Kariesh cheese	+	+		
141	Kariesh cheese	+		+	
172	Kariesh cheese	+		+	
186	Kariesh cheese	+		+	
92	Hand swab	+	+		
127	Hand swab	+	+		
129	Hand swab	+	+		
130	Hand swab	+	+		
204	Hand swab	+		+	
225	Hand swab	+	+		
228	Hand swab	+		+	

32	Milk	+	+			
33	Milk	+	+			
36	Milk	+		+		
38	Milk	+	+			
86	Milk	+	+			
108	Milk	+		+		
117	Milk	+		+		
119	Milk	+		+		
122	Milk	+	+			
212	Milk	+	+			
213	Milk	+	+			
215	Milk	+		+		
136	Pharyngeal swab	+				+
157	Pharyngeal swab	+				+
166	Pharyngeal swab	+				+
241	Pharyngeal swab	+	+			
244	Pharyngeal swab	+	+			
245	Pharyngeal swab	+	+			
257	Pharyngeal swab	+		+		
252	Vaginal swab	+	+			
269	Vaginal swab	+	+			
270	Vaginal swab	+	+			
271	Vaginal swab	+	+			

Occurrence of ScpB, Rib, and Lmb virulence genes in different examined samples

Upon streptococcal infection, virulence factors enable the bacteria to adapt to host environments and develop survival strategies, including biofilm formation, which contributes to disease manifestation. Group B *Streptococcus* (GBS) expresses a variety of surface-associated and secreted virulence factors that facilitate interactions with host cells while inhibiting innate immune responses. The nature of GBS infections is influenced by various virulence genes, such as *gbs67* (promoting adherence and invasion), *cylE* (enhancing invasion of host cells), *cfb* (forming pores in host cell membranes), *scpB* (aiding in adherence and preventing neutrophil access), *lmb* (promoting adherence to laminin), and

pavA (enhancing binding to fibronectin) [84].

In the current study, the virulence genes *scpB*, *rib*, and *lmb* were detected in both screened *S. agalactiae* isolates (100%) from milk and cheese. Additionally, these genes were found in 66.7%, 66.7%, and 100% of the three *S. agalactiae* isolates from various human samples. In contrast, the *scpB* gene was not detected in *S. dysgalactiae* isolates from either milk or cheese but was found in both screened human isolates. The *rib* gene was detected in 0%, 100%, and 100% of the screened *S. dysgalactiae* isolates from milk, cheese, and humans, respectively. Furthermore, the *lmb* gene was found in 100%, 100%, and 50% of the screened *S. dysgalactiae* isolates from milk, cheese, and humans (Table 4). Moreover, Table (4) results clearly demonstrate that there were no significant distinctions among various sources in terms of the presence of *scpB, rib,* or *lmb* virulence genes in the representative isolates of *S. agalactiae* and *S. dysgalactiae* ($p>0.05$). Nonetheless, within each source, nonsignificant variations were noted for *S. agalactiae*. In contrast, noteworthy differences were observed for *S. dysgalactiae* concerning all the examined genes ($p<0.05$ or <0.001). Notably, *scpB* and *Rib* were entirely absent in milk samples, and *scpB* was not detected in the mentioned isolate from cheese samples. Conversely, approximately 50% of the *Lmb* virulence genes were found in human samples as depicted in Figure 2.

