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Antibacterial Resistance and Bacterial Load in Milk Exposed to Dog Saliva in Sudan

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ABSTRACT

Background and Objective: Dogs saliva contains several types of bacteria, some of which are pathogenic to humans. Dogs contribute to many zoonotic diseases that may be transmitted by saliva. Dog bites and dog scratches are common sources of diseases among humans, particularly in children who use to play with dogs. This study aimed to identify and characterize bacteria present in dog saliva, quantify bacterial load in milk contaminated by dog saliva and assess bacterial susceptibility to twelve antibiotics. **Materials and Methods:** Two experiments were conducted: The first involved 150 swabs of police dog saliva in Bori and the second used 150 milk samples mixed with dog saliva from various areas. Bacteria were cultured on multiple media and tested against several antibiotics, with inhibition zones measured to determine sensitivity, intermediate response, or resistance. **Results:** Five bacterial species were identified: *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., *Gemella morbillorum* and *Bacillus* spp. *Staphylococcus aureus* was the predominant species. Co-trimoxazole showed the highest effectiveness, with 81.7% sensitivity, followed by Ciprofloxacin at 73.3%. The bacterial load increased with extended licking, with the highest counts in Gandahar and the lowest in Kafori. **Conclusion:** This study concludes that *Staphylococcus aureus*, is predominant in dog saliva, with bacterial load increasing as milk licking recurs. Co-Trimoxazole and Ciprofloxacin are the preferred treatments.

KEYWORDS

Dog saliva, bacterial load, antibiotic susceptibility, *Staphylococcus aureus*, co-trimoxazole, ciprofloxacin

INTRODUCTION

The interaction between humans and dogs is widespread, with dogs serving multiple roles, from pets to service animals. This close relationship poses potential health risks due to the transmission of pathogens via saliva. Previous studies have identified a diverse array of bacterial species in dog saliva. Key studies, such as those by Toth *et al.*[1] and Veir and Lappin[2], have documented the presence of *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp. and various Gram-negative bacteria. These studies highlight the potential for pathogenic bacteria to be present in dog saliva, which can pose a risk if transmitted to humans. Research by Johler *et al.*[3] indicated that food items contaminated with dog saliva could harbor high levels of bacteria, leading to food-borne illnesses. This is particularly concerning when considering dairy products, such as milk, which can provide an ideal growth medium for bacteria.

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Staphylococcus species, particularly *Staphylococcus aureus* and *Staphylococcus intermedius*, have been extensively studied due to their ability to cause a range of infections in humans and animals. Studies by Grudlewska-Buda *et al.*[4] and Cuny *et al.*[5] have shown that these bacteria can be resistant to multiple antibiotics, posing significant challenges in clinical settings. The presence of bacteria in dog saliva and their subsequent transfer to food items has been a topic of research, especially concerning the safety of consuming food contaminated by dog saliva. This study aims to isolate and characterize bacteria from dog saliva, quantify bacterial load in milk contaminated by dog saliva and assess the antibiotic susceptibility of isolated bacteria.

MATERIALS AND METHODS

Study area: Conducted across various locations (Tuti Island, Gandahar, EL-Thawrah, EL-Salha, Kafori, EL-Shuhada and Bori) in Khartoum from November, 2018 to November, 2021.

Study design: A descriptive cross-sectional study was conducted over three years, analyzing samples for bacterial contamination and antibiotic susceptibility.

Microbiological cultures and bacterial isolates

Sample collection: One hundred and fifty swabs from police dogs' saliva were collected at the police dog center in Bori. One hundred and fifty milk samples were mixed with dog saliva from domestic dogs in areas such as Elthawrah, Tuti Island, Gandahar, Elsalha, Kafori and Elshuhada.

Primary culture: Samples were cultured on blood agar and nutrient agar under aerobic conditions. Visual and microscopic examination for bacterial growth and characteristics[6-8].

Bacterial identification and count: Bacteria identified using conventional bacteriological methods and Colony-Forming Unit (CFU) counts[9].

Identification by biochemical tests[7]

For gram-positive bacteria:

- **Catalase test:** A culture sample was placed on a slide with a drop of 3% hydrogen peroxide[10]. The presence of gas bubbles indicated a positive result
- **Coagulase test:**
  - **Slide test:** A bacterial suspension in normal saline was mixed with undiluted human plasma[11]. Clumping within 5-10 sec indicated a positive result
  - **Tube test:** A diluted plasma sample was mixed with a broth culture and incubated at 37°C overnight[12]. Clot formation indicated a positive result
- **Voges-Proskauer test:** This test differentiates between *Staphylococcus aureus* and *Staphylococcus intermedius*. The broth media was inoculated, incubated and then treated with alpha-naphthol and KOH[10]. The development of a red color indicated a positive result
- Staining techniques[13]

Differentiation between bacteria: Novobiocin (5 µg/disk), basitracin (10 International Units) and optochin (5 µg/disk) disks were obtained from Sigma-Aldrich (Merck) and were used to differentiate bacterial species and observe hemolysis in blood agar[14]. The presence or absence of spores and colony pigmentation were also noted.

