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Article

Effect of environmental factors on bacterial flora of normal human skin

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SUMMARY

Human skin hosts an abundance of diverse microorganisms including bacteria, fungi and viruses that constitute the cutaneous microbiome. This microbiome is responsible for homeostasis, immune modulation, and protection from pathogens. The resident bacterial flora of the skin is generally innocuous in healthy individuals but can become a source of disease in those who are immunocompromised. The bacterial flora is implicated in infectious and non-infectious diseases of the skin such as non-healing ulcers, acne, and atopic and seborrheic dermatitis. Bacterial colonization of the skin depends on the characteristics of the local microenvironment – acidity, temperature, humidity, nutrient levels, etc. However, the effect of external environmental conditions on the composition of bacterial flora are unknown. We hypothesized that exposure to hot and humid environmental conditions could result in a microflora characterized by more pathogenic species of bacteria compared to that of people exposed to cooler and dryer conditions. The bacterial flora of the cubital fossa of two cohorts, one working in hot and humid conditions, and the other working in cold and dry conditions was analyzed by culture techniques. The most common species isolated in both groups was *Staphylococcus hominis ssp hominis*. *Staphylococcus saprophyticus* was found in individuals working in cold and dry environments while hemolytic coagulase-negative *Staphylococci*, which are known to be more pathogenic, were found only in individuals working in hot and humid environments. Although these differences were not statistically significant, we concluded that individuals exposed to different environmental conditions may have differences in their skin bacterial flora and hence, in their susceptibility to disease.

INTRODUCTION

The human skin hosts an abundance of diverse microorganisms including bacteria, fungi, and viruses that constitute the cutaneous microbiome. As the largest organ of the human body, skin harbors 10^3 bacteria per square centimeter, which adds up to millions of bacteria across the whole body (1, 2). A few minutes after birth, newborn skin begins getting colonized by bacteria. This initial colonization develops into a stable population as the child grows (2). The resident flora of the skin is generally innocuous in healthy individuals but can become a source of disease in immunocompromised individuals (3).

Skin bacteria have a profound significance on various aspects of an individual's life. They play a crucial role in health maintenance and disease causation by protecting the skin from pathogen colonization, antigen presentation, and immune modulation (4). The bacterial flora is thought to be instrumental in giving rise to infectious and non-infectious diseases of the skin such as non-healing ulcers, acne, atopic dermatitis and seborrheic dermatitis (4). Dysbiosis, an imbalance in the composition of the bacterial flora, is known to have profound consequences in mediating disease and ultimately, causing mortality in an individual (1, 3).

Other ways in which the bacterial flora of the skin interacts with the host and environment are still being elucidated (1, 3). Three possible interactions between bacteria and the host include a symbiotic relationship, where bacteria compete with pathogens for colonization, thereby preventing disease or by producing useful substances for the host; a pathogenic relationship, where bacteria cause disease; or a commensal relationship, where bacteria exist neutrally without causing harm or benefit (5).

Normal skin presents a harsh physical environment which is dry, nutrient-poor, and acidic. Bacterial colonization of the skin depends on the characteristics of the local microenvironment including acidity, temperature, humidity, sebum content, topography, ultraviolet light exposure, and nutrient levels (2, 6). Based on these factors, cutaneous microenvironments can be divided into three groups: sebaceous or oily, found on the face, chest, and back; moist, found on the elbow, back of knee, groin, axilla, perineum, and toe webs; and dry, found on volar forearm and palm. The variation in local conditions leads to differences in bacterial species found in each of these areas (2, 4). Skin sites with higher levels of moisture, higher body temperature, and higher surface lipid content, like the axilla, perineum, and toe webs, have a higher concentration of bacteria and often contain more gram-negative bacilli compared to drier and cooler sites such as legs, arms, and trunk (6).

