

PREVALENCE AND INFLUENCING FACTORS OF *DEMODEX* INFECTION AMONG COLLEGE STUDENTS IN ZHENGZHOU, PR CHINA

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Abstract. *Demodex* spp are ectoparasites living in human hair follicles and sebaceous glands, and are considered to play a pathogenic role in facial dermatoses such as acne vulgaris. This study investigated the prevalence and influencing factors of *Demodex* spp infection among college students in Zhengzhou, PR China. College students ($n = 500$) were enrolled and a cellophane tape method was used to determine *Demodex* infection after students washed their faces with cool ($n = 300$) or warm water ($n = 200$). Relevant factors were investigated by a questionnaire. The positive detection rate was 38% and 26% for samples collected from students washing with warm and cool water respectively. There is a significantly higher positive detection rate of *Demodex folliculorum* compared to *D. brevis* under both face washing conditions ($\chi^2_{\text{cool}} = 40.26$, $df = 1$, $p\text{-value}_{\text{cool}} < 0.0001$; $\chi^2_{\text{warm}} = 6.028$, $df = 1$, $p\text{-value}_{\text{warm}} = 0.014$). The rate of *Demodex* infection is significantly higher for students with oily skin compared to dry or a combination of oily and dry skin ($\chi^2_{\text{warm}} = 11.635$, $df = 2$, $p\text{-value}_{\text{warm}} = 0.003$). Our observations suggest that *Demodex* infection, particularly *D. folliculorum*, is fairly common among college students in Zhengzhou. Oily skin may increase susceptibility to *Demodex* infection, and washing the face with warm water before applying the cellophane tape method can improve the detection rate.

Keywords: *Demodex*, cellophane tape method, college student, skin type, washing face

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INTRODUCTION

Demodex spp are microscopic, obligate, elongated mites of the Demodicidae Family, Acari Order, and Arachnida Class (Eser *et al*, 2017). *D. brevis* and *D. folliculorum* are the only two species that can inhabit the human body (Ozer *et al*, 2012; Foley *et al*, 2021). *Demodex* pathogenicity is related to parasite burden, duration of parasitism and host immune response. *Demodex* infection is implicated in the occurrence of a wide range of clinical features, such as granulomatous rosacea, papulopustular rosacea, pityriasis folliculorum, and some isolated cases of blepharitis, folliculitis, hyperpigmentation, and inflammatory papule (Forton *et al*, 2005).

Demodex infection is very common, especially in the adult population. In patients with facial dermatosis or blepharitis, the positive rate of *Demodex* infestation can be as high as 52.0% and 62.4% respectively, while in healthy controls they are 2.6% and 24.3% respectively (Biernat *et al*, 2018; Aktaş Karabay and Aksu

Çerman, 2020). The prevalence of *D. folliculorum* infection in the eyelashes of healthy young males is 20.73% in Fujian Province, PR China (Ye *et al*, 2022).

The spread of *Demodex* infection occurs through close or direct contact with skin infested with mites or larvae, or through dust carrying the eggs. Beds are often considered the primary site for mite infestation (Tovey *et al*, 2013). Therefore, bedding and dormitory cleaning were included in the contributing factors in our work. *Demodex* colonizes hair follicles or sebaceous follicles, and persons with oily skin have increased amounts of *D. folliculorum* on the skin surface (Porta Guardia, 2015). In addition, the prevalence of *D. brevis* and *D. folliculorum* is higher in females than in males (Zhong *et al*, 2019).

Although facial problems are common among college students, no study, to date, has been conducted using a cellophane tape method (Kowalczyk *et al*, 2022) to explore the different positive detection rates of *Demodex* spp on the face following washing with cool or warm water. Thus, our

study determined the prevalence of *Demodex* spp following the two methods of face washing among college students in Zhengzhou, PR China according to sex, ethnicity, skin type, use of facial cleanser, diet, and sleep habits. This study aims to enhance college students' awareness of skin issues and their knowledge on *Demodex* spp, and to promote the establishment of good living habits.

MATERIALS AND METHODS

Study subjects

Participants were junior students ($n = 500$) residing in campus housings of Zhengzhou, Henan province, PR China, 19-22 years of age, comprising females ($n = 317$) and males ($n = 183$) who completed both the sample collection procedure and questionnaire survey.

Sample collection

A cellophane tape method was employed for collecting *Demodex* specimens (Kowalczyk *et al*, 2022). In brief, before sleep at night each participant washed face with either cool (15 ± 5 °C) or warm

(40 ± 5 °C) water. After drying the face using clean towels, a 3 cm long \times 1.2 cm wide piece of cellophane tape (Deli Group Co Ltd, Ningbo, Zhejiang, PR China) was pasted on the cheek, forehead and nose. The following morning, the tapes were removed, pressed onto slides, stored at 4 °C, and brought within 4 hours to the designated laboratory for examination under a light microscope (400 \times magnification). The procedure was repeated twice, with 2 sets of samples collected per participant.

Questionnaire

The questionnaire collected data on sex, ethnicity, skin type, facial cleanser usage, diet, and sleep habits. Skin type was defined based on a subjective assessment (Tao *et al*, 2014).

