#### Aerobic Microbial Skin Flora in Jeddah City, Saudi Arabia

#### Rajaa M. Milyani

Department of Biological Sciences, Faculty of Science, King Abdulaziz University.

#### Abstract

The aerobic microbial skin flora of 40 healthy subjects living in Jeddah city (Saudi Arabia) was determined. Two age groups: children and adults; including males and females were investigated. Seven sites were studied: forehead, axilla, chest, groin, leg, toe web and anterior nares. The skin was sampled by rubbing the chosen site with a surfactant substance (Tween 80) moistened cotton swab which was dipped back in the surfactant container and the resulted suspension was agitated for one minute.

Thirty three microbial species were isolated from the seven sites of the study group, in which Acinetobacter baumannii, Acinetobacter lwoffii, corynebacterium species and Staphylococcus (Staph.) aureus dominated among children (30% each). The most other prevalent isolates recovered were Alkaligenes species, Bacillus species, Chryseomonas luteola, Staph. epidermidis, Enterococcus faecalis and Staph. hominis (27.5% each). Organisms including Candida albicans, Enterobacter agglomerans, Escherichia coli, Flavobacterium meningosepticum, Klebsiella oxytoca, Micrococcus luteus, Micrococcus roseus, Micrococcus varians, Micrococcus species, Burkholderia cepacia, Stenotrophomonas maltophilia, Pseudomonas paucimobilis, Pseudomonas fluorescence, Pseudomonas species, Staph. capitis, Staph. cohnii, Staph. saprophyticus, Staph. simulans, Staph. warneri, Staph. xylosus, viridans-type streptococcus and yeasts were also found in different percentage. Higher isolation rates of Acinetobacter lwoffii, Staph. aureus, Alkaligenes species, Corynebacterium species, Chryseomonas luteola, Enterobacter agglomerans, Staph. epidermidis and other coagulase negative Staphylococci were noted in children from the seven sites. However, Chryseomonas luteola, and Pseudomonas species, were found only in the groin area among males. Otherwise, no significant differences were recorded in the isolation rates from each site separately in relation to age and sex. The role of the isolated microorganisms in endogenous, exogenous and nosocomial infections was emphasized.

#### Introduction

"Surprisingly little is known about the microflora of the skin and its role in skin common infections such as psoriasis and eczema and even less known of the flora in rare diseases". A statement that had been written in 1974 bv Noble and Somerville and regrettably, the same can be applied to our knowledge in 1990s, not only to the skin flora but also to the whole microflora of man, in a country that

receives millions of people for religious purposes, from all over the world at almost all the year around namely: Saudi Arabia. The importance of such knowledge is certainly obvious, particularly in preventing exogenous and or endogenous infections. Milyani *et. al.* (1987) stressed upon the medical significance of such topic and the lack of information amongst Saudi subjects, and took a step towards that by studying the throat microbial flora in Jeddah city as a start. Since then our knowledge have accumulated, from other parts of the world, and the role played by the normal microbial flora in different infections became clearly apparent (Maibach and Aly 1981; Noble 1983; Boyce et. al. (1990). Though, this phenomenon has gained attention as early as 1843 by Oliver Wendell Holmes who was known to teach at Boston that puerperal fever was caused by germs on the hands of physicians and midwives which were transmitted to the vaginas of women during internal examination. Few took these ideas seriously until Joseph Lister in 1865 used carbolic acid to disinfect the skin of the operation site and the operator's hands (Sethna, 1978). Furthermore, and above all Prophet Mohammad peace be upon him, before more than 14 century, taught the world to wash their bodies, faces, rinse the mouth, clear with water the inside of the nose (sniffing), wash hands, forearms to the elbows and feet to the ankles (around five times a day); the wisdom behind that is obviously to rid the body of any pathogens, transient minimize the number of microbial flora to a balanced state and thus preventing the mischief of these organisms that may act as opportunistic pathogens causing different diseases infections and (Milyani, 1998).

With the advent of antibiotics, cytotoxic and immunosuppressive drugs, in parallel to the increase in compromised patients, allowed the socalled innocent normal flora to establish itself and emerge as opportunistic pathogen if not as a true pathogen causing high rates of morbidity and mortality (Mandell *et. al.*, 2000).

The aim of the present study was to determine the microbial flora of the skin among children and adults, in Jeddah city, highlighting its medical significance and to discuss the role of the isolated organisms in endogenous, exogenous and nosocomial infections.

#### Materials And Methods

**Subjects**: 40 apparently healthy 22 females and 18 males consisted of two age groups. 1 -12 years (24 children before menarche and puberty); and 13 -17 years (16 early adults after puberty). None had been receiving antibiotics for nine weeks or using deodorants. Axillary hair was not removed.

Media Used: Five growth media were used: nutrient agar + 0.5% glucose + 0.5% Tween80, for the isolation of skin lipophilic organisms; blood agar + crystal violet 1:666.666; was used to select *Streptococcus pyogenes*. (Milyani, 1976); Tinsdale agar base; MacConkey agar and Cystine-Lactose-Deficient agar (Oxoid Ltd, London).

Sampling method and culture: A modified standardized swabbing method by Selwyn and Ellis (1972) was used. It involved rubbing thoroughly an area of  $2 \text{ cm}^2$  of the chosen site with moderate pressure, for one minute with a sterile cotton-wool swab which had been moistened with a sterile solution of 0.5 % Tween 80 (Fluka) in 0.075M phosphate buffer at pH 7.9. The swab was dipped back into a sterile 10ml test tube containing 2ml of the sampling solution (Tween 80, the surfactant substance) and the suspension was mechanically agitated for one minute on a Rotamixer. Whereas, anterior nares were sampled by, rotating the Tween 80 moistened cotton swab inside the nares for one minute. Culturing the samples was within one to two hours of collection . The following procedure was carried out for culturing: the cotton swab was removed from the tube and streaked evenly all over the surface of each growth medium (dipping it in the suspension each time prior to inoculation of the new medium). The inoculated plates were then incubated aerobically at  $37^{\circ}$ C for 48 - 72 hours.

Samples were collected within five months when ambient temperature varied between 37°C - 45°C.

#### **Identification:**

Commercial identification kits were used: API Staph., API 20E (Analytab Product, Plainview, N.Y) and Spectrum 10 System (ABL, Austin, Texas); In general, identification was carried out according to Koneman *et.al.* (1997).

**Statistical analysis**: The data were analyzed using Fisher's exact test and Pearson chi-square (each when applicable) to determine the significant differences between the microbial isolation rates in relation to age, sex, seven sites and each site separately.

#### **Results:**

The isolation rates of different organisms recovered from the total seven sites of the skin and from each site separately, in relation to age and sex are demonstrated in Tables 1- 16 and Figure 1. Figures 2-7 show the isolation rates of some of the isolates in each site.

Thirty three microbial species were isolated from the seven sites of the study group including Gram positive and Gram negative microorganisms: the Gram positive comprised 20 species while the Gram negative comprised 13 species. Acinetobacter (A.) baumannii, Acinetobacter (A.) lwoffii, Corynebacspecies and Staphylococcus terium dominated (Staph.) aureus among children comprising 30% of each isolate. The other microbial isolates followed in prevalence among the same group, were Alkaligenes species, Bacillus species, Chryseomonas (C.) luteola, Staph. epidermidis,

Enterococcus (E.) faecalis and Staph. hominis giving an incidence of 27.5% each (Table 1 and Figure 1). Organisms including Candida (C.) albicans, Enterobacter agglomerans, (E.) Escherichia (E.) coli, Flavobacterium (F.) meningosepticum, Klebsiella (K.) *Micrococcus* oxytoca, luteus. Micrococcus roseus, Micrococcus varians. *Micrococcus* species. Burkholderia  $(B_{\cdot})$ cepacia, *Stenotrophomonas* maltophilia, (S.)Pseudomonas (P.) paucimobilis, P. fluorescence. Pseudomonas species. Staph. capitis, Staph. cohnii, Staph. haemolyticus, Staph. saprophyticus, Staph. simulans, Staph. warneri, Staph. xylosus, viridas-type streptococcus and yeasts were also found in different percentage among both age groups, males and females (Table 1 and 2). On the other hand, significant differences between children and adults were found in the isolation rates of A. lwoffii (P= 0.004), Alkaligenes species (P= 0.027), Corynebacterium species (P=0.015), C. luteola (P=0.027), E. agglomerans (P=0.032), Staph. aureus (P=0.015), Staph. capitis (P= 0.032), Staph. cohnii Staph. epidermidis (P= (P = 0.027),0.012), Staph. haemolyticus (P= 0.032), Staph. hominis (P= 0.012), Staph. simulans (P= 0.027), Staph. warneri (P= 0.027) and Staph. xylosus (P=0.027) from the seven sites (Table 1). However, in relation to sex, significant differences were evident only in the isolation rates of C. luteola (P=0.013), and P. species (P=0.013) in the groin area among males (Table 12). Otherwise, no significant differences were recorded in the isolation rates from each site separately in relation to age and sex (Tables 3-16 and Figures 2-7).