Table 1: Standard zone of inhibition to different antibiotics

Antibiotic disk	Disk potency	Zone of inhibition (diameter in mm)		
		Resistant	Intermediate	Sensitive
Linezolid (LZ)	30 mcg	20 or less	-	21 or more
Ciprofloxacin (CIP)	5 mcg	15 or less	16-20	21 or more
Roxithromycin (RF)	15 mcg	9 or less	10-20	21 or more
Ampicillin (AS)	10 mcg	11 or less	12-14	15 or more
Cefotaxime (CF)	30 mcg	14 or less	15-22	23 or more
CoTrimexothazol (BA)	25 mcg	10 or less	11-15	16 or more
Tetracycline (TE)	30 mcg	14 or less	15-18	19 or more
Cephalexin (PR)	30 mcg	14 or less	15-17	18 or more
Cloxacillin (CX)	5 mcg	10 or less	11-12	13 or more
Gentamicin (GM)	10 mcg	12 r less	13-14	15 or more
Levofloxacin (LE)	5 mcg	13 or less	14-16	17 or more
Lincomycin (LM)	2 mcg	14 or less	15-20	21 or more

Source: Company of Axium Laboratories, Paharganj, New Delhi

Table 2: Percentages of gender of dogs from all areas

Gender	Frequency	Percentage
Male	162	54
Female	138	46
Total	300	100

Antimicrobial susceptibitity: Antibiotic susceptibility tested against twelve antibiotics, including Ampicillin, Co-Trimoxazole, Cephalexin, Tetracycline, Cefotaxime, Ciprofloxacin, Levofloxacin, Gentamycin, Lincomycin, Linezoled, Roxithromycin and Cloxacillin. Pure cultures from nutrient agar were inoculated into nutrient broth and incubated until light turbidity appeared. The suspension was spread on Mueller-Hinton agar and antibiotic discs were placed on the medium. The plates were incubated at 37°C for 18-24 hrs and the zones of inhibition were measured to determine sensitivity or resistance[15]. The standard zone of inhibition to different antibiotics was shown in Table 1. The antibiotic disks and their corresponding zones of inhibition were used to classify bacteria as resistant, intermediate or sensitive based on a standard interpretative chart.

RESULTS

In the study samples were collected from male and female dogs of different ages, the males had the highest percentage as shown in Table 2.

One hundred and fifty milk mixed with saliva samples were collected from different areas including Tuti Island, Elshuhada, Elthawrah, Kafori, Gandahar and Elsalha and 150 swab samples were collected from the saliva of police dogs from Bori in Khartoum state shown in Table 3-9.

Staphylococcus aureus had the highest percentage in all areas. In Tuti Island, *S. aureus* had the highest percentage. In Gandahar, *S. aureus* and *B. lentus* were the highest percentages. In Elthawrah and Elsalha, *S. aureus* and *S. intermedius* were the highest percentages. In Kafori, *S. aureus* and *B. badius* were the highest percentages. In Elshuhada,*S. aureus* and *S. sciuri* were the highest percentages. In the Police Dogs Center in Bori, *S. aureus* and *S. intermedius* had the highest percentage. *Staphylococcus aureus* was the highest percentage (Table 3-10).

The count of CFU bacteria due to licking milk three times was carried out for studied bacteria (Table 11). The highest load of bacteria was in samples which were collected from Gandahar (lick one was 7.0×10^6, lick two was 8.5×10^6 and lick three was 9.2×10^6), while the lowest load of bacteria was in samples which were collected from Kafori (lick one was 1.2×10^6, lick two was 2.3×10^6 and lick three was 3.4×10^6).

Table 3: Types of bacteria isolated from milk mixed with saliva of dogs at Tuti Island

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
1	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
2	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
3	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
4	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
5	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
6	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
7	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
8	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
9	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
10	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
11	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
12	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
13	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
14	G+ve Rods	+ve	-ve	+ve	Motile	*Bacillus*
15	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
16	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
17	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
18	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
19	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
20	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*

VP: Voges Proskauer

Table 4: Types of bacteria isolated from milk mixed with saliva of dogs at Gandahar

