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ORIGINAL ARTICLE

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Phenotypic and Genotypic Characterization of *Streptococci* Associated with Clinical Bovine Mastitis

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Abstract

This study was carried out on a total of 550 lactating animals; 310 and 240 cows and buffaloes, respectively which were examined for signs of clinical mastitis (swelling, hotness, redness, and apparent milk change) from different dairy farms and veterinary units located at El-Fayoum Governorate during the period from May 2017 to November 2017. Clinical examination proved that out of these animals**,** a total of 126 animals (87 cattle and 39 buffaloes) were found with clinical mastitis. *Streptococcus* species were recovered from 73 animals including; 29(39.7%) and 44(60.0%) cows and buffaloes, respectively. Furthermore, out of the 73 *Streptococci* isolates recovered from cows and buffaloes; there were 10(13.7%) and 15(20.5%) *S. agalactiae*, 5(6.8%) and 10(23.7%) *S. dysgalactiae*, 8 (10.6%) and 7 (13.7%) *S. uberis*, 3(4.1%) and 10(13.7 %) *E. fecalis* and 3(4.1%) and 2(2.7%) *S. lactarius*, respectively. Anti-microbial susceptibility testing showed that the highest resistance was recorded against penicillin, gentamicin, streptomycin, and doxycycline (100%). Conversely, the highest sensitivity was recorded against ceftriaxone, ciprofloxacin, and sulfamethoxazole-trimethoprim (100%). Biofilm formation capacity was phenotypically assessed on YESCA CR agar medium and showed that all examined *S. agalactiae* and *S. dysgalactiae* were strong biofilm producers, meanwhile, 78%, 50%, and 75% of *S. uberis*, *S. lactarius,* and *E. fecalis* were biofilm positive isolates respectively. Application of PCR technique revealed that enterotoxins producing genes; *sed, seb* were found in 20% and 80% of isolates, in order. Biofilm-associated genes; *fnb*A and *ica*A genes were detected in 90% and 70%, respectively. Resistance genes*; mec*A and *bla*Z, genes were possessed in 90% and 70% of isolates, respectively.

Keywords

Biofilm, Bovine mastitis, Resistance Genes, *Streptococci,* Virulence Genes

1. Introduction

Bovine mastitis is considered to be one of the most important health problems in dairy cattle and one of the most costly diseases to dairy producers. The inflammatory reaction primarily occurs in response to bacterial infection that impairs milk quality **(Petrovski et al., 2006; Halasa et al., 2007).** This disease also accounts for the highest proportion of antimicrobial usage in a dairy farm **(Kuipers et al., 2016; Stevens et al., 2016)** and increase of costs of disease control. It is estimated that 60 to 70% of all antimicrobials used on dairy farms were applied for the prevention and control of mastitis, of which roughly half is related to the treatment of clinical mastitis **(Kuipers et al., 2016; Stevens et al., 2016).**

Bovine mastitis occurs in two different clinical forms; subclinical and clinical mastitis. Clinical mastitis manifests with visible changes to the milk in the form of clots or flakes and clinical signs include fever, redness, pain, and swelling of the udder and lymph nodes **(Adkins and Middleton 2018).** However, some cases of bovine mastitis resolve themselves and most cases resolve after standard antibiotic treatment **(Ruegg, 2017).**

Mastitis in dairy cattle is a globally widespread disease that is responsible for large economic losses each year due to lower milk yield and reduced milk quality **(Thomas et al., 2015).**

Genus *Streptococcus* is one of the most frequently reported etiological causes; where the pathogens are classified as both contagious (e.g., *S. agalactiae*) and environmental (e.g., *S. uberis*) **(Neiwert et al., 2014; Pieterse and Todorov, 2010).** Moreover *S. dysgalactiae*, *S. agalactiae*, and *S. uberis* are common causes of clinical mastitis **(Minst et al., 2012).**

Streptococcal mastitis had been detected increasingly in dairy farms over the last decades. Infection with some strains can induce mild subclinical inflammation whilst others induce severe inflammation and clinical infections of the bovine udder. It represents the leading pathogen in a growing amount of dairy herds **(Kromker et al., 2014; Günther et al., 2016).** *S. uberis* have appeared to be the pathogen causing intra-mammary infections in dairy cattle **(Teklemariam et al., 2015).**