Nonetheless, as shown in Figure 2 and 3 within the age range 1-12 and 13 -17 years the carriage rate of *Staph*. *aureus* among children and adults, males and females, was found to be 5% and 0% in the forehead, 10% and 0% in the anterior nares, 2.5% and 5% in the axilla (both age groups, females and males respectively), whereas, in the chest area it was 7.5% and 0% in children and adults and 2.5% and 7.5% in females and males respectively. On the other hand, the carriage rate in the groin was 10% in children, 2.5% in adults, 7.5% in females and 5% in males; in the leg 10% in children and 0% in adults, 2.5% in females and 7.5% in males; finally in the toe webs, 12.5% in children and 2.5% in adults, whereas, the same carriage rate of 7.5% was noted in both females and males. Turning to other isolates, the incidence of *A. baumannii* and *A. lwoffii* in the seven sites of the skin is shown in Figures 4 and 5 in relation to age. Whereas, Figures 6-7 recorded the isolation rate of *C. albicans* among both age groups and sex.

 Table 1: The isolation rates of different organisms recovered from the seven sites of the skin in relation to age

Missohieliseletes	age '	age 1-12*		1-12* age 13-7		1-12* age 13-17**		3-17**	n volvoð	
Microbial Isolates	Freq	%	freq	%	p-value <sup>4</sup>	p-value©				
01- Acinetobacter baumannii	12	30	4	10		.114				
02- Acinetobacter Iwoffii	12	30	1	2.5		.004 b				
03- Alkaligenes species	11	27.5	2	5		.027 b				
04- Bacillus species	6	15	3	7.5	.717					
05- Candida albicans	7	17.5	4	10	1.00					
06- Corynebacterium species	12	30	2	5		.015 b				
07- Chryseomonas luteola	11	27.5	2	5		.027 b				
08- Enterobacter agglomerans	9	22.5	1	2.5	.032 b					
09- Escherichia coli	6	15	2	5	.439					
10- Flavobacterium meningosepticum	6	15	4	10	1.00					
11- Klebsiella oxytoca	8	20	4	10	.729					
12- Micrococcus luteus	8	20	1	2.5	.061					
13- Micrococcus roseus	8	20	2	5	.263					
14- Micrococcus species	6	15	2	5	.439					
15- Micrococcus varians	7	17.5	1	2.5	.114					
16- Burkholderia cepacia	9	22.5	3	7.5	.297					
17- Stenotrophomonas maltophilia	8	20	4	10	.729					
18- Pseudomonas paucimobilis	3	7.5	3	7.5	.668					
19- Pseudomonas fluorescens	7	17.5	1	2.5	.114					
20- Pseudomonas species	7	17.5	5	12.5	1.00					
21- Staph. aureus	12	30	2	5		.015 b				
22- Staph. capitis	9	22.5	1	2.5	.032 b					
23- Staph. cohnii	10	25	1	2.5	.027 b					
24- Staph. epidermidis	11	27.5	1	2.5	.012 b					
25- Enterococcus faecalis	11	27.5	3	7.5		.079				
26- Staph. haemolyticus	9	22.5	1	2.5	.032 b					
27- Staph. hominis	11	27.5	1	2.5	.012 b					
28- Staph. saprophyticus	8	20	2	5	.263					
29- Staph. simulans	10	25	1	2.5	.027 b					
30 -Staph. warneri	10	25	1	2.5	.027 b					
31- S.xylosus	10	25	1	2.5	.027 b					
32- Viridans- type streptococci	10	25	2	5	.079					
33- Yeasts	7	17.5	3	7.5	.711					

a. Using Fisher's Exact test (Exact Sig. 2 sided), using  $\alpha$ =0.05

b. The test is significant (otherwise the test is not significant).

©.Using Pearson Chi-square test (Asymp.sig.2-sided).

\* Age 1- 12 years were 24 subjects

Mierobial isolatos	Fem	Females*		Males**		n valuo®
Microbial Isolates	Freq	%	freq	%	p-value∝	p-value©
01- Acinetobacter baumannii	6	15	10	25		.069
02- Acinetobacter Iwoffii	6	15	7	17.5		.435
03- Alkaligenes species	7	17.5	6	15		.919
04- Bacillus species	5	12.5	4	10	1.00	
05- Candida albicans	6	15	5	12.5	1.00	
06- Corynebacterium species	10	25	4	10		.125
07- Chryseomonas luteola	6	15	7	17.5		.435
08- Enterobacter agglomerans	6	15	4	10	1.00	
09- Escherichia coli	^	10	4	10	1.00	
10- Flavobacterium meningosepticum	6	15	4	10	1.00	
11- Klebsiella oxytoca	9	22.5	3	7.5		.096
12- Micrococcus luteus	6	15	3	7.5	.476	
13- Micrococcus roseus	6	15	4	10	1.00	
14- Micrococcus species	^	10	4	10	1.00	
15- Micrococcus varians	5	12.5	3	7.5	.709	
16- Burkholderia cepacia	6	15	6	15		.677
17- Stenotrophomonas maltophilia	8	20	4	10		.332
18- Pseudomonas paucimobilis	1	2.5	5	12.5	.073	
19- Pseudomonas fluorescens	3	7.5	5	12.5	.430	
20- Pseudomonas species	^	10	8	20		.071
21- Staph. aureus	8	20	6	15		.842
22- Staph. capitis	6	15	4	10	1.00	
23- Staph. cohnii	7	17.5	4	10	.723	
24- Staph. epidermidis	5	12.5	7	17.5		.267
25- Enterococcus faecalis	8	20	6	15		.842
26- Staph. haemolyticus	7	17.5	3	7.5	.464	
27- Staph. hominis	8	20	4	10		.332
28- Staph. saprophyticus	6	15	4	10	1.00	
29- Staph. simulans	8	20	3	7.5	.286	
30 -Staph. warneri	6	15	5	12.5	1.00	
31- S.xylosus	^	10	7	17.5	.173	
32- Viridans- type streptococci	8	20	4	10		.332
33- Yeasts	6	15	4	10	1.00	

### Table 2: The isolation rates of different organisms recovered from seven sites of the skin in relation to sex

All tests are not significant by using  $\alpha$ =0.05 - a. Using Fisher's Exact test (Exact Sig. 2 sided)

©.Using Pearson Chi-square test (Asymp.sig.2-sided).

Microbial isolates	Age 1-12*		Age 1	n-value <sup>a</sup>	
Microbial Isolates	freq	%	freq	%	p-value
01- Acinetobacter baumannii	4	10	0	0	.136
02- Acinetobacter Iwoffii	2	5	0	0	.508
03- Alkaligenes species	3	7.5	1	2.5	.638
04- Bacillus species	0	0	1	2.5	.400
05- Candida albicans	3	7.5	0	0	.262
06- Corynebacterium species	3	7.5	0	0	.262
07- Chryseomonas luteola	1	2.5	0	0	1.00
08- Enterobacter agglomerans	3	7.5	0	0	.262
09- Escherichia coli	3	7.5	1	2.5	.638
10- Flavobacterium meningosepticum	4	10	1	2.5	.631
11- Klebsiella oxytoca	3	7.5	1	2.5	.638
12- Micrococcus luteus	6	15	1	2.5	.210
13- Micrococcus roseus	4	10	0	0	.136
14- Micrococcus species	2	5	1	2.5	1.00
15- Micrococcus varians	5	12.5	1	2.5	.373
16- Burkholderia cepacia	1	2.5	0	0	1.00
17- Stenotrophomonas maltophilia	1	2.5	1	2.5	1.00
18- Pseudomonas paucimobilis	2	5	1	2.5	1.00
19- Pseudomonas fluorescens	2	5	0	0	.508
20- Pseudomonas species	3	7.5	1	2.5	.638
21- Staph. aureus	2	5	0	0	.508
22- Staph. capitis	2	5	1	2.5	1.00
23- Staph. cohnii	4	10	0	0	.136
24- Staph. epidermidis	3	7.5	0	0	.262
25- Enterococcus faecalis	4	10	0	0	.136
26- Staph. haemolyticus	4	10	1	2.5	.631
27- Staph. hominis	4	10	0	0	.136
28- Staph. saprophyticus	2	5	0	0	.508
29- Staph. simulans	3	7.5	0	0	.262
30 -Staph. warneri	3	7.5	0	0	.262
31- S.xylosus	2	5	1	2.5	1.00
32- Viridans- type streptococci	3	7.5	0	0	.262
33- Yeasts	1	2.5	3	7.5	.283