Statistical analysis

Data were analyzed using the χ^2 test to determine differences, with a p -value ≤ 0.05 considered statistically significant. Statistical analysis was performed using a Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM, Armonk, NY).

Ethical consideration

The study protocol was approved by the Zhengzhou University Life Science Ethics Committee (no. ZZUIRB 2023–217). Prior signed informed consent was obtained from each participant.

RESULTS

The detection of *Demodex* spp was determined using an optical microscope (Fig 1). Participating college students residing in campus housings in Zhengzhou were divided into 2 groups prior to testing for *Demodex*: one group washed their faces with cool water and the other with warm water. The overall prevalence of *Demodex* infection on the faces of college students was 31% (156/500).

The general characteristics of participants ($n = 300$) who washed their faces with cool water prior to *Demodex* detection are that there are more women, and a relatively large number of people have good living habits, although more people prefer spicy food and staying up late (Table 1). The total positive detection rate of *Demodex* spp

among participants who washed their faces with cool water was 26% ($n = 79$). The positive detection rate of *D. folliculorum* (94%) is significantly higher than that of *D. brevis* (23%) ($\chi^2 = 40.26$, $df = 1$, p -value < 0.0001) (Table 2). There are no significant differences in gender, ethnicity, skin type, facial cleanser usage, diet (consumption of spicy food), and sleep habit (after midnight) of *Demodex*-infected participants who washed their faces with cool water (Table 2). The prevalence of *Demodex* infection among students with oily skin (43%) is not significantly different from those with a combination of oily and dry skin (51%) but higher from those with dry skin (6%) ($\chi^2 = 1.149$, $df = 2$, p -value = 0.563) (Table 2).

The general characteristics of participants ($n = 200$) who washed their faces with warm water are the same as participants who washed their faces with warm water (Table 3). The total positive detection rate of *Demodex* spp among participants who washed their faces with warm water was 39% ($n = 77$) (Table 3). The positive detection rate of

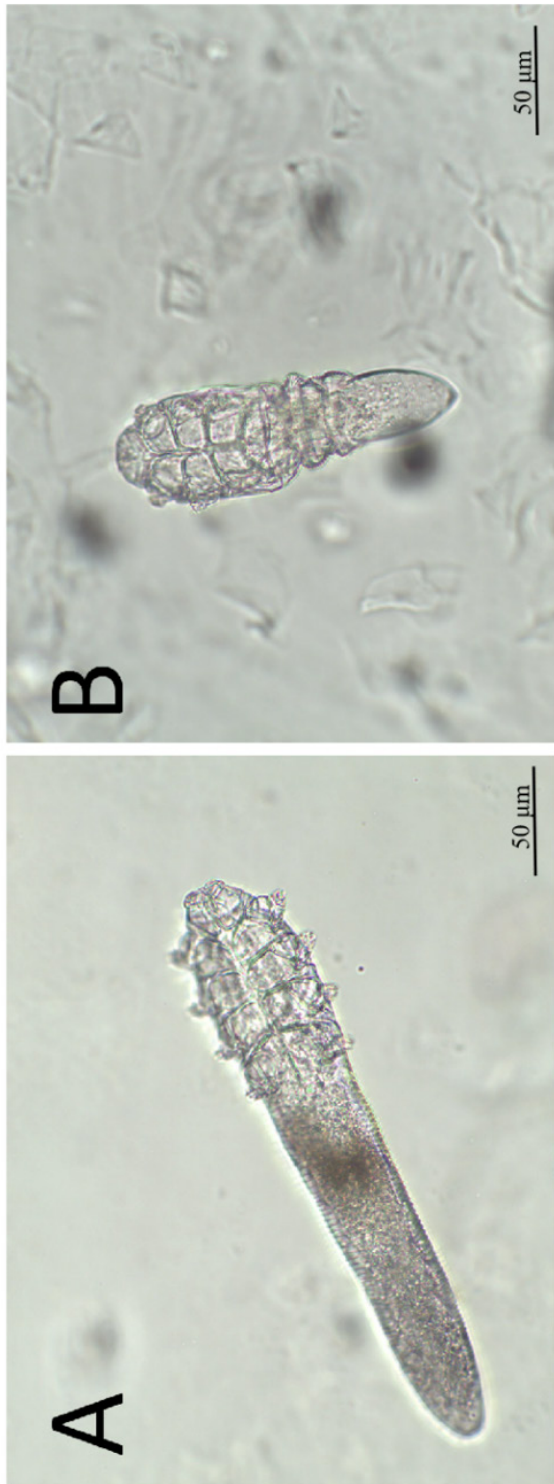


Fig1 - *Demodex* spp observed under an optical microscope (400× magnification)

A: *D. folliculorum*; B: *D. brevis*

µm: micrometer

Table 1

General characteristics of participants who washed their faces with cool water
(N = 300)