Despite the increasing pressure to reduce antimicrobial use in food-producing animals, antimicrobials still have important benefits for animal health and are still an essential tool in mastitis control programs **(Middleton et al., 2014).** Failure or success of treatment of clinical mastitis depends on several factors including those related to the cow such as age, stage of lactation and clinical mastitis history, and those related to the pathogen such as virulence and antimicrobial susceptibility **(Sol et al., 2000; Taponen et al., 2003; Barkema et al., 2006).** Antimicrobial resistance surveillance studies are imperative to choose the most appropriate therapies and to reduce the risk for further development and spread of antimicrobial resistance through lateral transfer of resistance genes or direct transfer of resistant pathogens. Some information is available on the prevalence of phenotypic antimicrobial resistance in *S. dysgalactiae* **(Bengtsson et al., 2009; McDougall et al., 2014)** though no studies have comprehensively investigated the genotypic antimicrobial resistance in *S. dysgalactiae*.

S. uberis is an important pathogen that has been implicated in bovine mastitis **(Reinoso et al., 2011).** Others, however, have proposed several virulence traits that may be related to the ability of *S. uberis* to cause mastitis due to the ability to form biofilm **(Varhimo et al., 2011).**

'Biofilm' is described as matrix-enclosed microbial accretions that could adhere each to organic or non-organic surfaces. By generating various types of substances, the formation of biofilm is a significant factor in the pathogenesis of several diseases in animals **(Melchior et al., 2006; Costerton and Stoodley, 2004).**

By forming such a structure, bacteria are more likely to survive in an environment that is hostile to them, and after invading an organism, they can be better protected against the action of the host immune system while becoming less sensitive to the activity of antibiotics or disinfectants **(Felipe et al., 2017).** In addition to the ability to produce biofilm, *Streptococcus* spp. possesses other invasiveness factors, as CAMP thing factor **(Reinoso et al., 2011).**
To date several studies have evaluated the ability of *Streptococcus* spp. to produce biofilms in addition to the presence of virulence factors in bacteria isolated from dairy animals suffering from mastitis in different countries **(Boonyayatra et al., 2016; Ebrahimi et al., 2013).**
Molecular diagnostic methods revealed that *Streptococcus* spp. can be subdivided into many different types with different epidemiological properties **(Kromker et al., 2014).**

Despite the severe economic impact because of the high prevalence of *Streptococcus* spp. in many well-managed dairy herds, virulence factors related to pathogenesis were not well understood and constituted a main impediment for the improvement of techniques to manipulate this important mastitis pathogen **(Oliver et al., 1998).** Several putative virulence related genes of *Streptococcus* spp. have been described. Among these, adherence to and invasion of epithelial cells mediated by *S. uberis* specific adhesion molecule (SUAM) **(Almeida et al., 2013)** and CAMP factor **(Jiang et al., 1996)** have been found.

The aim of this work was to determine the incidence rate of Streptococcus infection among mastitic cows and buffaloes among dairy farms based on both phenotypic and genotypic assays. Also, detection of some phenotypic virulence characteristics and some putative virulence associated genes in the isolated *Streptococcus* strains towards a better understanding of their pathogenesis and epidemiology as mastitis causing pathogens. Additionally, antibiotic susceptibility of the isolated *Streptococcus spp.* was investigated using disk diffusion method.

2. Materials and Methods

2.1. Animals

A total of 550 lactating animals; 310 cows and 240 buffaloes from different dairy farms and veterinary units located in El-Fayoum Governorate were examined for signs of clinical mastitis (swelling, hotness, redness and apparent milk change) during the period from May 2017 to November 2017. The udder of each animal was examined according to the guidelines of the National Mastitis Council before sampling for the presence of clinical mastitis.

2.2. Milk Samples Collection

A total of 126 mastitis milk samples were collected aseptically from clinically mastitic animals; 87 cows and 38 buffaloes. The sampling procedure was performed as recommended by **Quinn et al., (2002)** for aseptic collection of milk samples.