## Table 3: The isolation rates of different organisms recovered from forehead in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha{=}0.05$ 

\* Age 1- 12 years were 24 subjects

Microbial isolates	Fem	Females		Males		
Inicrobial Isolates	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	2	5	2	5	1.00	
02- Acinetobacter Iwoffii	1	2.5	1	2.5	1.00	
03- Alkaligenes species	2	5	2	5	1.00	
04- Bacillus species	0	0	1	2.5	.450	
05- Candida albicans	2	5	1	2.5	1.00	
06- Corynebacterium species	2	5	1	2.5	1.00	
07- Chryseomonas luteola	0	0	1	2.5	.450	
08- Enterobacter agglomerans	2	5	1	2.5	1.00	
09- Escherichia coli	1	2.5	3	7.5	.310	
10- Flavobacterium meningosepticum	4	10	1	2.5	.355	
11- Klebsiella oxytoca	3	7.5	1	2.5	.613	
12- Micrococcus luteus	4	10	3	7.5	1.00	
13- Micrococcus roseus	2	5	2	5	1.00	
14- Micrococcus species	2	5	1	2.5	1.00	
15- Micrococcus varians	3	7.5	3	7.5	1.00	
16- Burkholderia cepacia	1	2.5	0	0	1.00	
17- Stenotrophomonas maltophilia	1	2.5	1	2.5	1.00	
18- Pseudomonas paucimobilis	1	2.5	2	5	.579	
19- Pseudomonas fluorescens	0	0	2	5	.196	
20- Pseudomonas species	2	5	2	5	1.00	
21- Staph. aureus	2	5	0	0	.492	
22- Staph. capitis	2	5	1	2.5	1.00	
23- Staph. cohnii	2	5	2	5	1.00	
24- Staph. epidermidis	2	5	1	2.5	1.00	
25- Enterococcus faecalis	4	10	0	0	.114	
26- Staph. haemolyticus	2	5	3	7.5	.642	
27- Staph. hominis	3	7.5	1	2.5	.613	
28- Staph. saprophyticus	1	2.5	1	2.5	1.00	
29- Staph. simulans	1	2.5	2	5	.579	
30 -Staph. warneri	2	5	1	2.5	1.00	
31- S.xylosus	1	2.5	2	5	.579	
32- Viridans- type streptococci	2	5	1	2.5	1.00	
33- Yeasts	2	5	2	5	1.00	

# Table 4: The isolation rates of different organisms recovered from forehead in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha{=}0.05$ 

Microbial isolates	age 1-12*		age 13-17**		n-valua <sup>a</sup>	
Microbial Isolates	freq	%	freq	%	p-value"	
01- Acinetobacter baumannii	5	12.5	1	2.5	.373	
02- Acinetobacter Iwoffii	3	7.5	0	0	.262	
03- Alkaligenes species	4	10	1	2.5	.631	
04- Bacillus species	3	7.5	0	0	.262	
05- Candida albicans	4	10	0	0	.136	
06- Corynebacterium species	1	2.5	0	0	1.00	
07- Chryseomonas luteola	2	5	0	0	.508	
08- Enterobacter agglomerans	2	5	0	0	.508	
09- Escherichia coli	1	2.5	0	0	1.00	
10- Flavobacterium meningosepticum	2	5	0	0	.508	
11- Klebsiella oxytoca	1	2.5	0	0	1.00	
12- Micrococcus luteus	3	7.5	0	0	.262	
13- Micrococcus roseus	2	5	1	2.5	1.00	
14- Micrococcus species	3	7.5	1	2.5	.638	
15- Micrococcus varians	0	0	0	0	h	
16- Burkholderia cepacia	4	10	0	0	.136	
17- Stenotrophomonas maltophilia	3	7.5	1	2.5	.638	
18- Pseudomonas paucimobilis	1	2.5	0	0	1.00	
19- Pseudomonas fluorescens	1	2.5	0	0	1.00	
20- Pseudomonas species	1	2.5	2	5	.553	
21- Staph. aureus	4	10	0	0	.136	
22- Staph. capitis	1	2.5	0	0	1.00	
23- Staph. cohnii	3	7.5	0	0	.262	
24- Staph. epidermidis	3	7.5	0	0	.262	
25- Enterococcus faecalis	2	5	0	0	.508	
26- Staph. haemolyticus	2	5	0	0	.508	
27- Staph. hominis	4	10	1	2.5	.631	
28- Staph. saprophyticus	3	7.5	0	0	.262	
29- Staph. simulans	5	12.5	0	0	.071	
30 -Staph. warneri	2	5	1	2.5	1.00	
31- S.xylosus	3	7.5	0	0	.262	
32- Viridans- type streptococci	3	7.5	0	0	.262	
33- Yeasts	2	5	0	0	.508	

#### Table 5: The isolation rates of different organisms recovered from the anterior nares in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because ANTERIOR NARES are a constant.

\* Age 1- 12 years were 24 subjects

Microbial isolates	Fem	Females		Males		
WICTODIAL ISOlates	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	4	10	2	5	.673	
02- Acinetobacter Iwoffii	2	5	1	2.5	1.00	
03- Alkaligenes species	3	7.5	2	5	1.00	
04- Bacillus species	2	5	1	2.5	1.00	
05- Candida albicans	3	7.5	1	2.5	.613	
06- Corynebacterium species	1	2.5	0	0	1.00	
07- Chryseomonas luteola	2	5	0	0	.492	
08- Enterobacter agglomerans	2	5	0	0	.492	
09- Escherichia coli	1	2.5	0	0	1.00	
10- Flavobacterium meningosepticum	2	5	0	0	.492	
11- Klebsiella oxytoca	1	2.5	0	0	1.00	
12- Micrococcus luteus	2	5	1	2.5	1.00	
13- Micrococcus roseus	1	2.5	2	5	.579	
14- Micrococcus species	2	5	2	5	1.00	
15- Micrococcus varians	0	0	0	0	h	
16- Burkholderia cepacia	4	10	0	0	.114	
17- Stenotrophomonas maltophilia	3	7.5	1	2.5	.613	
18- Pseudomonas paucimobilis	1	2.5	0	0	1.00	
19- Pseudomonas fluorescens	1	2.5	0	0	1.00	
20- Pseudomonas species	2	5	1	2.5	1.00	
21- Staph. aureus	4	10	0	0	.114	
22- Staph. capitis	1	2.5	0	0	1.00	
23- Staph. cohnii	2	5	1	2.5	1.00	
24- Staph. epidermidis	1	2.5	2	5	.579	
25- Enterococcus faecalis	2	5	0	0	.492	
26- Staph. haemolyticus	2	5	0	0	.492	
27- Staph. hominis	4	10	1	2.5	.355	
28- Staph. saprophyticus	2	5	1	2.5	1.00	
29- Staph. simulans	3	7.5	2	5	1.00	
30 -Staph. warneri	2	5	1	2.5	1.00	
31- S.xylosus	1	2.5	2	5	.579	
32- Viridans- type streptococci	3	7.5	0	0	.238	
33- Yeasts	2	5	0	0	.492	

## Table 6: The isolation rates of different organisms recovered from the anterior nares in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha{=}0.05$ 

h. No statistics are computed because ANTERIOR NARES are a constant.

Microbial isolates	age	age 1-12*		age 13-17**		
	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	1	2.5	2	5	.553	
02- Acinetobacter Iwoffii	8	20	1	2.5	.061	
03- Alkaligenes species	2	5	1	2.5	1.00	
04- Bacillus species	2	5	0	0	.508	
05- Candida albicans	1	2.5	0	0	1.00	
06- Corynebacterium species	4	10	0	0	.136	
07- Chryseomonas luteola	1	2.5	1	2.5	1.00	
08- Enterobacter agglomerans	5	12.5	0	0	.071	
09- Escherichia coli	4	10	0	0	.136	
10- Flavobacterium meningosepticum	4	10	1	2.5	.631	
11- Klebsiella oxytoca	1	2.5	4	10	.138	
12- Micrococcus luteus	3	7.5	0	0	.262	
13- Micrococcus roseus	3	7.5	1	2.5	.638	
14- Micrococcus species	1	2.5	0	0	1.00	
15- Micrococcus varians	5	12.5	0	0	.071	
16- Burkholderia cepacia	4	10	0	0	.136	
17- Stenotrophomonas maltophilia	3	7.5	1	2.5	.638	
18- Pseudomonas paucimobilis	3	7.5	2	5	1.00	
19- Pseudomonas fluorescens	2	5	0	0	.508	
20- Pseudomonas species	2	5	1	2.5	1.00	
21- Staph. aureus	1	2.5	2	5	.553	
22- Staph. capitis	4	10	1	2.5	.631	
23- Staph. cohnii	3	7.5	0	0	.262	
24- Staph. epidermidis	0	0	0	0	h	
25- Enterococcus faecalis	2	5	1	2.5	1.00	
26- Staph. haemolyticus	3	7.5	0	0	.262	
27- Staph. hominis	4	10	1	2.5	.631	
28- Staph. saprophyticus	3	7.5	0	0	.262	
29- Staph. simulans	3	7.5	0	0	.262	
30 -Staph. warneri	4	10	1	2.5	.631	
31- S.xylosus	1	2.5	0	0	1.00	
32- Viridans- type streptococci	5	12.5	0	0	.071	
33- Yeasts	3	7.5	1	2.5	.638	

## Table 7: The isolation rates of different organisms recovered from the axilla in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because AXILLA is a constant.