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
21	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
22	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
23	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
24	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
25	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
26	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
27	G+ve Rods	+ve	-ve	+ve	Motile	*Bacillus*
28	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
29	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
30	G+ve Rods	+ve	-ve	+ve	Motile	*Bacillus*
31	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
32	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
33	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
34	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
35	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
36	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
37	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
38	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
39	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
40	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
41	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
42	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
43	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
44	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
45	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
46	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
47	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
48	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
49	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
50	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
51	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
52	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
53	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
54	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
55	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
56	G+ve cocci	-ve	-ve	-ve	Non motile	*Gemella morbillorum*
57	G+ve cocci	-ve	-ve	-ve	Non motile	*Gemella morbillorum*
58	G+ve cocci	+ve	+ve	-ve	Non motile	*Micrococcus*
59	G+ve cocci	-ve	-ve	-ve	Non motile	*Gemella morbillorum*
60	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*

Table 5: Types of bacteria isolated from milk mixed with saliva of dogs at Elthawrah

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
61	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
62	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
63	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
64	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
65	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
66	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
67	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
68	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
69	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
70	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
71	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
72	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
73	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
74	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
75	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
76	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
77	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
78	G+ve cocci	+ve	-ve	-ve	Non motile	*Streptococcus*
79	G+ve cocci	+ve	-ve	-ve	Non motile	*Streptococcus*
80	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
81	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*

Table 6: Types of bacteria isolated from milk mixed with saliva of dogs at Elsalha

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
82	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
83	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
84	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
85	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
87	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
88	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
89	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
90	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
91	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
92	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
93	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
94	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
95	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
96	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
97	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
98	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
99	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
100	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
101	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
102	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
103	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
104	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
105	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
106	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
107	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
108	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
109	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
110	G+ve Rods	+ve	-ve	-ve	Motile	Bacillus
111	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
112	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
113	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
114	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
115	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
116	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*

Table 7: Types of bacteria isolated from milk mixed with saliva of dogs at Kafori

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
117	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
118	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
119	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
120	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
121	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
122	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
123	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*
124	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
125	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
126	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
127	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
128	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
129	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
130	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
131	G+ve Rods	+ve	-ve	-ve	Motile	*Bacillus*

Table 8: Types of bacteria isolated from milk mixed with saliva of dogs at Elshuhada

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
132	G+ve cocci	+ve	+ve	+ve	Non motile	*Micrococcus*
133	G+ve cocci	+ve	+ve	+ve	Non motile	*Micrococcus*
134	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
135	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
136	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
137	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
138	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
139	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
140	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
141	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
142	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
143	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
144	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
145	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
146	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
147	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
148	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
149	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
150	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*

Table 9: Types of bacteria isolated from saliva of dogs at Bori

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
1	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
2	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
3	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
4	G+ve cocci	+ve	+ve	+ve	Non motile	*Micrococcus*
5	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
6	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
7	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
8	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
9	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
10	G+ve cocci	+ve	+ve	+ve	Non motile	*Micrococcus*
11	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
12	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
13	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
14	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
15	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
16	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
17	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*

Table 9: Continue

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
18	G+ve Rods	+ve	+ve	-ve	Motile	*Bacilllus*
19	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
20	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
21	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
22	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
23	G+ve Rods	+ve	+ve	+ve	Motile	*Bacilllus*
24	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
25	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
26	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
27	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
28	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
29	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
30	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
31	G+ve Rods	+ve	+ve	-ve	Motile	*Bacilllus*
32	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
33	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
34	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
35	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
36	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
37	G+ve cocci	+ve	+ve	-ve	Non motile	Micrococcus
38	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
39	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
40	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
41	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
42	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
43	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
44	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
45	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
46	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
47	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
48	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
49	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
50	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
51	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
52	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
53	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
54	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
55	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
56	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
57	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
58	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
59	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
60	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
61	G+ve cocci	+ve	-ve	+ve	Non motile	*Micrococcus*
62	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
63	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
64	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
65	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
66	G+ve cocci	+ve	-ve	-ve	Non motile	*Micrococcus*
67	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
68	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
69	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
70	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
71	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
72	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
73	G+ve Rods	+ve	+ve	+ve	Motile	*Bacilllus*

Table 9: Continue

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
74	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
75	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
76	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
77	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
78	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
79	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
80	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
81	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
82	G+ve Rods	+ve	-ve	+ve	Motile	*Bacilllus*
83	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
84	G+ve Rods	+ve	-ve	-ve	Motile	*Bacilllus*
85	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
86	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
87	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
88	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
89	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
90	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
91	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
92	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
93	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
94	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
95	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
96	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
97	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
98	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
99	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
100	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
101	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
102	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
103	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
104	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
105	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
106	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
107	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
108	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
109	G+ve cocci	-ve	-ve	-ve	Non motile	*Streptococcus*
110	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
111	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
112	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
113	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
114	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
115	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
116	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
117	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
118	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
119	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
120	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
121	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
122	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
123	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
124	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
125	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
126	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
127	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
128	G+ve cocci	-ve	+ve	-ve	Non motile	*Streptococcus*
129	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*