A previous study on the effects of environmental factors such as ambient temperature and humidity identified quantitative differences in bacterial prevalence with a greater bacterial load in warmer and more humid conditions (4). Further work has shown that occupational conditions can have a profound impact on the human skin microbiome (7). A study conducted in Texas found a significantly higher microbial skin population on the axilla, back, and feet among the participants who worked in hot and humid environmental conditions (laborers and coast guards) as compared to medical personnel, who predominantly work in controlled

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environmental conditions (8). However, they did not find any significant differences in the species isolated under different ambient conditions (8). A study conducted in Gambia in 2019 found that the prevalence of bacterial skin infections in children (most commonly caused by *Staphylococcus aureus* and *Streptococcus pyogenes*) increased significantly during the rainy season (9). This suggests that higher heat and humidity create conditions for colonization of skin and survival of more pathogenic bacteria. Hence, it is plausible that the composition of skin's bacterial flora is influenced by the particular set of environmental conditions that people are exposed to due to occupational choices, economic challenges, or other reasons. This would have implications for their health and susceptibility to disease as dysbiosis would predispose them to more serious infections in case of immunocompromise.

We hypothesized that individuals exposed to hot and humid environmental conditions will exhibit a skin microbiome with a higher proportion of potentially pathogenic bacterial species compared to those living in cooler and drier environments. This would lead to dysbiosis, increasing the incidence of diseases and mortality in an individual (1, 3). We investigated the influence of external environmental factors – ambient temperature and humidity, on the bacterial flora of normal human skin. Since bacterial flora plays an integral part in both infectious and non-infectious diseases, it is important to understand the factors that affect its composition. We also sought to understand whether environmental conditions contribute to an increase in drug-resistance in the normal bacterial flora of the skin. We collected skin swabs from the cubital fossa of 20 individuals (divided into two groups) to elucidate the normal bacterial composition of human skin and compare sensitivity of bacteria in both groups to clinically relevant antibiotics. We found that more pathogenic bacteria (*Staphylococcus hemolyticus*) were present in the hot and humid group, whereas the cold and dry group had a prevalence of more benign species (*Staphylococcus saprophyticus*). These differences indicate that differences in environmental conditions may affect individual susceptibility to disease with individuals in hot and humid conditions being more predisposed than those in cooler and drier conditions.

RESULTS

Environmental variables

Our study had two cohorts that allowed us to find out how ambient temperature and humidity affected the composition of the bacterial skin flora. One cohort was exposed to hot and humid conditions (security guards and construction workers) and the other cohort worked in a cold and dry (air-conditioned) environment (laboratory personnel and pharmacy assistants). Cubital fossa skin swabs were collected from individuals in each cohort and species identification was done by bacterial culture methods.

To ensure our two cohorts were distributed equally, we stratified the demographic information of all participants from both groups: "hot and humid" and "cold and dry". The ambient temperature and humidity data for both groups of participants were analyzed to ensure significant difference between the environment among the two groups. Further, the skin site temperature of all participants from both groups was measured **(Table 1)**.

The average age of participants in the hot and humid group was 34.7 years (σ = 12.83 years), while the average age of participants in the cold and dry group was 33.4 years (σ = 12.80 years). There was no significant difference in the age distribution between the two groups (p = 0.823). The average ambient temperature in hot and humid group was 30.4°C (σ = 0.44°C), and in the cold and dry group, it was 24.2°C (σ = 0.35°C). The ambient temperature between the two groups was significantly different (p = 0.00). The average

Hot and Humid					
Sample number	**Age (Years)**	**Sex (M/F)**	**Ambient temperature (°C)**	**Relative Humidity (%)**	**Skin surface temperature (°C)**
S01	51	M	29.7	75	32
S02	35	M	30.6	76	32.2
S03	40	M	30.6	76	32.4
S04	26	M	30.6	76	32
S05	56	M	30.5	80	32.4
S06	20	M	30.5	80	32.7
S07	29	F	30.5	80	31.8
S08	18	M	29.7	65	32.4
S09	28	F	30.4	75.4	31.9
S10	44	M	31.2	71	31.1
Cold and Dry					
Sample number	**Age (Years)**	**Sex (M/F)**	**Ambient temperature (°C)**	**Relative Humidity (%)**	**Skin surface temperature (°C)**
S11	59	F	23.8	49	31.7
S12	23	F	23.8	49	33.4
S13	46	M	23.8	49	28.1
S14	28	M	24.2	45	33.2
S15	23	M	24.2	45	30.9
S16	48	F	24.2	45	33.1
S17	27	M	23.8	49	31.7
S18	30	M	24.6	55	31.2
S19	23	M	24.6	55	32.4
S20	27	F	24.6	55	31.8

Table 1: Participant data of both experimental groups. Data was collected for age, gender, ambient temperature, relative humidity, and skin surface temperature at the time of swabbing for participants in both groups.