\* Age 1- 12 years were 24 subjects

Microbial isolates	Female		Males		a ulev-a	
Microbial Isolales	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	1	2.5	2	5	.579	
02- Acinetobacter Iwoffii	^	10	5	12.5	.705	
03- Alkaligenes species	2	5	1	2.5	1.00	
04- Bacillus species	1	2.5	1	2.5	1.00	
05- Candida albicans	0	0	1	2.5	.450	
06- Corynebacterium species	2	5	2	5	1.00	
07- Chryseomonas luteola	2	5	0	0	.492	
08- Enterobacter agglomerans	2	5	3	7.5	.642	
09- Escherichia coli	3	7.5	1	2.5	.613	
10- Flavobacterium meningosepticum	3	7.5	2	5	1.00	
11- Klebsiella oxytoca	3	7.5	2	5	1.00	
12- Micrococcus luteus	2	5	1	2.5	1.00	
13- Micrococcus roseus	3	7.5	1	2.5	.613	
14- Micrococcus species	0	0	1	2.5	.450	
15- Micrococcus varians	3	7.5	2	5	1.00	
16- Burkholderia cepacia	2	5	2	5	1.00	
17- Stenotrophomonas maltophilia	2	5	2	5	1.00	
18- Pseudomonas paucimobilis	1	2.5	4	10	.155	
19- Pseudomonas fluorescens	0	0	2	5	.196	
20- Pseudomonas species	0	0	3	7.5	.083	
21- Staph. aureus	1	2.5	2	5	.579	
22- Staph. capitis	3	7.5	2	5	1.00	
23- Staph. cohnii	2	5	1	2.5	1.00	
24- Staph. epidermidis	0	0	0	0	h	
25- Enterococcus faecalis	3	7.5	0	0	.238	
26- Staph. haemolyticus	1	2.5	2	5	.579	
27- Staph. hominis	2	5	3	7.5	.642	
28- Staph. saprophyticus	1	2.5	2	5	.579	
29- Staph. simulans	1	2.5	2	5	.579	
30 -Staph. warneri	2	5	3	7.5	.642	
31- S.xylosus	1	2.5	0	0	1.00	
32- Viridans- type streptococci	4	10	1	2.5	.355	
33- Yeasts	4	10	0	0	.114	

### Table 8: The isolation rates of different organisms recovered from the axilla in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because AXILLA is a constant.

Microbial isolates	age	age 1-12*		3-17**	n-value <sup>a</sup>	
	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	2	5	2	5	1.00	
02- Acinetobacter Iwoffii	2	5	0	0	.508	
03- Alkaligenes species	4	10	1	2.5	.631	
04- Bacillus species	2	5	2	5	1.00	
05- Candida albicans	2	5	1	2.5	1.00	
06- Corynebacterium species	1	2.5	0	0	1.00	
07- Chryseomonas luteola	1	2.5	0	0	1.00	
08- Enterobacter agglomerans	4	10	0	0	.136	
09- Escherichia coli	3	7.5	0	0	.262	
10- Flavobacterium meningosepticum	1	2.5	3	7.5	.283	
11- Klebsiella oxytoca	3	7.5	0	0	.262	
12- Micrococcus luteus	2	5	1	2.5	1.00	
13- Micrococcus roseus	3	7.5	1	2.5	.638	
14- Micrococcus species	1	2.5	2	5	.553	
15- Micrococcus varians	3	7.5	0	0	.262	
16- Burkholderia cepacia	4	10	0	0	.136	
17- Stenotrophomonas maltophilia	3	7.5	3	7.5	.668	
18- Pseudomonas paucimobilis	0	0	0	0	h	
19- Pseudomonas fluorescens	1	2.5	1	2.5	1.00	
20- Pseudomonas species	2	5	1	2.5	1.00	
21- Staph. aureus	4	10	0	0	.136	
22- Staph. capitis	3	7.5	0	0	.262	
23- Staph. cohnii	3	7.5	1	2.5	.638	
24- Staph. epidermidis	3	7.5	0	0	.262	
25- Enterococcus faecalis	4	10	0	0	.136	
26- Staph. haemolyticus	1	2.5	0	0	1.00	
27- Staph. hominis	4	10	0	0	.136	
28- Staph. saprophyticus	4	10	0	0	.136	
29- Staph. simulans	1	2.5	0	0	1.00	
30 -Staph. warneri	2	5	0	0	.508	
31- S.xylosus	5	12.5	0	0	.071	
32- Viridans- type streptococci	3	7.5	0	0	.262	
33- Yeasts	3	7.5	2	5	1.00	

## Table 9: The isolation rates of different organisms recovered from the chest skin in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because CHEST is a constant.

\* Age 1- 12 years were 24 subjects

Microbial isolates	Fem	Females		Males		
Microbial Isolales	freq	%	freq	%	p-value-	
01- Acinetobacter baumannii	2	5	2	5	1.00	
02- Acinetobacter Iwoffii	2	5	0	0	.492	
03- Alkaligenes species	2	5	3	7.5	.642	
04- Bacillus species	3	7.5	1	2.5	.613	
05- Candida albicans	2	5	1	2.5	1.00	
06- Corynebacterium species	1	2.5	0	0	1.00	
07- Chryseomonas luteola	1	2.5	0	0	1.00	
08- Enterobacter agglomerans	3	7.5	1	2.5	.613	
09- Escherichia coli	2	5	1	2.5	1.00	
10- Flavobacterium meningosepticum	2	5	2	5	1.00	
11- Klebsiella oxytoca	3	7.5	0	0	.238	
12- Micrococcus luteus	2	5	1	2.5	1.00	
13- Micrococcus roseus	1	2.5	3	7.5	.310	
14- Micrococcus species	3	7.5	0	0	.238	
15- Micrococcus varians	2	5	1	2.5	1.00	
16- Burkholderia cepacia	1	2.5	3	7.5	.310	
17- Stenotrophomonas maltophilia	4	10	2	5	.673	
18- Pseudomonas paucimobilis	0	0	0	0	h	
19- Pseudomonas fluorescens	2	5	0	0	.492	
20- Pseudomonas species	2	5	1	2.5	1.00	
21- Staph. aureus	1	2.5	3	7.5	.310	
22- Staph. capitis	1	2.5	2	5	.579	
23- Staph. cohnii	3	7.5	1	2.5	.613	
24- Staph. epidermidis	1	2.5	2	5	.579	
25- Enterococcus faecalis	3	7.5	1	2.5	.613	
26- Staph. haemolyticus	1	2.5	0	0	1.00	
27- Staph. hominis	3	7.5	1	2.5	.613	
28- Staph. saprophyticus	3	7.5	1	2.5	.613	
29- Staph. simulans	1	2.5	0	0	1.00	
30 -Staph. warneri	2	5	0	0	.492	
31- S.xylosus	1	2.5	4	10	.155	
32- Viridans- type streptococci	2	5	1	2.5	1.00	
33- Yeasts	2	5	3	7.5	.642	

## Table 10: The isolation rates of different organisms recovered from the chest skin in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha{=}0.05$ 

h. No statistics are computed because CHEST is a constant.