Table 9: Continue

No.	Gram stain	Catalase	Coagulase	VP test	Motility	Species
130	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
131	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
132	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*
133	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
134	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
135	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
136	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
137	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
138	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
139	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
140	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
141	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
142	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
143	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
144	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
145	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
146	G+ve Rods	+ve	-ve	+ve	Motille	*Bacilllus*
147	G+ve Rods	+ve	-ve	+ve	Motille	*Bacilllus*
148	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
149	G+ve cocci	+ve	+ve	-ve	Non motile	*Staphylococcus*
150	G+ve cocci	+ve	+ve	+ve	Non motile	*Staphylococcus*

Table 10: Percentage of bacterial species isolated from milk mixed with saliva from different samples

Bacterial species	Tuti Island (20)	Bori (150)	Gandahar (40)	EL-Shuhada (19)	EL-Thawrah (21)	EL-Salha (34)	Kafori (15)
Staphylococcus intermedius	1 (5%)	18 (12%)	4 (10%)	1 (5.26%)	5 (23.81%)	5 (14.71%)	2 (17.33%)
Staphylococcus aureus	5 (25%)	47 (31.33%)	11 (27.5%)	5 (26.32%)	9 (42.86%)	14 (41.18%)	7 (46.67%)
Staphylococcus epidermidis	1 (5%)	11 (7.33%)	3 (7.5%)	2 (10.53%)	0	3 (8.82%)	0
Staphylococcus sciuri	1 (5%)	5 (3.33%)	3 (7.5%)	4 (21.05%)	2 (9.52%)	3 (8.82%)	0
Staphylococcus caseolyticus	0	7 (4.67%)	3 (7.5%)	2 (10.53%)	0	0	00
Streptococcus pneumoniae	3 (15%)	10 (6.67%)	2 (5%)	1 (5.26%)	1 (4.76%)	1 (2.94%)	1 (6.67%)
Streptococcus pyogenes	0	7 (4.67%)	2 (5%)	2 (10.53%)	2 (9.52%)	1 (2.94%)	00
Streptococcus sanguis	0	5 (3.33%)	2 (10%)	0	0	0	
Bacillus badius	2 (10%)	9 (6%)	1 (2.5%)	0	0	2 (5.88%)	3 (20%)
Bacillus subtilis	2 (10%)	9 (6%)	0	0	0	0	0
Bacillus lentus	2 (10%)	7 (4.67%)	5 (12.5%)	0	0	1 (2.94%)	1 (6.67%)
Micrococcus agilis	2 (10%)	1 (0.67%)	1 (2.5%)	1 (5.26%)	1 (4.76%)	0	0
Micrococcus kristinae	1 (5%)	2 (1.33%)	3 (7.5%)	0	1 (4.76%)	0	1 (6.67%)
M. mucilaginosus	0	4 (2.67%)	0	0	0	1 (2.94%)	0
Micrococcus lylae	0	1 (0.67%)	0	1 (5.26%)	0	3 (8.82%)	0
Gemella morbillorum	0	7 (4.67%)	3 (7.5%)	0	0	0	0

Novobiocin, Basitracin and Optochin were used to differentiate between some bacteria shown in Table 12, VP test and the position of spore was used to differentiate between *Bacillus* spp. as shown in Table 13. In species of *M. kristinae* the pigmentation of colonies was yellow colour, *M. mucilaginosus* had no pigmentation in colonies, *M. agilis* appeared red colour, while *M. lylae* showed cream colour in the surface of colonies, presented in Table 14.

Various bacterial species were tested against various antibiotics as shown in Table 15 Co-Trimexothazol showed the best antibiotic against different species of bacteria as 245 isolates were sensitive (81.7%) from the total isolates. Ciprofloxacin followed Co-Trimexothazol in a percentage of sensitivity, 220 isolates were sensitive to Ciprofloxacin (73.3%) from the total isolates. While Cloxacillin showed the lowest percentage against different bacteria as 14 isolates were sensitive (4.7%) from the total isolates.