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relative humidity in hot and humid group was 75.4 % (σ = 4.62%), and in the cold and dry group, it was 49.6% (σ = 4.11%). The ambient humidity between the two groups was also significantly different (p = 0.00). The average skin site surface temperature recorded for the hot and humid group was 32.1°C (σ = 0.45°C), whereas for the cold and dry group it was 31.75°C (σ = 1.54°C). The skin surface temperature variance was not statistically significant (p = 0.512).

Bacterial identification

We tested 10 isolates each in the hot and humid group and in the cold and dry group. All samples in both groups showed bacterial growth. Morphological examination revealed growth of a single organism on each plate. Upon Gram staining and observation under the microscope, we concluded that all the isolates in each group were gram-positive cocci. In both groups, all the isolates were catalase-positive and coagulase-negative **(Table 2 and 3)**.

Automated testing using the VITEK GP card found that in the hot and humid group, 70% of the isolates were *S. hominis ssp hominis*, 20% were *S. hemolyticus* and 10% were *Kocuria kristinae*. The same testing in the cold and dry group found that 70% of the isolates identified were *S. hominis ssp hominis,* 20% were *S. saprophyticus* and 10% were *Staphylococcus warneri* **(Table 4)**. Statistical testing revealed that there is no significant difference in the bacterial composition between the hot and humid group and cold and dry group (X^2 = 6.000, df = 4, p = 0.199).

Antibiotic sensitivity testing

All 20 isolates underwent antibiotic sensitivity testing using the automatic VITEK platform and the manual Kirby-Bauer disk diffusion method. Isolates were tested for sensitivity against 17 clinically relevant drugs **(Table 5)**. Based on these results, the average percentage sensitivity of the four isolates – *S. hominis* (hot and humid), *S. hominis* (cold and dry), *S. saprophyticus*, *S. hemolyticus* – was calculated for each drug **(Figure 1)**. Using the figure, each isolate was classified as either highly sensitive (>70% sensitive), moderately sensitive (30-70% sensitive) or resistant (<30% sensitive) to each drug **(Table 6)**. All isolates were resistant against benzylpenicillin and oxacillin, and moderately sensitive to ciprofloxacin and levofloxacin. All isolates were highly sensitive to 6 drugs: linezolid, daptomycin, vancomycin, tigecycline, rifampicin, and minocycline. *S. hemolyticus* was resistant to the maximum number of drugs (n=6), while *S. hominis* (hot and humid) was resistant to the least number of drugs (n= 3) **(Table 6)**.

When comparing sensitivity against all 17 drugs, S. *hominis* isolates in the cold and dry group were 63.0% (σ = 37.5%) sensitive while those in the hot and humid group were 68.0% (σ = 33.5%) sensitive. However, this difference was not significant (p = 0.705). Against all 17 drugs, *Staphylococcus hemolyticus* isolates were 60.3% (σ = 42.4%) sensitive, while S. *saprophyticus* isolates were 55.9% (σ = 43.8%) sensitive.

DISCUSSION

Our study was conducted in North India which experiences a summer characterized by high ambient temperature and humidity. Humidity greater than 60% (which was found in the hot and humid group) has significant health impacts and is associated with an increased incidence of fungal infections of the skin, rashes, intertrigo, etc. (10). Certain groups of individuals are exposed to high temperature and humidity conditions for long periods due to their occupations. We studied whether these external influences affect the composition of the bacterial flora on human skin.

While the ambient temperature and humidity conditions in the two groups were significantly different, the skin surface temperature between the two groups was statistically insignificant. This shows that the human body can maintain its temperature across a range of external conditions and that, differences in surface body temperature at the same body site (cubital fossa) do not account for the differences in bacterial flora.