2.3. Cultivation of Milk Samples

Fresh milk samples were centrifuged at 3000 rpm for 15 min. The cream layer and supernatant fluid were discarded. Loopful from the sediment was taken and cultivated into tryptone soya broth then incubated at 37°C for 18-24 hrs. Then, loopful from turbid tryptone soya broth was streaked onto sodium azide crystal violet blood agar (Oxoid) and MacConkey's bile salt lactose agar media (Oxoid) and incubated at 37°C for 24-48 hrs.

2.4. Morphological Examination (Quinn et al., 2002)

Suspected *Streptococci* isolates were identified primary as Gram-positive cocci arranged either singly or in chains like. Pure isolates were subjected to further biochemical identification.

2.5. Biochemical Identification of *Streptococcus spp.*

Biochemical tests as catalase, aesculin hydrolysis CAMP test, and sugar fermentation tests beside other colonial characteristics; hemolysis on 5% sheep blood agar and growth on MacConkey agar according to **Quinn et al., (2002)** and confirmed by VITEK-2-compact-SYSTEM.

2.6. Antimicrobial Susceptibility Testing of the Isolates

All isolates were examined for their antimicrobial sensitivity (AMS) to 14 different antimicrobial discs. The used antimicrobial discs (Oxoid, Basing Stoke, UK) were illustrated in **Table (1).** The disc diffusion technique was applied according to the Clinical and Laboratory Standards Institute **(CLSI, 2018).**

Table (1). Interpretation values of growth inhibition zone of disc used in the antimicrobial susceptibility testing of the isolates.

Antimicrobial Class	Antimicrobial Type	Symbol	Disc Content (µg)	Interpretation		
				susceptible	intermediate	resistant
β –lactams	Penicillins	Ampicillin	AM	10	≥ 29	-	≤ 28
		Amoxicillin	AML	10	≥ 20	11-19	≤ 10
	β – lactamase stable	Amoxicillin – clavulanic acid	AMC	30	20	-	19
	Cephalosporines	Ceftriaxone	CTR	30	23	20-22	19
	Fluoroquinolones	Ciprofloxacin	CIP	5	21	16-20	15
		Ofloxacin	OFX	5	18	15-17	14
	Lincosamides	Clindamycin	DA	2	21	15-20	14
	Aminoglycosides	Gentamycin	GEN	10	15	13-14	12
		Kanamycin	K	30	18	14-17	13
		Apramycin	APR	15	17	15-16	14
		Streptomycin	S	10	15	12-14	11
	Potentiated Sulphonamides	sulfamethoxazole-trimethoprim	STX	1.25/23.75	16	11-15	10
	Fosfomycin	Fosfomycin	FO	200	16	13-15	12
	Tetracycline	Doxycycline	DO	30	16	13-15	12

2.7. Detection of Slime Production on YESCA CR Agar Plate (Zhou et al., 2013)

The bacterial colonies were examined for the biofilm formation by streaking onto YESCA Congo red agar (CRA) and incubated at 37°C for 24 hrs. The red color of bacterial colonies indicated positive biofilm formation, while the pink or white of the bacterial colonies indicated negative biofilm formation.

2.8. Polymerase Chain Reaction for Detection of Some Selected Genes

PCR was applied on 10 selected different *Streptococcus* isolates to determine 6 genes; 2 enterotoxins producing genes (*sed* and *seb*), 2 biofilm-associated genes (*ica*A and *fnb*A) and 2 resistance genes (*mec*A and *bla*Z). The primer sequences, size of generated products and cycling conditions used in PCR amplification were illustrated in **Tables (2, 3).**

Table (2): Oligonucleotide primers of some selected genes used in PCR.

Primers			Primer sequence (5'-3')	Amplified product	Reference
Enterotoxin genes	*Sed*	F	*CCAATAATAGGAGAAAATAAAAG*	278 bp	Mehrotra et al., (2000)
		R	*ATTGGTATTTTTTTTCGTTC*		
	Seb	F	*GTATGGTGGTGTAACTGAGC*	164 bp	
		R	*CCAAATAGTGACGAGTTAGG*		
Biofilm genes	*ica*A	F	*CCT AAC TAA CGA AAG GTA G*	1315 bp	Ciftci et al., (2009)
		R	*AAG ATA TAG CGA TAA GTG C*		
	*fnb*A	F	*CAT AAA TTG GGA GCA GCA TCA*	127 bp	Vancraeynest et al., (2004)
		R	*ATC AGC AGC TGA ATT CCC ATT*		
Resistance genes	*mec*A	F	*GTA GAA ATG ACT GAA CGT CCG ATA A*	310 bp	McClure et al., (2006)
		R	*CCA ATT CCA CAT TGT TTC GGT CTA A*		
	*bla*Z	F	*ACTTCAACACCTGCTGCTTTC*	173 bp	Duran et al., (2012)
		R	*TGACCACTTTTATCAGCAACC*		

Table (3). Cycling conditions of the different primers during PCR.