Table 11: Number of the CFU/mL from milk after licking for three times

Sample No.	Lick one (CFU/mL)	Lick two (CFU/mL)	Lick three (CFU/mL)
Tuti Island			
1	3.6×10^6	5.0×10^6	6.0×10^6
2	3.5×10^6	6.0×10^6	7.5×10^6
3	4.0×10^6	5.8×10^6	7.0×10^6
4	4.9×10^6	7.3×10^6	8.0×10^6
5	6.0×10^6	8.0×10^6	8.7×10^6
6	4.0×10^6	6.0×10^6	7.5×10^6
7	2.5×10^6	3.8×10^6	5.0×10^6
8	1.5×10^6	2.9×10^6	3.7×10^6
9	1.4×10^6	2.1×10^6	3.0×10^6
10	2.2×10^6	3.1×10^6	4.4×10^6
11	1.8×10^6	2.5×10^6	3.4×10^6
12	2.6×10^6	3.2×10^6	4.8×10^6
13	3.8×10^6	5.6×10^6	$6.4x10^6$
14	2.6×10^6	3.2×10^6	4.0×10^6
15	2.9×10^6	3.7×10^6	4.6×10^6
16	3.8×10^6	5.1×10^6	6.0×10^6
17	1.7×10^6	3.0×10^6	4.5×10^6
18	4.9×10^6	6.4×10^6	7.2×10^6
19	1.6×10^6	2.5×10^6	3.5×10^6
20	2.2×10^6	3.7×10^6	4.0×10^6
Gandahar			
21	3.2×10^6	5.0×10^6	6.3×10^6
22	5.5×10^6	7.3×10^6	8.0×10^6
23	2.4×10^6	4.0×10^6	5.9×10^6
24	3.3×10^6	5.9×10^6	7.2×10^6
25	4.0×10^6	5.3×10^6	6.0×10^6
26	7.0×10^6	8.5×10^6	9.2×10^6
27	3.3×10^6	4.0×10^6	5.5×10^6
28	2.3×10^6	3.9×10^6	5.6×10^6
29	3.8×10^6	5.0×10^6	7.5×10^6
30	1.5×10^6	3.0×10^6	4.9×10^6
31	2.4×10^6	3.7×10^6	4.5×10^6
32	1.9×10^6	2.7×10^6	5.7×10^6
33	2.6×10^6	4.0×10^6	6.2×10^6
34	2.0×10^6	3.1×10^6	4.9×10^6
35	1.7×10^6	3.0×10^6	4.3×10^6
36	3.7×10^6	6.8×10^6	9.0×10^6
37	3.8×10^6	5.0×10^6	6.7×10^6
38	2.1×10^6	3.2×10^6	5.0×10^6
39	4.0×10^6	5.6×10^6	7.2×10^6
40	3.1×10^6	4.4×10^6	6.4×10^6
41	2.9×10^6	3.9×10^6	5.0×10^6
42	3.3×10^6	4.7×10^6	6.0×10^6
43	2.9×10^6	3.8×10^6	5.0×10^6
44	3.6×10^6	5.6×10^6	7.4×10^6
45	2.0×10^6	3.3×10^6	4.0×10^6
46	1.4×10^6	2.3×10^6	3.6×10^6
47	1.3×10^6	2.5×10^6	4.1×10^6
48	2.0×10^6	2.9×10^6	4.0×10^6
49	2.9×10^6	4.8×10^6	7.0×10^6
50	2.6×10^6	3.5×10^6	4.8×10^6
51	2.4×10^6	3.5×10^6	6.0×10^6
52	1.5×10^6	2.2×10^6	4.0×10^6
53	3.7×10^6	5.2×10^6	7.0×10^6
54	3.8×10^6	5.0×10^6	6.0×10^6
55	4.0×10^6	5.5×10^6	6.0×10^6
56	1.9×10^6	2.3×10^6	3.3×10^6
57	2.9×10^6	4.0×10^6	5.5×10^6
58	2.3×10^6	3.0×10^6	4.1×10^6
59	2.0×10^6	2.9×10^6	3.4×10^6
60	2.9×10^6	4.0×10^6	6.0×10^6