95% of all the species identified in our study were coagulase-negative *Staphylococci* (CoNS), and the most common isolate in both groups was *S. hominis ssp hominis*, accounting for 70% of isolates. However, the sample size in

Hot and Humid Group					
Sample no.	Color	Shape	Size	Hemolytic	Organism Identified
S01	Dirty white	Spherical cocci in clusters	~1mm	No	*Staphylococcus hominis ssp hominis*
S02	Dirty white	Dense spherical cocci	<1mm	Yes	*Staphylococcus haemolyticus*
S03	Dirty white	Spherical cocci in clusters	~1mm	No	*Staphylococcus hominis ssp hominis*
S04	Dirty white	Spherical cocci in clusters	<1mm	No	*Staphylococcus hominis ssp hominis*
S05	Dirty white	Spherical cocci in dense irregular clusters	~1mm	No	*Kocuria kristinae*
S06	Dirty white	Spherical cocci in dense clusters	<1mm	No	*Staphylococcus hominis ssp hominis*
S07	Dirty white	Dense spherical cocci	~1mm	Yes	*Staphylococcus haemolyticus*
S08	Dirty white	Spherical cocci in clusters	~1mm	No	*Staphylococcus hominis ssp hominis*
S09	Dirty white	Spherical cocci	<1mm	No	*Staphylococcus hominis ssp hominis*
S10	Dirty white	Spherical cocci in clusters	~1mm	No	*Staphylococcus hominis ssp hominis*

Table 2: Differences in microbial and biochemical analysis of the hot and humid group. Colony morphology – color, shape, size and hemolysis – was determined by observation and the organism identified (determined using the VITEK platform) for participants in the hot and humid group. All organisms tested positive for the catalase test, negative for the coagulase test, and positive for the Gram stain test (these results were similar for each organism).

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Cold and Dry Group					
Sample no.	**Color**	**Shape**	**Size**	**Hemolytic**	**Organism Identified**
S11	Dirty white	Spherical cocci in pairs or loose aggregates	~1mm	No	*Staphylococcus saprophyticus*
S12	Dirty white	Spherical cocci	~1mm	No	*Staphylococcus hominis ssp hominis*
S13	Dirty white	Spherical cocci in pairs or loose aggregates	< 1mm	No	*Staphylococcus saprophyticus*
S14	Dirty white	Spherical cocci, not dense	~1mm	No	*Staphylococcus hominis ssp hominis*
S15	Dirty white	Spherical cocci, not dense	~1mm	No	*Staphylococcus hominis ssp hominis*
S16	Dirty white	Spherical cocci in pairs	~1mm	No	*Staphylococcus hominis ssp hominis*
S17	Dirty white	Spherical cocci, not dense	~1mm	No	*Staphylococcus hominis ssp hominis*
S18	Dirty white	Spherical cocci, not dense	~1mm	No	*Staphylococcus hominis ssp hominis*
S19	Dirty white	Spherical cocci, not dense	~1mm	No	*Staphylococcus hominis ssp hominis*
S20	Dirty white	Spherical cocci in dense clusters	~1mm	No	*Staphylococcus warneri*

Table 3: Differences in microbial and biochemical analysis of the cold and dry group. Colony morphology – color, shape, size and hemolysis – was determined by observation and the organism identified (determined using the VITEK platform) for participants in the cold and dry group. All organisms tested positive for the catalase test, negative for the coagulase test, and positive for the Gram stain test (these results were similar for each organism).

our study was small and only a single skin site was sampled. Repeating this study on a larger population and different body sites to obtain a larger data set would strengthen the power of our study and allow us to present our conclusions with a higher confidence level.

Studies have shown that *Propionibacterinaceae* are the most common isolates from the volar skin surface of the forearm, followed by *Corynebacterianeae*, *Micrococcineae*, *Staphylococcus,* and *Streptococcus* (2, 11). However, our findings are quite different, with the most common isolate being *Staphylococcus*. A study found that *Staphylococcus epidermidis* is the most common CoNS isolated from the skin of healthy individuals (6). However, none of the isolates in our study was *S. epidermidis*. We used culture-dependent methods to isolate the bacteria and VITEK's automated platform to identify the species isolated. Modern methods using high-throughput sequencing could have provided a better picture of the bacterial flora on skin (4). Alternatively, the study could have also been repeated with more diverse agars and incubation under anaerobic conditions to isolate a wider range of species (12).