Gene	Primary denaturing	secondary denaturing	Annealing	Extension	No. of cycles	Final extension
Sed	94˚C / 5 min.	94˚C / 30 sec.	57˚C / 40 sec.	72˚C / 45 sec.	35	72˚C / 10 min.
Seb	94˚C / 5 min.	94˚C / 30 sec.	57˚C / 40 sec.	72˚C / 45 sec.	35	72˚C / 10 min.
*ica*A	94˚C / 5 min.	94˚C / 30 sec.	49˚C / 1 min.	72˚C / 1 min.	35	72˚C / 12 min.
*fnb*A	94˚C / 5 min.	94˚C / 30 sec.	50˚C / 30 sec.	72˚C / 30 sec.	35	72˚C / 7 min.
*mec*A	94˚C / 5 min.	94˚C / 30 sec.	50˚C / 30 sec.	72˚C / 30 sec.	35	72˚C / 7 min.
sta	94˚C / 5 min.	94˚C / 30 sec.	54˚C / 30 sec.	72˚C / 45 sec.	35	72˚C / 7 min.

3. Results

3.1. Prevalence and Distribution of *Streptococcus* Isolates in Clinically Mastitic Animals

Out of 126 collected milk samples, a total of 73 *Streptococcus spp*. were recovered with a total prevalence of 57.9%; distributed as 29(39.7%) and 44(60.3%) isolates from clinically mastitic cows and buffaloes, respectively **(Table, 4).**

3.2. Identification and Distribution of *Streptococcus* Isolates

Streptococcus isolates (*n*=73) were identified using traditional methods including morphological, colonial and biochemical characteristics **(Table, 5)** and further identification by VITEK-2- COMPACT-SYSTEM®.

3.3. Prevalence and Distribution of *Streptococcus* Isolates among Examined Animals

The results represented that out of 73 *Streptococcus* spp. isolates from cows and buffaloes; there were 10(13.7%) and 15(20.5%) *S. agalactiae*, 5(6.8%) and 10(23.7%) *S. dysgalactiae*, 8(10.6%) and 7(13.7%) *S. uberis*, 3(4.1%) and 10(13.7 %) *E. fecalis* and 3(4.1%) and 2(2.7%) *S. lactarius*, respectively **(Table, 6).**

3.4. Results of Antimicrobial Susceptibility Test of *Streptococcus* Isolates using Disc Diffusion Method

All isolates were tested for *in-vitro* susceptibility test against 14 antimicrobial agents of 9 different antimicrobial classes of important veterinary significance. The results showed high resistance to the tested antimicrobials with varying degrees. Penicillins, gentamicin, streptomycin, and doxycycline had the highest resistance (100%), followed by kanamycin and fosfomycin. In contrast, ceftriaxone, ciprofloxacin, and sulfamethoxazole-trimethoprim of the tested *Streptococcus* isolates had the highest sensitivity (100%), followed by ofloxacin, clindamycin, and apramycin.

3.5. Prevalence of Biofilm Formation among Isolated *Streptococci*

All examined *S. agalactiae* and *S. dysgalactiae* were strong biofilm producers using YESCA CRA medium, meanwhile, 78%, 50% and 75% of *S. uberis*, *S. lactarius* and *E. fecalis* were biofilm positive isolates respectively **(Table, 7).**

3.6. Results of PCR for Detection of Virulence and Resistance Genes in *Streptococcus* Isolates

As shown in **Table (8)** and **Figs. (1-6),** the results of PCR revealed that enterotoxins producing genes; *sed* and *seb*, were found in 20% and 80% of the tested isolates, respectively **(Figs. 1, 2).** Biofilm-associated genes; *fnb*A and *ica*A, were found in 90% and 70%, respectively **(Figs. 3, 4).** Resistance genes; *mec*A *and bla*Z, were possessed in 90% and 70% of the tested isolates, respectively **(Figs. 5, 6).**

Table (4). Prevalence and distribution of *Streptococcus* isolates among the clinically mastitic dairy animals.