Table 11: Continue

Sample No.	Lick one (CFU/mL)	Lick two (CFU/mL)	Lick three (CFU/mL)
ELthawrah			
61	3.5×10^6	4.2×10^6	6.6×10^6
62	5.0×10^6	6.3×10^6	7.5×10^6
63	1.7×10^6	2.5×10^6	4.0×10^6
64	3.0×10^6	4.5×10^6	6.0×10^6
65	2.1×10^6	3.0×10^6	5.4×10^6
66	3.7×10^6	4.9×10^6	6.0×10^6
67	1.5×10^6	2.3×10^6	3.8×10^6
68	3.1×10^6	4.0×10^6	5.2×10^6
69	2.7×10^6	3.3×10^6	4.5×10^6
70	2.2×10^6	3.9×10^6	5.0×10^6
71	4.0×10^6	5.3×10^6	6.9×10^6
72	1.4×10^6	2.5×10^6	3.7×10^6
73	3.1×10^6	4.8×10^6	5.9×10^6
74	1.5×10^6	2.9×10^6	3.5×10^6
75	3.7×10^6	4.5×10^6	6.0×10^6
76	2.8×10^6	4.9×10^6	6.8×10^6
77	2.7×10^6	5.5×10^6	7.0×10^6
78	2.4×10^6	3.6×10^6	5.0×10^6
79	1.3×10^6	2.1×10^6	3.0×10^6
80	3.0×10^6	4.5×10^6	5.7×10^6
81	3.7×10^6	4.9×10^6	6.0×10^6
ELsalha			
82	4.0×10^6	5.5×10^6	7.0×10^6
83	1.9×10^6	2.8×10^6	4.1×10^6
84	3.5×10^6	4.3×10^6	6.0×10^6
85	1.2×10^6	2.2×10^6	3.2×10^6
86	3.6×10^6	5.6×10^6	6.7×10^6
87	2.9×10^6	3.5×10^6	4.2×10^6
88	1.3×10^6	2.1×10^6	3.2×10^6
89	3.8×10^6	5.3×10^6	6.3×10^6
90	1.4×10^6	2.8×10^6	4.0×10^6
91	3.9×10^6	5.1×10^6	6.0×10^6
92	4.9×10^6	5.6×10^6	7.0×10^6
93	1.6×10^6	2.3×10^6	4.7×10^6
94	4.7×10^6	6.2×10^6	7.3×10^6
95	5.0×10^6	6.6×10^6	7.5×10^6
96	1.5×10^6	2.5×10^6	3.8×10^6
97	2.1×10^6	3.0×10^6	4.2×10^6
98	1.8×10^6	2.7×10^6	3.9×10^6
99	2.5×10^6	3.6×10^6	5.0×10^6
100	3.4×10^6	$4.5x10^6$	5.8×10^6
101	2.3×10^6	3.1×10^6	4.0×10^6
102	2.6×10^6	3.0×10^6	4.2×10^6
103	1.7×10^6	2.3×10^6	3.1×10^6
104	2.5×10^6	3.1×10^6	4.0×10^6
105	5.3×10^6	6.2×10^6	7.0×10^6
106	3.0×10^6	4.1×10^6	5.4×10^6
107	1.3×10^6	2.2×10^6	3.5×10^6
108	2.6×10^6	3.9×10^6	5.0×10^6
109	5.0×10^6	6.3×10^6	7.1×10^6
110	1.6×10^6	2.7×10^6	3.5×10^6
111	2.0×10^6	3.5×10^6	4.2×10^6
112	3.5×10^6	4.3×10^6	5.5×10^6
113	2.2×10^6	3.0×10^6	4.7×10^6
114	1.9×10^6	2.7×10^6	3.2×10^6
115	2.4×10^6	3.5×10^6	5.1×10^6

Table 11: Continue

Sample No.	Lick one (CFU/mL)	Lick two (CFU/mL)	Lick three (CFU/mL)
Kafori			
116	1.2×10^6	2.3×10^6	3.4×10^6
117	3.3×10^6	4.0×10^6	5.3×10^6
118	5.1×10^6	5.9×10^6	6.2×10^6
120	5.0×10^6	6.6×10^6	7.3×10^6
121	2.1×10^6	3.4×10^6	5.0×10^6
122	1.6×10^6	2.5×10^6	3.4×10^6
123	1.8×10^6	2.8×10^6	3.6×10^6
124	2.0×10^6	3.8×10^6	5.0×10^6
125	3.3×10^6	4.0×10^6	5.5×10^6
126	1.7×10^6	2.9×10^6	3.8×10^6
127	2.6×10^6	3.2×10^6	4.5×10^6
128	5.0×10^6	6.2×10^6	7.3×10^6
129	3.3×10^6	4.0×10^6	5.5×10^6
130	2.3×10^6	3.2×10^6	4.0×10^6
131	2.5×10^6	3.3×10^6	4.3×10^6
ELshuhada			
132	4.5×10^6	5.2×10^6	6.1×10^6
133	1.7×10^6	2.9×10^6	3.8×10^6
134	3.1×10^6	4.0×10^6	5.2×10^6
135	2.4×10^6	3.5×10^6	4.8×10^6
136	1.9×10^6	2.8×10^6	3.6×10^6
137	3.2×10^6	4.5×10^6	5.9×10^6
138	2.5×10^6	3.7×10^6	5.1×10^6
139	4.4×10^6	5.4×10^6	6.7×10^6
140	2.4×10^6	3.5×10^6	5.0×10^6
141	2.1×10^6	3.2×10^6	4.3×10^6
142	3.5×10^6	4.3×10^6	5.5×10^6
143	3.2×10^6	4.0×10^6	5.3×10^6
144	2.2×10^6	3.5×10^6	4.5×10^6
145	4.0×10^6	5.2×10^6	6.6×10^6
146	6.1×10^6	7.3×10^6	8.2×10^6
147	7.0×10^6	8.5×10^6	9.2×10^6
148	2.7×10^6	3.5×10^6	4.4×10^6
149	4.2×10^6	5.3×10^6	6.1×10^6
150	1.8×10^6	2.6×10^6	3.3×10^6

Table 12: Some antibiotics used to differentiate some bacterial species and observe appearing of haemolysis