	Hot and Humid	**Cold and Dry**	**Total**
Staphylococcus hominis ssp hominis	7	7	14
Staphylococcus haemolyticus	2	0	2
Staphylococcus saprophyticus	0	2	2
Staphylococcus warneri	0	1	1
Kocuria kristinae	1	0	1
Total	10	10	Grand total: 20

Table 4: Contingency table of isolated organisms for chi-square calculation. Organisms were identified to the species level using the VITEK platform. In the hot and humid group, 70% of the participants were found to harbor *S. hominis ssp hominis*, 20% had *S. hemolyticus* while 10% had *K. kristinae*. In the cold and dry group, 70% of the participants were found to harbor *S. hominis ssp hominis,* 20% had *S. saprophyticus*, while 10% had *S. warneri.* A chi-square value of 6.000 was calculated indicating there was no significant difference among the species isolated in both groups.

A recent study conducted in an Indian population on the changing epidemiology of the normal flora of human skin concluded that the most common isolate from the cubital fossa was *S. hominis* (13). The results of our study were similar with *S. hominis ssp hominis* accounting for 70% of the samples studied. Additionally, there have been increasing reports of *S. hominis* being isolated from septicaemia related to blood donation/transfusion and other intravenous lines, indicating its presence in the bacterial flora (14). These differences in bacterial composition may indicate regional or racial differences, or a widespread change in bacterial flora of skin mediated through pollution, antibiotic abuse, and climate change, all of which need further investigation.

While *S. hominis* was the most common isolate in both groups, the hot and humid group differed from the cold and dry group due to the isolation of hemolytic *Staphylococci* in the former. Hemolytic CoNS are one of the most commonly implicated agents of staphylococcal infections, especially blood infections, and are known to have acquired multi-drug resistance against available antimicrobial agents, including glycopeptides such as vancomycin, teicoplanin, gentamicin, erythromycin, etc. (15, 16). We also found that *S. hemolyticus* was resistant to erythromycin; however, it was sensitive to vancomycin, teicoplanin, and gentamicin. The results could potentially differ because only two samples were studied, thus only providing a limited picture of antibiotic resistance. Although not as virulent as *S. aureus*, it is a very hardy and resilient organism and is known to cause nosocomial infections with high antibiotic resistance (17). The presence of pathogenic *S. hemolyticus* as a part of normal bacterial flora in individuals exposed to a hot and humid environment may predispose them to infection.

In addition, we found that 20% of individuals in the cold and dry group had *S. saprophyticus* as part of their skin flora. The non-hemolytic *S. saprophyticus* is a benign organism and is found in the normal genitourinary flora of young sexually active women where it is commonly associated with uncomplicated urinary tract infections (UTIs) (18). Hence it is considered a less virulent species than *S. hemolyticus*

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Sample number	Species Name	Benzylpenicillin	Oxacillin	Gentamicin	Ciprofloxacin	Levofloxacin	Erythromycin	Clindamycin	Linezolid	Daptomycin	Teicoplanin	Vancomycin	Tigecycline	Rifampicin	Trimethoprim/sulfamethoxazol	Minocycline	Azithromycin	Cefoxitin
Hot and Humid Group																		
01	*S. hominis*	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	S	S
02	*S. hemolyticus*	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	R	R
03	*S. hominis*	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
04	*S. hominis*	R	R	S	R	R	R	S	S	S	S	S	S	S	R	S	R	R
05	*Kocuria kristinae*	-	-	-	-	-	-	-	S	-	R	S	-	S	-	S	S	S
06	*S. hominis*	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	I	R
07	*S. hemolyticus*	R	R	S	S	S	R	S	S	S	S	S	S	S	S	S	R	I
08	*S. hominis*	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	S	S
09	*S. hominis*	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	I	R
10	*S. hominis*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	R
Cold and Dry Group																		
11	*S. saprophyticus*	R	R	S	S	S	R	R	S	S	I	S	S	S	S	S	R	R
12	*S. hominis*	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	R	S
13	*S. saprophyticus*	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	I
14	*S. hominis*	R	R	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S
15	*S. hominis*	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	R
16	*S. hominis*	R	R	S	S	S	R	R	S	S	S	S	S	S	R	S	R	R
17	*S. hominis*	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S
18	*S. hominis*	R	R	S	S	S	R	S	S	S	S	S	S	S	S	S	R	R
19	*S. hominis*	R	R	S	R	R	R	S	S	S	S	S	S	S	R	S	R	R
20	*S. warneri*	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S	R	R

Table 5: Antibiotic sensitivity of isolates from both groups. Antibiotic sensitivity of all 20 isolates was tested automatically (using the VITEK platform) and manually (using the Kirby-Bauer disk diffusion method and HIMEDIA's zone of inhibition chart). "S" (green) corresponds to sensitive, "I" (yellow) corresponds to intermediate sensitivity, and "R" (red) corresponds to resistance.