Microbial isolates	age	age 1-12*		age 13-17**		
MICTODIAL ISOIALES	freq	%	freq	%	p-value*	
01- Acinetobacter baumannii	4	10	2	5	1.00	
02- Acinetobacter Iwoffii	1	2.5	0	0	1.00	
03- Alkaligenes species	2	5	0	0	.508	
04- Bacillus species	1	2.5	0	0	1.00	
05- Candida albicans	4	10	3	7.5	1.00	
06- Corynebacterium species	5	12.5	2	5	.681	
07- Chryseomonas luteola	4	10	1	2.5	.631	
08- Enterobacter agglomerans	6	15	1	2.5	.210	
09- Escherichia coli	0	0	0	0	h	
10- Flavobacterium meningosepticum	2	5	1	2.5	1.00	
11- Klebsiella oxytoca	5	12.5	0	0	.071	
12- Micrococcus luteus	4	10	0	0	.136	
13- Micrococcus roseus	2	5	0	0	.508	
14- Micrococcus species	1	2.5	0	0	1.00	
15- Micrococcus varians	3	7.5	0	0	.262	
16- Burkholderia cepacia	2	5	1	2.5	1.00	
17- Stenotrophomonas maltophilia	3	7.5	0	0	.262	
18- Pseudomonas paucimobilis	0	0	1	2.5	.400	
19- Pseudomonas fluorescens	1	2.5	0	0	1.00	
20- Pseudomonas species	3	7.5	2	5	1.00	
21- Staph. aureus	4	10	1	2.5	.631	
22- Staph. capitis	2	5	1	2.5	1.00	
23- Staph. cohnii	2	5	0	0	.508	
24- Staph. epidermidis	3	7.5	0	0	.262	
25- Enterococcus faecalis	4	10	2	5	1.00	
26- Staph. haemolyticus	3	7.5	0	0	.262	
27- Staph. hominis	2	5	0	0	.508	
28- Staph. saprophyticus	3	7.5	2	5	1.00	
29- Staph. simulans	6	15	1	2.5	.210	
30 -Staph. warneri	4	10	0	0	.136	
31- S.xylosus	1	2.5	0	0	1.00	
32- Viridans- type streptococci	4	10	2	5	1.00	
33- Yeasts	0	0	0	0	h	

## Table 11: The isolation rates of different organisms recovered from the groin in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because GROIN is a constant.

\* Age 1- 12 years were 24 subjects

Microbial isolates	Fem	Females		les	a ulev-a	
WICTODIAL ISOlaleS	freq	%	freq	%	p-value.	
01- Acinetobacter baumannii	2	5	4	10	.381	
02- Acinetobacter Iwoffii	0	0	1	2.5	.450	
03- Alkaligenes species	2	5	0	0	.492	
04- Bacillus species	1	2.5	0	0	1.00	
05- Candida albicans	3	7.5	4	10	.680	
06- Corynebacterium species	4	10	3	7.5	1.00	
07- Chryseomonas luteola	0	0	5	12.5	.013 b	
08- Enterobacter agglomerans	3	7.5	4	10	.680	
09- Escherichia coli	0	0	0	0	h	
10- Flavobacterium meningosepticum	1	2.5	2	5	.579	
11- Klebsiella oxytoca	3	7.5	2	5	1.00	
12- Micrococcus luteus	2	5	2	5	1.00	
13- Micrococcus roseus	1	2.5	1	2.5	1.00	
14- Micrococcus species	0	0	1	2.5	.450	
15- Micrococcus varians	1	2.5	2	5	.579	
16- Burkholderia cepacia	2	5	1	2.5	1.00	
17- Stenotrophomonas maltophilia	2	5	1	2.5	1.00	
18- Pseudomonas paucimobilis	0	0	1	2.5	.450	
19- Pseudomonas fluorescens	0	0	1	2.5	.450	
20- Pseudomonas species	0	0	5	12.5	.013 b	
21- Staph. aureus	3	7.5	2	5	1.00	
22- Staph. capitis	3	7.5	0	0	.238	
23- Staph. cohnii	1	2.5	1	2.5	1.00	
24- Staph. epidermidis	1	2.5	2	5	.579	
25- Enterococcus faecalis	1	2.5	5	12.5	.073	
26- Staph. haemolyticus	2	5	1	2.5	1.00	
27- Staph. hominis	2	5	0	0	.492	
28- Staph. saprophyticus	3	7.5	2	5	1.00	
29- Staph. simulans	6	15	1	2.5	.105	
30 -Staph. warneri	2	5	2	5	1.00	
31- S.xylosus	1	2.5	0	0	1.00	
32- Viridans- type streptococci	4	10	2	5	.673	
33- Yeasts	0	0	0	0	h	

## Table 12: The isolation rates of different organisms recovered from the groin in relation to sex

a. Using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha {=} 0.05$ 

b. The test is significant (otherwise the test is not significant).

h. No statistics are computed because GROIN is a constant.

Microbial isolates	age 1-12*		age 13-17**		n volue?
	freq	%	freq	%	p-value"
01- Acinetobacter baumannii	3	7.5	1	2.5	.638
02- Acinetobacter Iwoffii	3	7.5	0	0	.262
03- Alkaligenes species	4	10	0	0	.136
04- Bacillus species	0	0	0	0	h
05- Candida albicans	0	0	1	2.5	.400
06- Corynebacterium species	2	5	0	0	.508
07- Chryseomonas luteola	2	5	0	0	.508
08- Enterobacter agglomerans	2	5	0	0	.508
09- Escherichia coli	0	0	0	0	h
10- Flavobacterium meningosepticum	3	7.5	1	2.5	.638
11- Klebsiella oxytoca	1	2.5	1	2.5	1.00
12- Micrococcus luteus	3	7.5	0	0	.262
13- Micrococcus roseus	4	10	0	0	.136
14- Micrococcus species	3	7.5	0	0	.262
15- Micrococcus varians	2	5	0	0	.508
16- Burkholderia cepacia	1	2.5	2	5	.553
17- Stenotrophomonas maltophilia	1	2.5	1	2.5	1.00
18- Pseudomonas paucimobilis	0	0	0	0	h
19- Pseudomonas fluorescens	2	5	1	2.5	1.00
20- Pseudomonas species	2	5	1	2.5	1.00
21- Staph. aureus	4	10	0	0	.136
22- Staph. capitis	1	2.5	0	0	1.00
23- Staph. cohnii	3	7.5	1	2.5	.638
24- Staph. epidermidis	3	7.5	0	0	.262
25- Enterococcus faecalis	3	7.5	1	2.5	.638
26- Staph. haemolyticus	4	10	0	0	.136
27- Staph. hominis	3	7.5	0	0	.262
28- Staph. saprophyticus	2	5	0	0	.508
29- Staph. simulans	3	7.5	0	0	.262
30 -Staph. warneri	5	12.5	0	0	.071
31- S.xylosus	3	7.5	0	0	.262
32- Viridans- type streptococci	1	2.5	1	2.5	1.00
33- Yeasts	2	5	0	0	.508

### Table 13: The isolation rates of different organisms recovered from the leg in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because LEG is a constant.

\* Age 1- 12 years were 24 subjects

\*\* Age 13 -17 years were 16 subjects\* Age 1- 12 years were 24 subjects

Microbial isolates	Females		Males		n voluo <sup>a</sup>
	freq	%	freq	%	p-value-
01- Acinetobacter baumannii	2	5	2	5	1.00
02- Acinetobacter Iwoffii	3	7.5	0	0	.238
03- Alkaligenes species	3	7.5	1	2.5	.613
04- Bacillus species	0	0	0	0	h
05- Candida albicans	1	2.5	0	0	1.00
06- Corynebacterium species	2	5	0	0	.492
07- Chryseomonas luteola	2	5	0	0	.492
08- Enterobacter agglomerans	2	5	0	0	.492
09- Escherichia coli	0	0	0	0	h
10- Flavobacterium meningosepticum	3	7.5	1	2.5	.613
11- Klebsiella oxytoca	2	5	0	0	.492
12- Micrococcus luteus	2	5	1	2.5	1.00
13- Micrococcus roseus	3	7.5	1	2.5	.613
14- Micrococcus species	1	2.5	2	5	.579
15- Micrococcus varians	1	2.5	1	2.5	1.00
16- Burkholderia cepacia	3	7.5	0	0	.238
17- Stenotrophomonas maltophilia	1	2.5	1	2.5	1.00
18- Pseudomonas paucimobilis	0	0	0	0	h
19- Pseudomonas fluorescens	2	5	1	2.5	1.00
20- Pseudomonas species	1	2.5	2	5	.579
21- Staph. aureus	1	2.5	3	7.5	.310
22- Staph. capitis	1	2.5	0	0	1.00
23- Staph. cohnii	2	5	2	5	1.00
24- Staph. epidermidis	2	5	1	2.5	1.00
25- Enterococcus faecalis	3	7.5	1	2.5	.613
26- Staph. haemolyticus	3	7.5	1	2.5	.613
27- Staph. hominis	2	5	1	2.5	1.00
28- Staph. saprophyticus	2	5	0	0	.492
29- Staph. simulans	2	5	1	2.5	1.00
30 -Staph. warneri	4	10	1	2.5	.355
31- S.xylosus	3	7.5	0	0	.238
32- Viridans- type streptococci	2	5	0	0	.492
33- Yeasts	2	5	0	0	.492

### Table 14: The isolation rates of different organisms recovered from the leg in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

h. No statistics are computed because LEG is a constant.