Genus	Novobiocin	Basitracin	Optochin	Haemolysis
Staphylococcus caseolyticus	S	-	-	-
Staphylococcus sciuri	R	-	-	-
Streptococcus pyogenes	-	S	R	Beta haemolysis
Streptococcus pneumoniae	-	S	S	Alpha haemolysis

S: Sensitive and R: Resistant

Table 13: VP test, position and shape of spore used to differentiate some species of *Bacilli*

Genus	VP test	Position and shape of spore
Bacillus badius	-ve	Central and oval
Bacillus subtilis	+ve	Central and oval
Bacillus lentus	-ve	Terminal and oval

Table 14: Genus of *Micrococcus* spp showed different pigmentation on the surface of colonies

Genus	Pigmentation of colonies
M. kristinnae	Yellow
M. mucilaginosus	-
Megachile agilis	Red
Micrococcus lylae	Cream

Table 15: Sensitivity percentage of antibiotic disk against various bacterial species

Antimicrobial agent	Resistant	Intermediate	Sensitive
Ampicillin (10 µg)	80 (26.6%)	20 (6.7%)	200 (66.7%)
Co-Trimexothazol (25 µg)	34 (11.3%)	21 (7.0%)	245 (81.7%)
Cephalexin (30 µg)	55 (18.3%)	37 (12.3%)	208 (69.3%)
Tetracycline (30 µg)	55 (18.3%)	53 (17.7%)	192 (64%)
Cefotaxime ((30 µg)	38 (12.7%)	95 (31.7%)	167 (55.7%)
Ciprofloxacin (5 µg)	22 (7.3%)	58 (19.3%)	220 (73.3%)
Levofloxacin (5 µg)	38 (12.7%)	45 (15%)	217 (72.3%)
Gentamycin (10 µg)	62 (20.7%)	42 (14%)	196 (65.3%)
Lincomycin (2 µg)	89 (29.7%)	78 (26%)	133 (44.3%)
Linezoled (30 µg)	114 (38%)	7 (2.3%)	179 (59.7%)
Roxithromycin (15 µg)	76 (25.3%)	105 (35%)	119 (39.7%)
Cloxacillin (5 µg)	281 (93.7%)	5 (1.7%)	14 (4.7%)

DISCUSSION

This study's findings aligned with previous literature that highlights the presence of Gram-positive bacteria, particularly *Staphylococcus aureus*, in canine oral flora. Similar studies have identified *S. aureus* as a significant bacterium in dog saliva, emphasizing its potential as a source of contamination in various environments, including food sources[16]. The identification of Co-Trimoxazole and Ciprofloxacin as effective treatments for these bacterial isolates also resonates with existing research, which suggests that these antibiotics are generally effective against Gram-positive bacteria, including *S. aureus*[17].

The results obtained in this study reveal that a significant amount of bacteria, more than 1×10^5 CFU/mL, are introduced into sterile milk licked by a dog three times. Furthermore, the types of bacteria introduced can be harmful to human health. This study evaluated all isolated bacterial genera against different antibiotics. *Staphylococcus* spp. and *Streptococcus* spp. were isolated from dog saliva, aligned with findings by Georges and Adesiyun[18], who recorded that dog bites to children between the ages of 8-12 in Trinidad presented risk factors and preventive strategies for reducing injuries. Most studies on dog injuries derive from hospital data, primarily from emergency departments, with the most common injury sites being the face, head and neck, especially in children under five years old. Bacteria such as *Staphylococcus* spp. *Streptococcus* spp. and *Pasteurella multocida* were isolated from dog bite wounds.

This study isolated *S. aureus* from dog saliva, similar to findings by Robertson *et al.*[19], who reported dogs as main reservoirs of many infective stages of parasites transmissible to humans and other domestic animals. There is evidence of resistant organism transfer between animals and people, highlighting dogs' public health significance due to their close companionship with humans. In the United States, up to 43 million (36.5%) households own a dog. Saliva, a biological fluid, offers advantages as a diagnostic medium over blood due to non-invasive, simple collection and repeated sampling without discomfort to the patient. In human medicine, saliva is gaining attention as an alternative to blood analysis. In dogs, analytes like C-reactive protein, cortisol, alpha-amylase, adenosine deaminase or muscle enzymes have been measured in saliva[20].