(isolated in the hot and humid group). Although our results found *S. saprophyticus* to be more multi-drug resistant than *S. hominis* and *S. hemolyticus*, with only a 55% sensitivity to the 17 drugs it was tested against, the difference between the sensitivity of *S. hemolyticus* and *S. saprophyticus* was statistically insignificant. The distribution of males and females among the groups was not equal, thus the results may favor bacteria that grow more commonly on the skin of males in the hot and humid group, and those that grow more commonly on the skin of females, such as *S. saprophyticus*, in the cold and dry group (18, 19).

S. hominis, the most frequently isolated commensal in our study, is an odor producing bacteria that releases lantebiotics, which have a role in preventing colonization of skin by opportunistic pathogens like *S. aureus* (20). The molecular mechanism by which *S. hominis* inhibits the pathogenic action of *S. aureus* has been deduced (20). Research is ongoing about the role of pathogenic bacteria like *S. aureus* in atopic dermatitis and the use of *S. hominis* as a treatment modality (6, 21). It has been found that an imbalance in the composition of the microbiome, characterized by decreased diversity and higher prevalence of pathogenic bacteria like *S. aureus*, correlates with the severity of disease in patients with atopic dermatitis (21). Additionally, treatment through the application of commensal organisms such as *S. hominis* or *Roseomonas mucosa* on the skin has shown reduced severity of atopic dermatitis in patients. (21).

Antibiotic sensitivity analysis showed that all species of *Staphylococcus* isolated were resistant to benzylpenicillin and oxacillin. This result coincides with a recent study in Korea which used a LC-MS method for rapidly determining the resistance of *S. aureus* to benzylpenicillin (22). All species of *Staphylococcus* isolated were highly sensitive to linezolid, daptomycin, teicoplanin, vancomycin, tigecycline, rifampicin,

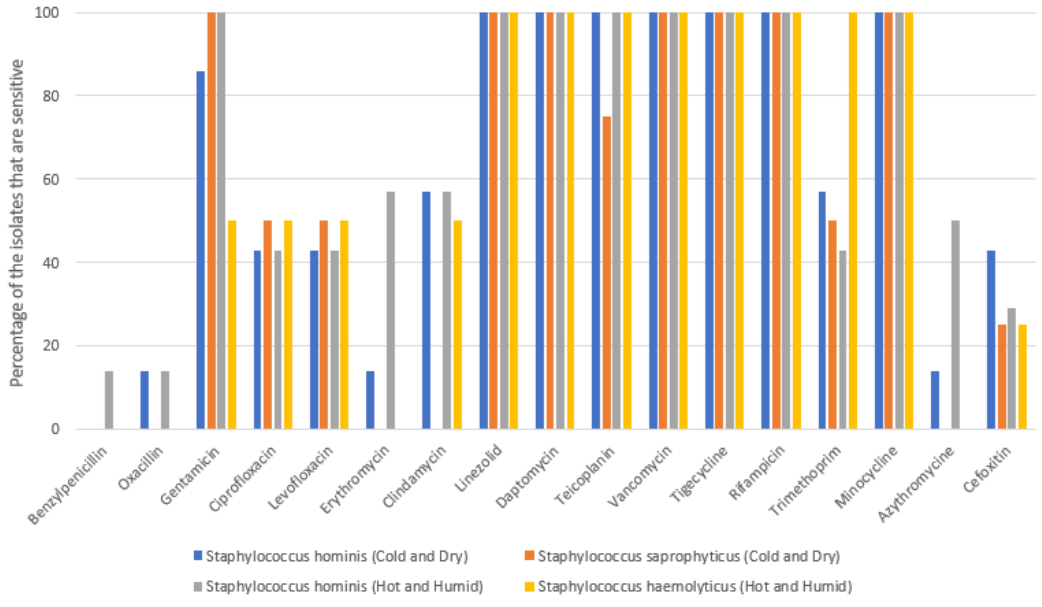

Figure 1: Percentage of isolated species in both groups that were sensitive to clinically relevant antibiotics. The sensitivity of isolates to clinically relevant antibiotics was determined automatically (using the VITEK platform) and manually (using the Kirby-Bauer disk diffusion method). In the cold and dry group, there were seven isolates for *S. hominis* (blue) and two isolates for *S. saprophyticus* (orange). In the hot and humid group, there were seven isolates for *S. hominis* (grey) and two isolates for *S. hemolyticus* (yellow). To create the graph, sensitivity was counted as 1, intermediate sensitivity as 0.5 and resistance as 0.