Collected milk samples		*Streptococcus* isolates *S. aureus*	
Examined animals	No. of samples	No.	%
Cattle	87	29	39.7
Buffaloes	39	44	60.3
Total	126	73	57.9

%: was calculated according to the corresponding number (No.) of samples.

Table (5). Results of colonial and biochemical identification of *Streptococci spp.*

Streptococcus spp.	*S. agalactiae*	*S. dysgalactiae*	*S. uberis*	*E. fecalis*	*S. lactarius*
Catalase test	-	-	-	-	-
Aesculin hydrolysis	-	-	+	+	-
CAMP test	+	-	-	-	-
Hemolysis	+(β)	+(β)	(α)	- (γ)	+ (α)
Growth on MacConkey	-	-	-	+	-
Inulin Fermentation	-	-	+	-	-
Lactose Fermentation	+	+	+	+	-
Mannitol Fermentation	-	-	+	+	-
Trehalose Fermentation	+	+	+	+	+
Sorbitol Fermentation	-	-	+	+	-

Table (6). Distribution of Streptococcus spp. among examined animals.

Milk samples from examined animals	*S. agalactiae*		*S. dysgalactiae*		*S. uberis*		*E. fecalis*		*S. lactarius*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cattle	10	13.7	5	6.8	8	10.6	3	4.1	3	4.1
Buffaloes	15	20.5	10	13.7	7	13.7	10	13.7	2	2.7
Total (73)	25	34.2	15	20.5	15	20.5	13	17.8	5	6.8

Table (7). Results of biofilm formation among *Streptococcus* isolates.

Streptococcus isolates	No. of examined isolates	The percentage of biofilm-forming bacteria	
		No.	%
S. agalactiae	6	5	100
S. dysgalactiae	17	17	100
S. uberis	23	18	78
S. lactarius	11	5	50
E. fecalis	16	12	75
Total	73	57	78

Table (8). Detection of the virulence genes in *Streptococcus* spp. isolated from clinical mastitic animals.

Tested *Streptococcus* Spp. (No.)	Enterotoxins				Biofilm				Resistance			
	sed		*seb*		*ica*A		*fnb*A		*mec*A		*bla*Z	
S. agalactiae (1)	0	0%	1	100%	0	0%	1	100%	1	100%	0	0%
S. dysgalactiae (3)	2	66.7%	3	100%	3	100%	2	66.7%	2	66.7%	2	66.7%
S. uberis (3)	0	0%	2	66.7%	3	100%	3	100%	3	100 %	3	100 %
E. fecalis (3)	0	0%	2	66.7%	1	33.3%	3	100%	3	100 %	2	66.7%
Total (10)	2	20	8	80	7	70	9	90	9	90	7	70

%: was calculated according to the corresponding number of tested isolates.

Fig (1). Agarose gel electrophoresis showing the amplification of sed gene at amplicon of 278 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (2, 3): A positive result.

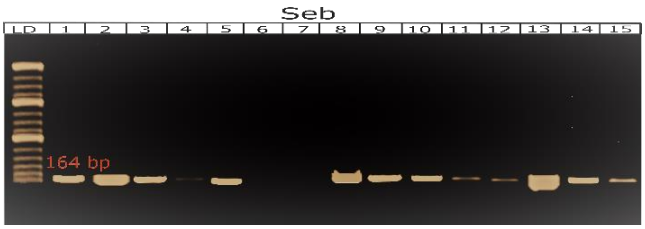

Fig (2). Agarose gel electrophoresis showing the amplification of Seb gene at amplicon of 164 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (1-3, 5, 8-10): A positive result.