Microbial isolates	age 1-12*		age 13-17**		
	freq	%	freq	%	p-value-
01- Acinetobacter baumannii	1	2.5	0	0	1.00
02- Acinetobacter Iwoffii	3	7.5	0	0	.262
03- Alkaligenes species	1	2.5	0	0	1.00
04- Bacillus species	1	2.5	2	5	.553
05- Candida albicans	3	7.5	3	7.5	.668
06- Corynebacterium species	3	7.5	0	0	.262
07- Chryseomonas luteola	5	12.5	0	0	.071
08- Enterobacter agglomerans	1	2.5	0	0	1.00
09- Escherichia coli	3	7.5	2	5	1.00
10- Flavobacterium meningosepticum	3	7.5	1	2.5	.638
11- Klebsiella oxytoca	4	10	0	0	.136
12- Micrococcus luteus	4	10	0	0	.136
13- Micrococcus roseus	3	7.5	0	0	.262
14- Micrococcus species	1	2.5	1	2.5	1.00
15- Micrococcus varians	4	10	1	2.5	.631
16- Burkholderia cepacia	6	15	0	0	.064
17- Stenotrophomonas maltophilia	3	7.5	1	2.5	.638
18- Pseudomonas paucimobilis	2	5	0	0	.508
19- Pseudomonas fluorescens	3	7.5	1	2.5	.638
20- Pseudomonas species	2	5	1	2.5	1.00
21- Staph. aureus	5	12.5	1	2.5	.373
22- Staph. capitis	5	12.5	0	0	.071
23- Staph. cohnii	3	7.5	0	0	.262
24- Staph. epidermidis	3	7.5	1	2.5	.638
25- Enterococcus faecalis	4	10	0	0	.136
26- Staph. haemolyticus	4	10	1	2.5	.631
27- Staph. hominis	1	2.5	0	0	1.00
28- Staph. saprophyticus	2	5	0	0	.508
29- Staph. simulans	2	5	0	0	.508
30 -Staph. warneri	2	5	1	2.5	1.00
31- S.xylosus	2	5	0	0	.508
32- Viridans- type streptococci	2	5	0	0	.508
33- Yeasts	2	5	0	0	.508

## Table 15: The isolation rates of different organisms recovered from the toe webs in relation to age

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha$ =0.05

\* Age 1- 12 years were 24 subjects

Microbial isolates	Females		Males		
	freq	%	freq	%	p-value"
01- Acinetobacter baumannii	1	2.5	0	0	1.00
02- Acinetobacter Iwoffii	1	2.5	2	5	.579
03- Alkaligenes species	0	0	1	2.5	.450
04- Bacillus species	1	2.5	2	5	.579
05- Candida albicans	3	7.5	3	7.5	1.00
06- Corynebacterium species	2	5	1	2.5	1.00
07- Chryseomonas luteola	1	2.5	4	10	.155
08- Enterobacter agglomerans	0	0	1	2.5	.450
09- Escherichia coli	2	5	3	7.5	.642
10- Flavobacterium meningosepticum	2	5	2	5	1.00
11- Klebsiella oxytoca	2	5	2	5	1.00
12- Micrococcus luteus	1	2.5	3	7.5	.310
13- Micrococcus roseus	2	5	1	2.5	1.00
14- Micrococcus species	1	2.5	1	2.5	1.00
15- Micrococcus varians	3	7.5	2	5	1.00
16- Burkholderia cepacia	2	5	4	10	.381
17- Stenotrophomonas maltophilia	2	5	2	5	1.00
18- Pseudomonas paucimobilis	1	2.5	1	2.5	1.00
19- Pseudomonas fluorescens	1	2.5	3	7.5	.310
20- Pseudomonas species	0	0	3	7.5	.083
21- Staph. aureus	3	7.5	3	7.5	1.00
22- Staph. capitis	2	5	3	7.5	.642
23- Staph. cohnii	2	5	1	2.5	1.00
24- Staph. epidermidis	2	5	2	5	1.00
25- Enterococcus faecalis	2	5	2	5	1.00
26- Staph. haemolyticus	2	5	3	7.5	.642
27- Staph. hominis	1	2.5	0	0	1.00
28- Staph. saprophyticus	1	2.5	1	2.5	1.00
29- Staph. simulans	0	0	2	5	.196
30 -Staph. warneri	1	2.5	2	5	.579
31- S.xylosus	0	0	2	5	.196
32- Viridans- type streptococci	1	2.5	1	2.5	1.00
33- Yeasts	1	2.5	1	2.5	1.00

### Table 16: The isolation rates of different organisms recovered from the toe webs in relation to sex

a. All tests are not significant by using Fisher's Exact test (Exact Sig. 2 sided) using  $\alpha{=}0.05$ 



Figure 1 The isolation rates of different organisms recovered from the seven sites of the skin in relation to age

\* The microbial isolates are numbered as shown in Tables 1-16



Figure 2: Isolation rates of *Staph aureus* among children and adults from seven skin sites



Figure 3: Isolation rates of *Staph. aureus* among females and males from seven skin sites



Figure 4: Isolation rates of *Acinetobacter baumannii* among children and adults from seven skin sites



Figure5: Isolation rates of *Acinetobacter Iwoffii* among children and adults from seven skin sites



Figure 6: Isolation rates of *Candida albicans* among children and adults from seven skin sites



Figure7: Isolation rates of *Candida albicans* among females and males from seven skin sites

#### Discussion

### Reproducibility of the sampling method:

are different Although there methods for the investigation of skin microflora, rubbing technique was used for sampling the skin in the present study as a qualitative method rather than quantitative. Moistening the cotton swab with 0.5% Tween 80 and scrub bing the skin with moderate pressure for one minute, gave reproducible results. Agitation of the samples in addition to using a surfactant substance (Tween 80) allowed the removal of bacteria from the skin and the dispersal of the majority of microorganisms held on the cotton swab; moreover this procedure had lead to the breakdown of bacterial aggregates, thus achieving an optimal recovery of organisms (DeCoursey et.al., 1956; Milyani and Selwyn, 1976). In the standardized swabbing method by Selwyn and Ellis (1972), Triton X-100 was used as a surfactant substance. However, Tween 80 was replaced in

this study since Triton X-100 was found to have to some extent bactericidal activity if there was a delay in processing the specimen for more than three hours. The lipophilic Corvnebacterium species are destroyed (Noble and Somerville, 1974). Furthermore, the variety of media and identification techniques used in the present work allowed adequate isolation and identification of various species and a higher yield of organisms.

#### Incidence of the isolated microorganisms:

Thirty three species including Gram positive and Gram negative microorganisms have been isolated from the seven sites of the skin; the Gram positive microorganisms comprised 20 species while the Gram negative ones comprised 13 species. It is well known that Gram negative rods other than *Acinetobacter species* are rare on normal skin, and only occasionally are found in quantity in moist areas (Noble and Somerville, 1974). In the present study, the incidence of A. baumannii and A. lwoffii dominated among children within the seven sites, comprising 30% of each isolate. However, the isolation rates of these species varied in each separate site (Figures 4-5), and this is in agreement with researchers who isolated this different species from skin sites (Marples, 1965: Somerville, 1966: Noble and Somerville, 1974). Though, the role of Acinetobacter species as a ubiquitous opportunistic pathogen is now appreciated (Mandell, et.al., 2000). Surprisingly, other Gram negative rods isolates followed in prevalence among the same group within all sites with significant difference namely: Alkaligenes species, C. luteola (an incidence of 27.5% each); E. agglomerans (22.5%) and E. coli (15%). On the other hand, F. meningosepticum (15%); K. oxytoca (20%), B. cepacia (22.5%) and different species of Pseudomonas (7.5% - 17.5%)were isolated from children and adults and females and males but no significant difference was evident. Also, similar incidence was noted between different sites among the age groups. However, in relation to sex, significant differences were recorded only in the isolation rates of C. luteola and P. species (P=0.013) each) in the groin area among males; and this could be attributed to the micro-climate and humidity at this occluded area which favoured the establishment of these species. Differences were expected between children and adults especially in the microbial flora of the axilla, since axillary hair was not removed in addition to the presence of excessive sebum in adults, thus providing green a house atmosphere. Yet the isolation rates of microbial isolates were not significantly different at this site in spite of variations

hygienic habits, dietary habits, in different activities and hormonal factors between children before puberty and adults after puberty in both sexes which are known to play a role in the qualitative and quantitative composition of skin microbial flora (Marples, 1965; Noble and Somerville, 1974; Skinner and Carr, 1974). In this respect, Prophet Mohammad peace be upon him 14 century ago instructed us to remove axillary and pubic hair periodically.