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Sensitivity Level	Hot and Humid Group		Cold and Dry Group	
	Staphylococcus hominis	*Staphylococcus hemolyticus*	*Staphylococcus hominis*	*Staphylococcus saprophyticus*
Highly Sensitive	8	8	8	8
Moderately Sensitive	6	4	5	3
Resistant	3	5	4	6

Table 6: Summary of species sensitivity to antibiotics. Isolates in both groups were tested against 17 antibiotics both manually (using the Kirby-Bauer disk diffusion method) and automatically (using the VITEK platform). This table compares the number of antibiotics that a particular species was highly sensitive (more than 70% of isolates of that species were sensitive), moderately sensitive (if 30-70% of isolates of that species were sensitive) or resistant (if less than 30% of that species was sensitive).

and minocycline. Research on multi drug-resistant bacteria have shown *S. aureus* was sensitive to only a handful of drugs which included vancomycin, linezolid, tigecycline, and daptomycin – each of which also had 100% sensitivity in our study (23). Hence, these four drugs, along with teicoplanin and rifampicin, can be used to effectively treat staphylococcal infections due to their high sensitivity with many *Staphylococcus* species.

Upon comparing the antibiotic sensitivity of *S. hominis* in both groups, the difference was found to be insignificant. In addition, the antibiotic sensitivity difference between *S. hemolyticus* and *S. saprophyticus* was also insignificant. This suggests that the bacterial species found in this study were similar in terms of the degree of antibiotic resistance.

In conclusion, our study found that the most common bacterial species colonizing the normal cubital fossa skin was *S. hominis*. Hemolytic coagulase-negative *Staphylococci*, which are more pathogenic, were found to be more prevalent in individuals working in hot and humid environments, while *S. saprophyticus* was more common in individuals working in cold and dry environments. While these differences were not statistically significant, it is possible that environmental conditions may affect the composition of the skin microbiome with people exposed to hot and humid environments harboring more pathogenic bacteria. A possible extension of this study could be isolating the effect of distinct environmental factors – temperature and humidity – to better understand their role in affecting the bacterial composition of the skin flora. There was no significant difference among the antibiotic sensitivity patterns of the bacteria isolated in either group, and none of them were multi-drug resistant.

Our study had certain limitations. No survey was conducted; hence it is difficult to know what kind of ambient conditions the participants are normally exposed to outside of work, including their history of exposure in the last few months, skin products used, hygiene practices and frequency, all of which could be potential factors that could affect their skin microbiome. Additionally, there was no blinding in this study; all the samples were collected and processed by the author.
These findings have important implications in understanding risk factors for skin diseases and further developing prevention and treatment strategies for the same. Prolonged exposure to hot and humid environmental conditions through occupational exposure may predispose individuals to bacterial skin diseases. Also, cutaneous interventions in such patients might lead to bacteraemia with more pathogenic bacterial strains, warranting thorough antimicrobial prophylaxis. Moreover, this information can be used to define better occupational health and safety standards for workers. Although, the sample size and population in this study was limited, it adds important data about the North Indian population to the skin microbiome research field. More population-wide studies are needed to map the normal bacterial skin flora under different environmental conditions to better understand its role in health and disease.

MATERIALS AND METHODS

The study was conducted in a hospital in North India in July 2023. Institutional Review Board approval was obtained for the study. Twenty healthy volunteers were identified and divided into two groups.

Cohort assignment

Each group contained 10 volunteers who worked at least 8-hour shifts in their respective environment. The hot and humid group was defined as ambient temperature greater than 30°C and relative humidity greater than 70%. This group included security guards and construction workers. The cold and dry group was defined as ambient temperature not exceeding 25°C and relative humidity not exceeding 55%. This group included laboratory personnel and pharmacy assistants. The temperature ranges were chosen using the Environmental Protection Agency and World Health Organization recommendations for indoor temperature and humidity, with the cold and dry group corresponding to the ideal conditions (24, 25). The ambient temperature and humidity were measured at the time of sample collection using a Temperature and Humidity Display Meter. The age and sex of the participants were also noted.