This study detected various bacterial species among dogs kept as pets in Khartoum State. Samples were collected from milk mixed with dog saliva from Tuti Island, Elshuhada, Elthawrah, Kafori, Gandahar and Elsalha and direct saliva samples using sterile cotton swabs from Bori (police dogs). All isolated bacteria were Gram-positive, with some capable of causing numerous human diseases. The genus *Staphylococcus* exhibited the highest percentage, with 181 isolates (60.33%) of the total isolation. This genus displayed different shapes, colors and sizes. Five *Staphylococcus* species were fully identified: *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus sciuri*, *Staphylococcus epidermidis* and *Staphylococcus caseolyticus*. *Staphylococcus intermedius* was isolated from 18 samples of licked milk. Known to be carried in dog saliva, *Staphylococcus intermedius* poses a serious health hazard for dog owners and those consuming contaminated food. This finding aligned with Kempker *et al.*[21], who isolated *Staphylococcus intermedius* from a woman with a history of endoscopic pituitary edema resection presenting with foul-smelling nasal discharge. *Staphylococcus intermedius* is a potential human pathogen.

The results of this study, which isolated *Staphylococcus intermedius* from dog saliva, were substantiated by Kikuchi *et al.*[22], who reported a case of a 37 year-old female with *Staphylococcus intermedius* in a surgical wound infection. A month later, a case of mastoid cavity infection due to ear licking by a dog was reported. These cases emphasize the danger of consuming food contaminated by dog saliva carrying *Staphylococcus intermedius*. The association of human infection with *Staphylococcus intermedius* may be attributed to its status as normal dog skin flora[23]. Dog and cat bites are primary sources of bacterial diseases, some fatal[24]. Maurelli *et al.*[25] demonstrated that bodily contact significantly contributes to infection occurrence.

The genus *Micrococcus* showed 24 isolates (8%) from the total number, with a high percentage isolated from Bori. This study isolated *Micrococcus* spp. from dog saliva, substantiated by Abrahamian and Goldstein[26], who isolated *Micrococcus* spp., from dog bites. The genus *Streptococcus* showed 40 isolates (13.33%) from the total number, with a high percentage isolated from Bori. In this study, *Streptococcus pneumoniae*, *Streptococcus sanguis* and *Streptococcus pyogenes* isolation aligned with Fredrick *et al.*[26].

Three *Bacillus* species were isolated in this study: *Bacillus lentus*, *B. badius* and *B. subtilis*, comprising 43 isolates (14.33%) of the total. This genus showed different shapes, colors and sizes, with some having mucoid colonies. The study found *M. mucilaginosus* sensitivity to various antibiotics, similar to von Eiff *et al.*[27], who found *M. mucilaginosus* sensitivity in 63 isolates. Leyden *et al.*[28] counted aerobic bacteria colonies from dogs' moist areas, such as the axillae or toe web spaces, reaching 10^7 bacteria per cm^2, while dry areas like the forearm or trunk harbored fewer bacteria per cm^2, substantiating[28]. Anaerobic bacteria are present on human skin, with colony counts up to 10^6 bacteria per cm^2 [29]. Ribeiro *et al.*[30] reported that *Strep pyogenes* has significant importance as an opportunistic animal pathogen causing a variety of purulent infections. These infections pose a huge economic problem in livestock breeding, it is considred to be a part of the biota of skin and mucous membrane of the upper respiratory and urogenital tracts of animals.

According to Moosavy *et al.*[31], most animal bites to humans in the Saudi Arabia involve snakes, dogs, cats, rodents and foxes. Their study also identified Actinobacteria in dog saliva, mirroring the findings of Wilhem *et al.*[32], who detected Actinobacteria in the oral cavities of dogs. Additionally, Harvey[33] documented the isolation of Gram-positive bacteria from the mouths of dogs in various studies, highlighting that the prevalence of periodontal disease increases with age, affecting around 80% of dogs. The most common treatment for periodontal disease in dogs is scaling, which usually requires general anesthesia.

The study revealed a significant bacterial load, exceeding 1×10^5 CFU/mL, in the milk mixed with dog saliva. The isolated bacteria included *Staphylococcus aureus*, *Staphylococcus intermedius* and various *Streptococcus* species, which are known to cause infections in humans. The antimicrobial susceptibility testing showed that many of the isolated bacteria were resistant to multiple antibiotics.

CONCLUSION

The study concludes that saliva, particularly from dogs, contains pathogenic bacteria like *S. aureus*, making it a significant source of infection. The bacterial count in milk increased with repeated licking, highlighting the risk of pathogen transmission through saliva in food and drinks. Co-trimoxazole emerged as the most effective antibiotic against these bacteria, followed by Ciprofloxacin, while Fungistatin was recommended for preventing fungal contamination. Future recommendations include more extensive surveillance of antibiotic-resistant bacteria, public education on the risks of dog saliva and the implementation of preventive hygiene measures to reduce bacterial transmission.

SIGNIFICANCE STATEMENT

This study underscores the importance of understanding the microbial composition of dog saliva and its implications for public health. The isolation and characterization of bacteria from milk mixed with dog saliva reveal significant bacterial contamination and antimicrobial resistance, posing potential health risks. Continuous research and public health initiatives are crucial to address these concerns and safeguard human health.

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