In Brazil, Duarte *et al.* [85] detected the *lmb* and *scpB* genes respectively in 7 (8.2%) and 43 (50.5%) out of 85 *S. agalactiae* isolates from milk. Jain *et al.* [37] found the *scpB*, *rib*, and *lmb* genes respectively in 6 (22.2%), 7 (25.9%), and 8 (29.63%) *S. agalactiae* isolates from mastitic cows out of 27 examined. Beigverdi *et al.* [86] identified the *scpB* (97.6%) and *rib* (53.65%) genes in 41 *S. agalactiae* isolates from pregnant women in Iran. In Argentina, Hernandez *et al.* [18] detected the *rib* gene in 59% of 56 *S. agalactiae* isolates recovered from 1500 milk samples; however, the *lmb* and *scpB* genes were not found in any of the isolates. However, in China, *lmb and scpB* genes were respectively detected in 3.33% and 18.33% of 60 *S. dysgalactiae* isolates from 830 milk samples from Holstein cows with clinical mastitis [60]. Additionally, no *lmb* genes were detected in 105 *S. agalactiae* isolates from mastitic cow's milk samples in China [47]. In India, *lmb* and *rib* genes were detected in 85.7% and 38% of 21 *S. agalactiae* isolates recovered from patients with various infections [87]. Furthermore, 105 (95.5%), 30 (27.3%), and 2 (1.8%) out of 110 strains of clindamycin-resistant *S. agalactiae* isolated from two tertiary hospitals in China, harboured the *lmb, rib,* and *scpB* virulence genes, respectively [44]. Lastly, the *lmb* (57.53%) and *scpB* (16.43%) virulence genes were detected in 73 *Streptococcus* species isolated from raw milk samples collected from various Taiwanese dairy farms [84]. Our comparison with studies from different countries suggests that discrepancies may be due to geographical location among other factors such as the sources from which *Streptococcus* spp. has been isolated.

Table 4. Occurrence of *scpB*, *rib*, and *lmb virulence* genes in representative isolates of *S. agalactiae* and *S. dysgalactiae* from milk, cheese, and human samples.

Serotypes	Source	No. screened	Virulence genes [+ve (%)]		
			ScpB	*Rib*	*Lmb*
S. agalactiae	Milk	1	1 (100%)	1 (100%)	1 (100%)
	Cheese	1	1 (100%)	1 (100%)	1 (100%)
	Human	3	2 (66.7%)	2 (66.7%)	3 (100%)
	Total	5	4 (80%)	4 (80%)	5 (100%)
S. dysgalactiae	Milk	1	0%	0%	1 (100%)
	Cheese	1	0%	1 (100%)	1 (100%)
	Human	2	2 (100%)	2 (100%)	1 (50%)
	Total	4	2 (50%)	3 (75%)	3 (75%)
Total		9	6 (66.7%)	7 (77.8%)	8 (88.9%)

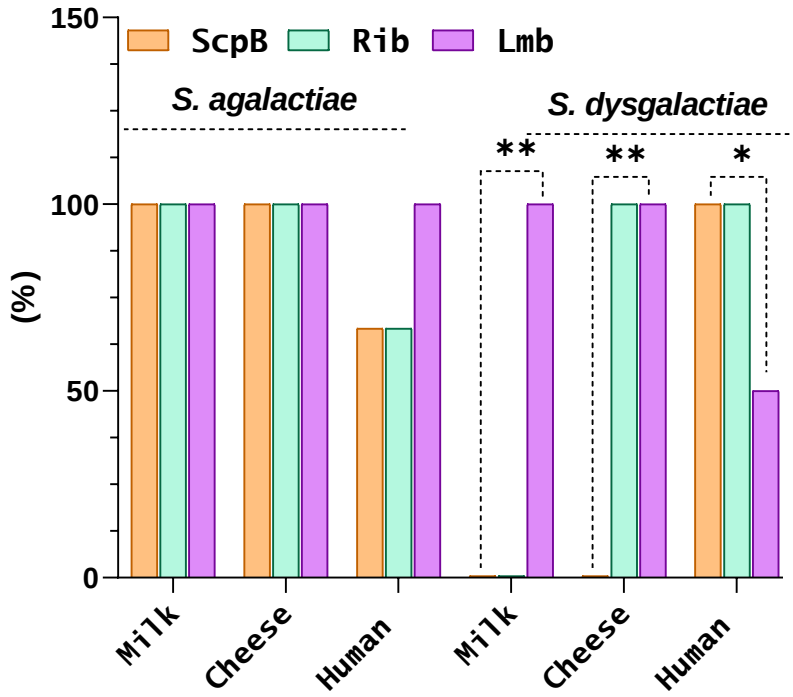

Figure 2: Occurrence of *scpB*, *rib*, and *lmb* virulence genes across different studies isolates

***p<0.05. **p<0.01 ***p<0.001**

Conclusion

In conclusion, *S. agalactiae* and *S. dysgalactiae* were prevalent among milk, cheese of dairy cows as well as hand, pharyngeal, and vaginal swabs of humans in Sharkia Governorate, Egypt suggests the potential for zoonotic transmission. The virulence genes *scpB, rib, lmb were* more prevalent in *S. agalactiae* isolates from milk and cheese than those from humans. On the contrary, *scpB, rib, and lmb* virulence genes were more prevalent in *S. dysgalactiae* isolates from humans than those from milk and cheese.