Sample collection

The dry swabbing method was used to obtain a skin surface sample from the cubital fossa - fold of elbow (26). A dry cotton swab was rotated slowly over a small, prepared region of 25 cm^3 on the skin at a 30° angle. The swab was immediately dipped in 2 ml sterile nutrient broth (HIMEDIA) and capped with a cotton plug. The surface temperature of the skin site at the time of taking swab was recorded with an infrared thermometer. The process was repeated for all the volunteers and each sample was carefully labelled.

Sample processing

Each cotton swab was struck onto one half of a Columbia blood agar (CBA) (an enriched and non-selective medium) and the other half left empty. The plates and swabs were placed in a 37°C incubator for enrichment. After 1 hour, the streaking was repeated on the empty half of the CBA plate. CBA plates were incubated in aerobic conditions for 24 hours at 37°C. Only a single type of bacterial colony grew in each plate after incubation. The plates were observed and their morphological features were recorded. Controls used for both nutrient broth and blood agar medium were satisfactory.

Heat fixed slides were prepared and stained with Gram's stain. Slides were observed under the microscope and any bacteria seen were identified as gram positive/negative. A slide catalase test was carried out by taking a drop of hydrogen peroxide on a slide using a dropper and mixing a single colony from the CBA plate. Bubbling indicated a positive result. Coagulase slide test was also carried out by adding one drop of normal saline on a slide, and mixing one

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colony from the CBA plate. A drop of plasma was added and the mixture was swirled to observe agglutination. Separation indicated a positive result.

Controls were made using standards procured from ATCC (American Type Culture Collection): *Escherichia coli* (cat number: 25922) and *S. aureus* (cat number: 25923). Bacterial species were sub-cultured aerobically on CBA and incubated for 24 hours at 37°C, to obtain fresh growth. Fresh colonies were mixed with a standard saline solution (0.9% NaCl) using sterile toothpicks until an inoculum density 1.5 x 10^8 CFU/ml (0.5 McFarland Standard) was reached (27, 28).

Samples were loaded into the Biomérieux VITEK 2 Compact machine. Based on the results of gram staining and biochemical tests conducted previously, "VITEK GP" and "VITEK 2 AST-P628" cards were chosen to identify the bacteria to the species level and measure their antibiotic sensitivity respectively (27). The drugs tested for sensitivity were benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tigecycline, rifampicin, trimethoprim. The species name identified by the VITEK platform as well as the level of sensitivity (sensitive, intermediate or resistant) of the isolates to each drug were recorded.

Antibiotic sensitivity testing was performed manually as well to test for drugs not on the VITEK cards using the Kirby-Bauer's disk diffusion method on Mueller-Hinton agar (HIMEDIA) (28). The previously prepared bacterial solutions were inoculated onto the medium using sterile cotton swabs. Commercially available antibiotic disks for minocycline, cefoxitin and azithromycin were impregnated by placing and gently pressing onto the surface of the agar. After 24 hours of incubation, the zone of inhibition was recorded and the samples were classified as sensitive, intermediate, or resistant using HIMEDIA's Zone Size Interpretative chart (29). Sensitive values were counted as 1, intermediate sensitivity as 0.5 and resistant as 0. The average of these values was taken and multiplied by 100 to obtain a value for the percentage sensitivity. These percentage values were also characterized as either highly sensitive (if greater than 70% of isolates were sensitive), moderately sensitive (if 30-70% of isolates were sensitive), and resistant (if less than 30% of isolates were sensitive).

Statistical Analysis

A two-tailed student t-test was used to identify if the difference between variables was significant. Values were considered significant when $p<0.05$ (confidence interval 95%). The test was used on ambient temperature and humidity (independent variables), age and skin site surface temperature (confounding variables), and antibiotic sensitivity of Staphylococcus hominis in both groups (dependent variable).

A chi-squared test was performed by creating a contingency table of the species identified in the hot and humid and the cold and dry group. The chi-square value (X^2) was compared with a confidence interval of 0.05 (95%) to determine significance.

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