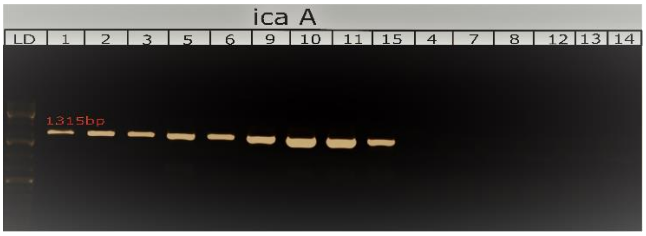

Fig (3). Agarose gel electrophoresis showing the amplification of icaA gene at amplicon of 131 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (1-3, 5-6, 9-10): A positive result.

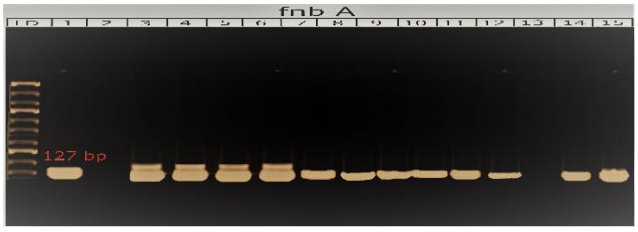

Fig (4). Agarose gel electrophoresis showing the amplification of fnbA gene at amplicon of 127 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (1, 3-10): A positive result.

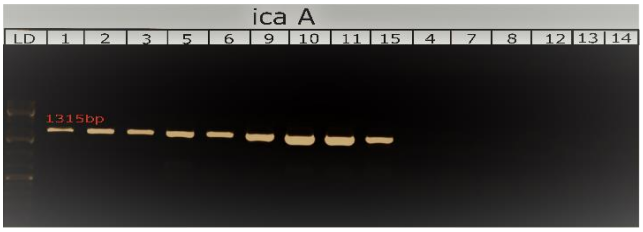

Fig. (5). Agarose gel electrophoresis showing the amplification of mecA gene at amplicon of 310 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (1, 3-10): A positive result.

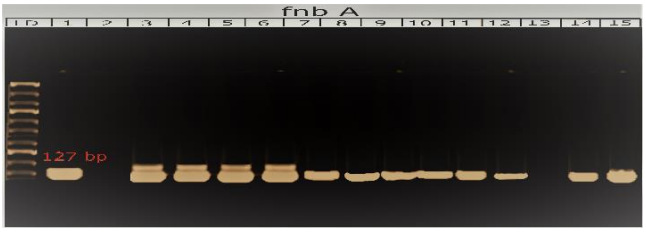

Fig. (6). Agarose gel electrophoresis showing the amplification of blaZ gene at amplicon of 173 bp. *LD: Molecular size ladder *Pos: Control positive *Lane (2-3, 5-9): A positive result.

4. Discussion

Streptococcus is a worldwide pathogen that causes intra-mammary infections in dairy cattle. *Streptococcus Spp.* has been described as an opportunistic pathogen that utilizes nutritional flexibility to adapt to a range of ecological positions, including the mammary gland **(Ward et al., 2009; Collado et al., 2016).** *S. agalactiae* was first identified in 1887 as a pathogen causing the mastitis **(Chen, 2019).**

Results of the prevalence and distribution of *Streptococcus* isolates from clinical mastitic animals presented that, out 73 of *Streptococcus* isolates; 29(39.7%) and 44(60.0%) isolates were recovered from clinically mastitic cows and buffaloes, respectively. This prevalence differs from that obtained by **Tariq et al., (2021)** who reported clinical mastitis in cattle as (20%) compared to buffaloes (11%) and in line with **Mustafa et al., (2011)** and **Abera et al., (2012)** who reported a prevalence of 40% and 30.3% of clinical mastitis in buffaloes and cattle, respectively.

Such variation in the prevalence might be attributed to the difference in herd management and the level of hygiene. Several hygienic and management practices can decrease the incidence as teat dipping before and after milking, washing milkers' hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method as well as treating clinical cases at early stage of infection **(Teklemariam et al., 2015).** The prevalence and distribution of *Streptococcus* spp. among examined animals cleared that, among the 73 *Streptococci* isolated in this study 15(20.5%), 25(34%), 15(20.5%), 13(17.8%) and 5(6.8%) were *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *E. fecalis* and *S. lactarius* respectively based on biochemical examination and confirmation by VITEK-2-compact- system.