Although, desiccation is known to be the prime reason for the failure of many organisms to survive on skin, such as the Gram negative rods vet. а considerable species numbers of these organisms have been isolated. However, it has been reported that increasing humidity lead to an immense increase in the bacterial population (Marples, 1965; Noble and Somerville, 1974); and these paradoxical results may be attributed to the humid and hot climate of Jeddah city which may have encouraged these organisms to survive on the skin of the study group. Factors such as occlusion (clothing), hormonal changes, nutrients, pH, body temperature and hygiene should not be ignored since they are indeed important in the diversity of microbial flora not only from time to time but also from site to site and within the same site (McBride et.al, 1975; McBride et.al, 1977). Moreover, most of the Gram negative rods that have been isolated in this study have not been isolated elsewhere as normal skin flora, but have been reported only as common from clinical specimens isolates especially from compromised patients (Noble, 1981; Koneman et.al., 1997; Mandell, et.al., 2000).

The Gram positive isolates consisted of rods and cocci, where *Corynebacterium species* (coryneforms) *and Bacillus species* represented the rods, while members of the Family Micrococcaceae, viridans-type streptococci and yeasts represented the coccal forms in the present work. The isolation of coryneforms from the skin is expected since it is well established that they are dominant residents of the skin flora and mucous surfaces. Coryneforms have been isolated from the seven skin sites in different percentage among the study group as well as by others (Marples, 1969b; Noble and Somerville, 1974; Maibach and Aly, 1981). Unfortunately, because of their varied characteristics and unavailable (local) simple identification methods, further identification up to the species level was hindered in this work. On the other hand the recovery of Bacillus species is considered as transient flora, nonetheless, it has been implicated in serious nosocomial infections and in intravenous drug abusers injection (Sliman, et. al., 1987: Richard, et.al., 1988: Mandell, et.al 2000)

The Family Micrococcaceae includes the genus Staphylococcus and the genus Micrococcus. The genus *Staphylococcus* comprises the coagulase negative staphylococci and the coagulase positive staphylococci, the former staphylococci are the second normal resident flora of the human skin, in addition to members of the genus (Leyden *et.al.*, Micrococcus 1981; Levden et.al., 1987). Different species of coagulase negative staphylococci, micrococcus and coagulase positive staphylococci namely: Staph. aureus have been isolated in the present work at different from the seven sites percentage as shown in all Tables. Other researchers have also reported findings (Kloos similar and Musselwhite, 1975; Maibach and Aly, 1981; Romero, et. al., 1990). However, Staph. aureus was not recovered from chest and legs of adults and surprisingly

also from forehead and anterior nares of both adults and males (Figures 2-3), though Milyani and Memish (1987) reported a carriage rate of 12% and 9% in the throat of children and adults in Jeddah city and from the anterior nares of female adults (Milyani, unpublished data). However, significant difference was not found when each site was studied separately at the present study, and this could be attributed to the rather small number of subjects investigated.

Among the streptococci, viridans type *streptococcus* was isolated at variable rates from different sites. Though, in other studies streptococci were not recovered frequently from skin (unpublished data). However, Noble (1981) reported that Alpha and Gamma -haemolytic streptococci are found well over the body especially in infants and other children. On the hand. Streptococcus pyogenes was not recovered in this work as expected. Apart from bacterial species. С. albicans (Figure 6-7) and yeasts were noted among the aerobic isolated organisms in different sites of the skin and at different isolation rates, this is also in accordance with Marples, (1965); Noble, (1981) and O'Connell et. al., (1995).

#### The role of the isolated organisms in endogenous, exogenous and nosocomial infections:

The diversity of microorganisms isolated from the skin in the present study highlights the importance of pursuing similar investigation on a wide scale; since it is known that every individual movement an makes dislodges epidermal fragments which act as 'rafts', carrying microorganisms to the surrounding habitat (Smith and Bruch, 1969). Thus, most of these isolates or the carriage of a true pathogen might be a potential risk for individual himself or the others.

especially, in the hospital environment. It has been documented that Coagulase negative staphylococci especially Staph. epidermidis are significant nosocomial pathogens complicating central venous catheters, indwelling prosthetic medical devices, intensive care units, catheter related septisaemia and a cause of infection of peritoneal dialysis catheters and postoperative wound infections Lowy, et. al., 1983; Andremont, et. al. 1988; Boyce, et. al., 1990; Kamath et. al., 1992; Mandell, et. el. 2000). It is obvious that the source of these organisms could be mainly from the skin or the body normal flora in addition to the hospital environment (Maibach and Aly, 1981; Benson, 2000). Staph. aureus, Micrococcus, Corynebacterium, Acinetobacter, Pseudomonas, a wide variety of Gram negative rods and C. albicans are also incriminated in a wide range of endogenous, exogenous and nosocomial infections particularly, in compromised patients (Flynn, et. el. 1987; Govan, et. al. 1996; Rangel-Frausto, et. al. 1999).

In view of the most common organisms encountered in three main hospitals in Jeddah city during two years (1995-1997), the predominant organisms reported were Staph. aureus, Staph. epidermidis, Staph. Haemolyticus, Staph. saprophyticus, Staph. warneri, Staph. capitis, E. faecalis, E. agglomerans, E. coli, Proteus, Serratia, C. luteola, S. maltophilia, F. meningosepticum, Klebsiella oxytoca, Klebsiella pneumoniae, B. cepacia, Pseudomonas species, viridans streptococci and yeasts. These organisms were incriminated in upper respiratory tract, urinary tract, postoperative wound and infections, bacteraemia, burn and infections related to indwelling medical devices and in intensive-care units (personal communication). Preventive measures should be undertaken and

strictly followed to reduce the morbidity and mortality associated with microbial infections. Basic hygiene, hand hygiene and care with catheter insertion and maintenance using aseptic techniques, are of all importance in prevention. Also, rapid diagnosis and aggressive effective treatment may reduce morbidity and mortality, bearing in mind the growing microbial resistance in hospitals, at different parts of the world (Bergogne-Berezin, et. al., 1993; Roberts, et. al., 2001; Wang, et. al., 2001).

#### Acknowlegment

I am indebted to King Abdulaziz University for the financial support of this project. I am also grateful to Professor W. C. Noble(St John's Hospital for Diseases of the Skin, London) for his valuable consultations at the beginning of this work. I also acknowledge the help of Miss Fatin Alharatani (B.Sc.) who carried out the technical work with patience and accuracy.

#### References

- 1. Andremont, A.; Paulet, R.; Nittenberg, G.; Hill, C. (1988) Value Of Semiquantitative Cultures of Blood Drawn through Catheter Hubs for Estimating the Risk of Catheter Tip Colonization in Cancer Patients. J. Clin. Microbio 26:2297-2299.
- 2. Benson, K. (2000) Bacterial Contamination of Blood Components. *Infect Med* 17(4): 248-250.
- 3. Bergogne-Berezin, E.; Decre, D.; Joly-Guillou, ML. (1993) Opportunistic nosocomial multiply resistant bacterial infections- their treatment and prevention. *J Antimicrob-Chemother*. 32 suppl A: 39-47.
- Boyce, M. J.; Bynoe, P. G.; Opal, M. S. (1990) A common-Source Outbreak of *Staphylococcus epidermidis* Infections among Patients Undergoing

Cardiac Surgery. J Infect Diseases 161: 493-499.

- De Coursey, J.D., McGuire, C.D., Otto, J. S. and Durant, R. C., (1956). T he role of flies in the transmission of non-enteric diseases in the Middle East, *Naval Medical Research Unit No.3, Cairo, Egypt, Report* pp. 1-4.
- Flynn, D.M.; Weinstein, R.A.; Nathan, C. et. el. (1987) Patients' endogenous flora as the source of "nosocomial" *Enterobacter* in cardiac surgery. J Infect Dis 156: 363-368.
- Govan, J.R.; Hughes, J.E.; Vandamme, P. (1996) Burkholderia cepacia: medical, taxonomic and ecological issues. J. Med Microbiol. 45(6): 395-407.
- Kamath, U.; Singer, C.; Isenberg, D. H. (1992) Clinical Significance of *Staphylococcus warneri* Bacteremia. *J. Clin Microbio* 30: 261-264.
- Kloos, W. E.; and Musselwhite, M. S. (1975) Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl. Microbiol.* 30: 81-395.
- Koneman, W. E.; Allen, D. S.; Janda, M. W. et. al. (1997) Color Atlas and Text Book of Diagnostic Microbiology. Lippincott- raven publishers.
- 11. Leyden, J, K. McGinley, E. Hoelzle, J. Labows, and kligman, A. (1981) The microbiology of the human axilla and its relationship to axillary odor. *J. Invest. Dermatol.* 77: 413-416.
- 12. Leyden, J, K. McGinley, K. Nordstrom, and G. Webster. (1987) Skin Microflora. J. Invest. Dermatol. 88(Suppl.): 65-72.
- **13. Lowy, D. F. and Hammer, M. S.** (1983) Staphylococcus epidermidis Infections. Annals of Internal Medicine 99: 834-839.
- 14. Maibach, H. I. and Aly Raza (1981) Skin Microbiology, Relevance to Clinical Infection. Springer-Verlag, New York
- 15. Maibach, H. T. and Hildick-Smith, G. (1965) Skin Bacteria and their Role in Infection. McGraw-Hill, New York.