Conflicts of Interest:

No conflicts of interest for publication of this paper

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الملخص العربى

مدى الانتشار والتوصيف الجزيئي لبعض جينات الضراوة في عزلات ستربتوكوكس أجالاكتيا وستربتوكوكس دسأجالاكتيا المعزولة من الحليب و منتجات الألبان والإنسان في محافظة الشرقية، مصر.

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في هذه الدراسة، تم تحديد أنواع من المكورات العقدية في 68.75% من عينات الحليب، و69.33% من الجبن، و80% من مسحات اليد، و77.5% من مسحات البلعوم، و45% من مسحات المهبل. من عينات الحليب، تم تحديد 37 عزلة من المكورات العقدية :ستربتوكوكس أجالاكتيا (37.83%) ، ستربتوكوكس دسأجالاكتيا (21.62%)، ستربتوكوكس أبريز(13.51%) ، ستربتوكوكس بيوجينز(8.11%) ، وستربتوكوكس نيمونى .(2.7%) أظهر تحليل مسحات اليد :ستربتوكوكس أجالاكتيا (40%)، ستربتوكوكس دسأجالاكتيا (10%) ، ستربتوكوكس نيمونى(10%) ، وكلا من ستربتوكوكس بيوجينز وستربتوكوكس أبريز (15% لكل منهما). بالنسبة لمسحات البلعوم، شملت 19 عزلة :ستربتوكوكس أجالاكتيا(15.79%) ، ستربتوكوكس دسأجالاكتيا (5.26%) ، ستربتوكوكس نيمونى(26.32%) ، وستربتوكوكس بيوجينز .(36.84%) تم تحقيق التأكيد الجزيئي لـ 34 عينة: 1 ستربتوكوكس أجالاكتيا و3 ستربتوكوكس دسأجالاكتيا من الجبن؛ 7 ستربتوكوكس أجالاكتيا و5 ستربتوكوكس دسأجالاكتيا من الحليب؛ 5 ستربتوكوكس أجالاكتيا و2 ستربتوكوكس دسجالاكتيا من مسحات اليد؛ 3 ستربتوكوكس أجالاكتيا و1 ستربتوكوكس دسجالاكتيا و3 ستربتوكوكس بيوجينز من مسحات البلعوم؛ و4 ستربتوكوكس أجالاكتيا من مسحات المهبل. أشارت النتائج إلى أن جينات الضراوة scpB و rib و lmb كانت موجودة في جميع عزلات ستربتوكوكس أجالاكتيا (100%) من الحليب والجبن. في العينات البشرية، تم اكتشاف scpB و rib في 66.7% من العزلات، وتم العثور على lmb في جميعها (100%). كان جين scpB غائبًا في عزلات ستربتوكوكس دسجالاكتيا من الحليب والجبن، ولكنه موجود في العزلات البشرية. لم يتم العثور على جين rib في عزلات الحليب، ولكنه موجود في جميع عزلات الجبن والبشر من ستربتوكوكس دسأجالاكتيا .تم العثور على جين lmb في جميع عزلات الحليب والجبن، ولكن فقط في 50% من عزلات البشر. في الختام، تم اكتشاف ستربتوكوكس أجالاكتيا وستربتوكوكس دسأجالاكتيا بشكل متكرر في منتجات الألبان والمسحات البشرية في محافظة الشرقية، مصر، مما يشير إلى احتمال انتقال العدوى من الحيوانات إلى البشر. كانت جينات الضراوة scpB و rib و lmb أكثر شيوعًا في ستربتوكوكس أجالاكتيا من الحليب والجبن مقارنة بتلك من البشر، بينما كانت هذه الجينات أكثر انتشارًا في ستربتوكوكس دسأجالاكتيا من البشر مقارنة بالحليب والجبن.