The prevalence rate was much lower than the 72.3% isolation of *S. dysgalactiae* from milk samples obtained by **Bi et al., (2016).** The prevalence of *S. dysgalactiae* from cases of clinical mastitis in our study was higher than those reported by **Gao et al., (2017),** 10.5% from clinical mastitis in China, **Whist et al., (2007),** 12.1% in Norway and **Leelahapongsathon et al., (2014)** 4.0% in Thailand. Regarding, *Streptococcus uberis* **(Bhat et al., 2017);** reported 4.35%.
Our results disagreed with the results of **(Amosun et al., 2010, Adesola 2012; Kia et al., 2014)** who reported that, the *S. uberis* strains were 48 out of 74 *Streptococcus spp.* (64.9%).

The results of antimicrobial susceptibility test of Streptococci isolates using disc diffusion method cleared that, high resistances were recorded against the tested antimicrobials with variable degrees. *Streptococcus* isolates showed complete resistances were recorded against penicillins, gentamicin, streptomycin and doxycycline while high resistances were found against kanamycin and fosfomycin. Conversely, complete sensitivities were recorded against ceftriaxone, ciprofloxacin and sulfamethoxazole-trimethoprim while high sensitivities were found against ofloxacin, clindamycin and apramycin.
This was not surprising as kanamycin and streptomycin are mainly active against Gram-negative bacteria and *S. dysgalactiae* belongs to the group of Gram-positive bacteria. An intrinsic resistance for most of Streptococcus spp. has also been described for sulfonamide and aminoglycosides **(Porter and Kaplan, 2011).** In contrast, in a study of bovine *S. dysgalactiae* isolates from New Zealand, the resistance to trimethoprim/sulfamethoxazole was only 17% **(McDougall et al., 2014).**

The enzymes and toxins produced by bacteria or the ability of bacteria to produce biofilm help microorganisms survive in infected tissues either through direct impact on host stromal cells or by affecting host defense mechanisms.

Our results on the prevalence of biofilm formation among isolated *Streptococci* cleared that all examined *S. agalactiae* and *S. dysgalactiae* were biofilm producing streptococci using YESCA agar medium, meanwhile, 78%, 50% and 75% of *S. uberis*, *S. lactarius* and *E. fecalis* were biofilm positive isolates respectively. This results matches with **(Ebrahimi et al., 2013)** in Iran who reported that among 31 *S. agalactiae* isolates, 28(90.3%) of strains were biofilm producers. Also, our results of molecular detection of virulence and resistance genes in *Streptococcus* isolates cleared that, ten different selected isolates of streptococci; 1 *S. agalactiae*, 3 *S. dysgalactiae*, 3 *S. uberis* and 3 *E. fecalis* were screened by RT-PCR and the results showed that enterotoxins producing genes; *sed* gene and *seb* gene were found in 20% and 80%, respectively. Biofilm-associated genes; *fnb*A and *ica*A genes were presented in 90% and 70% respectively. Resistance genes; *mec*A and *bla*Z genes were possessed in 90% and 70%.

5. Conclusion

Streptococcus species was recovered in a percentage of 57.9% from totally examined mastitis milk samples. Out of them; *S. agalactiae* isolated in (13.7%) and (20.5%) from cows and buffaloes, *S. dysgalactiae* (6.8%) and (23.7%), *S. uberis* (10.6%) and (13.7%), *E. fecalis* (4.1%) and (13.7%) and *S. lactarius* (4.1%) and (2.7%), respectively. Most of isolates were highly resistant to penicillin, gentamicin, streptomycin and doxycycline and highly sensitive to ceftriaxone, ciprofloxacin and sulfamethoxazole-trimethoprim. Biofilm formation capacity showed that *S. agalactiae* and *S. dysgalactiae* were strong biofilm producers, meanwhile, 78%, 50% and 75% of *S. uberis*, *S. lactarius* and *E. fecalis* were biofilm positive isolates respectively. Molecular detection of virulence genes revealed presence of *sed* gene and *seb* gene in 20% and 80%, respectively. Biofilm-associated genes; *fnb*A and *ica*A genes presented in 90% and 70% respectively. Resistance genes; *mec*A and *bla*Z genes possessed in 90% and 70%.

6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

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