- Mandell, L.G.; Bennett, E.J.; Dolin, R. (2000) Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Churchill Livingstone. New York, London.
- 17. Marples, M. I. (1965a) The Ecology of the Human Skin. Springfield, Illinois, Thomas.
- 18. Marples, R. R. (1969b) Diphtheroids of normal human skin. *Br. J. Derm.*, 81, supplement 1, 47-54.
- 19. McBri de, M. E., Duncan, W. C. and Knox, J. M. (1975) Physiological and environmental control of Gramnegative bacteria on skin. *Br. J. Derm.*, 93, 191-99.
- 20. McBride, M., Duncan, W. and Knox J. (1975) The Environment and the microbial ecology of human skin. *Appl Environ. Microbiol.* 33: 603-608.
- 21. Milyani, M. R. (1976) Studies on interactions of human skin microorganisms on solid surfaces. Ph.D. Thesis, University of London.
- 22. **Milyani, M. R. (1998)** Principles of Medical Bacteriology (Arabic Language). Bookshops, Publishing and distribution Co., Ltd. Jeddah, Saudi Arabia: PP 13-15; 219.
- 23. Milyani, M. R. and Selwyn, S. S. (1976) Errors in viable counts due to bacterial aggregations. *J. Clin. Path.* Abstract, 9.
- 24. Milyani, M. R. and Memish A. T. (1987) Studies on Throat Microbial Flora in Jeddah, Saudi Arabia, II. Occurrence of Pathogenic Bacteria in the Throat of Female School Children and University Students. *Researches Sci*, *K.A.U.* pp 123-128.
- 25. Milyani, R. M.; Memish A. T. and A. H. Salama (1987). Studies on Throat Microbial Flora in Jeddah, Saudi Arabia, I. In Relation to Age and Environmental Factors. *Researches Sci.*, *K.A.U.* pp 109-122.
- 26. Noble, W. C. (1983) Microbial Skin Disease: its epidemiology. Edward Arnold (publishers) Ltd.
- 27. Noble , W. C. and Somerville, D. A. (1974) Microbiology of Human Skin. W. B. Saunders Co. Ltd., London.

- 28. O'Connell, B.; Coleman, D.C.; Bennett, D. et. al. (1995) An epidemiological study of *Candida* 
  - p-value<sup>a</sup> age 13-17\*\* age 1-12\* Microbial isolates

p-value<sup>a</sup> age 13-17\*\* age 1-12\*

p-value<sup>a</sup> age 13-17\*\*

#### p-value<sup>a</sup>

species infection in cancer patients using genetic fingerprinting and morphotyping. *J Hosp Infect* 31(3): 2117-7.

- 29. Rangel-Frausto, M.S.; Wiblin, T.; Blumberg, H.M. et. al. (1999) National epidemiology of mycosis survey (NEMIS): variations in rates of blood stream infections due to Candida species in seven surgical intensive care units and six neonatal intensive care units. *Clin Infect Dis 29: 253-58*.
- Richard, V. ; Van der Auwera P, Smoek R, et. Nosocomail bacteremia caused by Bacillus species. *Eur J Clin Microbiol Infect Dis.* 1988; 7:783 – 785.

- 31. Roberts, S.A.; Findlat, R.; Lang S.D. (2001) Investigation of an outbreak of multi-drug resistant *Acinetobacter* es baumannii in an intensive care burns unit. J Hosp Infect. 48: 228-32.
  - 32. Romero, S. ; Witek, T.; Balish, E. (1990) Adherence of Skin Bacteria to Human Epithelial Cells. *J. Clin. Microbio.* 28: (1) 27-31.
  - 33. Selwyn, S. and Ellis, H. (1972) Skin bacteria and skin disinfection reconsidered. *Br. Med. J.*, *I*, 136-40.
  - 34. Sethna, N.T. (1978) In Vitro and In Vivo Studies of An Antibiotic-Producing Staphylococcus From Human skin. Ph.D. Thesis, University of London.
  - 35. Skinner, A. F. and Carr, G. J. (1974) The Normal Microbial Flora of Man. Academic Press. London. New York.
- 36. Sliman, R; Rehm, S; Shlaes, D. M. Serious infections caused by Bacillus species. Medicine. (1987); 66:218 – 223
- 37. Smith, F. W. and Bruch, M. (1969) Reduction of Microbiological Shedding in Clean Rooms. *Devs. Ind. Microbiol.* 10, 290.
- 38. Wang, JT; Chang, Sc; KoW, J. et. al.(2001) A hospital-acquired outbreak of methicillin-resistant *Staphylococcus* aureus infection initiated by a surgeon carrier. J Hosp Infect 47: 104-09.

Rajaa M. Milyani

لقد تم التعرف على الفلور الميكروبية الهوائية لجلد أربعين شخصاً يعيشون في مدينة جدة (المملكة العربية السعودية). وتضمنت الدراسة مجموعتين من الأعمار : أطفال وبالغين من الذكور والإناث، شملت سبعة مناطق: الجبين، الابط، الارب، الرجل، الجليدة بين أصابع القدم ثم فتحتي الأنف الأمامية. وقد أخذت عينات الجلد بدعك المنطقة بواسطة ماسحة قطنية مشبعة بمادة منظفة للجلد (توين 80) توضع بعدها في الوعاء المحتوي على هذه المادة ثم يخض العلق الناتج لمدة دقيقة وإحدة.

عزلت ثلاث وثلاثون نوعاً من السبع مناطق من المجموعة قيد الدر اسة حيث سادت بين الأطفال كل من أساينتوبكتر بوميناي، أساينتوبكتر لوفاي، كور اينبكتيريم، والمكورات العنقودية الذهبية ( بنسبة 30% لكل منها). ومن أكثر العز لات الأخرى شيوعاً: نوع من الكالاجينز، نوع من باسبللاس، كر ايز وموناس لاتيو لا، المكور ات العنقو دية الجلّدية، المكور ات المعوية البر ازية و المكور ات العنقودية هو ميناس (بنسبة 27% لكل منها). كما ظهرت مبكر وبات مثل: كانديدا البيكانز، إينتير وبكتر أجلو مبر انس، الإيشير بشيا القولونية، فلافوبكتيريم مينينجوسيبتيكم، كليبسيللا أوكزيتوكا، المكورات الدقيقة لوتيس، المكورات الدقيقة الوردية، المكورات الدقيقة فيرينس، نوع من المكورات الدقيقة، بيركهولديريا سيبيشيا، ستينوتر وفوموناس مالتوفيليا، سودوموناس بوسيموبيلاس، سودوموناس فلوريسانس، نوع من السودوموناس، المكورات العنقودية كابيتيس، المكورات العنقودية كوناي، المكور اتَّ العنقودية المتر ممة، المكور ات العنقودية سيميو لانس، المكور ات العنقودية. ورنيراي، المكورات العنقودية زايلوزيس، المكورات العقدية من الأنواع المخضرة ث و خمائر بنسب مختلفة. كما لوحظ لدى الأطفال في السبع مناطق نسب أعلى من: أسابنتوبكتر لوفاي، المكورات العنقودية الذهبية، نوع من الكالاجينز، كوراينبكتيريم، كرايزوموناس لاتيولًا، إينتيروبكتر أجلوميرانس، المكورات العنقودية الجلدية ومكورات عنقودية اخرى سالبة للكوأجيوليز. بينما وجد كل من كرايز وموناس لاتيو لا ونوع من السودوموناس في منطقة الإرب لدى الذكور فقط عدا ذلك لم تسجل أي فروقات معنوية في نسب العزل لكل منطقة على حدة بالنسبة للعمر والجنس

وقد تم التأكيد على دور الميكروبات المعزولة في العدوى الداخلية والخارجية والعدوى المكتسبة داخل المستشفيات.