Variable	Frequency <i>n</i> (%)
Sex	
Male	97 (32)
Female	203 (68)
Ethnicity	
Han	285 (95)
Others	15 (5)
Skin type	
Oily	124 (41)
Combination of oily and dry	148 (49)
Dry	28 (10)
Frequency of facial cleanser usage	
Often (twice a day)	201 (67)
Occasionally (more than once a week)	76 (25)
Never	23 (8)
Frequency of dormitory cleaning	
Rarely	83 (28)
Often (once a day)	51 (17)
Occasionally (twice a week)	166 (55)
Frequency of bedding cleaning	
Rarely	38 (13)
Often (once a day)	37 (12)
Occasionally (twice a week)	225 (75)

Table 1 (cont)

Variable	Frequency <i>n</i> (%)
Consumption of spicy food	
Occasionally	185 (62)
Often	84 (28)
Rarely	31 (10)
Sleep after midnight	
Rarely	10 (4)
Often (>3 times a week)	193 (64)
Occasionally (2-3 times a week)	97 (32)
<i>Demodex</i> infection	
Positive	79 (26)
Negative	221(74)

D. folliculorum (68%) is significantly higher than that of *D. brevis* (42%) ($\chi^2 = 6.028$, $df = 1$, p -value = 0.014) (Table 4). There are no significant differences in sex, ethnicity, facial cleanser usage, diet (consumption of spicy food), and sleep habit (after midnight) (Table 4). The prevalence of *Demodex* infection among students with oily skin (47%) is not significantly different from those with a combination of oily and dry skin (49%) but higher than those with dry skin (4%)

($\chi^2 = 11.635$, $df = 2$, p -value = 0.003) (Table 4). However, *Demodex* infection prevalence is significantly higher in students who used warm than cool water for washing their faces ($\chi^2 = 8.275$, $df = 1$, p -value = 0.004).

DISCUSSION

Demodex lives mostly in the hair follicle of the human face but is detected in various places of the human body, such as the chin, external ear canal,

Table 2

General characteristics of *Demodex*-infected participants who washed their faces with cool water (N = 79)

Variable	Frequency ^a n (%)	p-value ^b
Sex		0.898
Male	26 (33)	
Female	53 (67)	
Ethnicity		N/A
Han	78 (99)	
Others	1 (1)	
Skin type		0.563
Oily	34 (43)	
Combination of oily and dry	40 (51)	
Dry	5 (6)	
Frequency of facial cleanser use		0.957
Often (twice a day)	52 (66)	
Occasionally (more than once a week)	21 (26)	
Never	6 (8)	
Frequency of dormitory cleaning		0.650
Rarely	25 (32)	
Often (once a day)	13 (16)	
Occasionally (once a day)	41 (52)	
Frequency of bedding cleaning		0.106
Rarely	15 (19)	
Often (once a day)	11 (14)	
Occasionally (once a day)	53 (67)	

Table 2 (cont)

Variable	Frequency ^a n (%)	p-value ^b
Consumption of spicy food		0.996
Occasionally	49 (62)	
Often	22 (28)	
Rarely	8 (10)	
Sleep after midnight		0.732
Rarely	2 (3)	
Often (>3 times a week)	49 (62)	
Occasionally (2-3 times a week)	28 (35)	
<i>Demodex</i> species detected ^c		<0.0001
<i>D. brevis</i>	18 (23)	
<i>Demodex folliculorum</i>	74 (94)	
<i>D. brevis</i> infection according to sex (N = 23)		0.540
Male	7 (30)	
Female	11 (48)	
<i>D. folliculorum</i> infection according to sex (N = 74)		0.983
Male	24 (32)	
Female	50 (68)	

Note: All statistical analyses were conducted using the chi-square test.

^aN = 79 unless indicated otherwise; ^bSignificant when $p \leq 0.05$; ^cMixed infection were detected in some participants.

N/A: not applicable

Table 3

General characteristics of participants who washed their faces with warm water
(N = 200)

Variable	Frequency <i>n</i> (%)
Sex	
Male	86 (43)
Female	114 (57)
Ethnicity	
Han	190 (95)
Others	10 (5)
Skin type	
Oily	67 (34)
Combination of oily and dry	115 (57)
Dry	18 (9)
Frequency of facial cleanser usage	
Often (twice a day)	144 (72)
Occasionally (more than once a week)	42 (21)
Never	14 (7)
Consumption of spicy food	
Occasionally	142 (71)
Often	44 (22)
Rarely	14 (7)
Sleep after midnight	
Rarely	5 (3)
Often (>3 times a week)	148 (74)
Occasionally (2-3 times a week)	47 (23)
<i>Demodex</i> infection	
Positive	77 (39)
Negative	123 (61)

Table 4

General characteristics of *Demodex*-infected participants who washed their faces with warm water (N = 77)

Variable	Frequency ^a n (%)	p-value ^b
Sex		0.254
Male	37 (48)	
Female	40 (52)	
Ethnicity		N/A
Han	74 (96)	
Others	3 (4)	
Skin type		0.003
Oily	36 (47)	
Combination of oily and dry	38 (49)	
Dry skin	3 (4)	
Frequency of facial cleanser usage		0.886
Often (twice a day)	54 (70)	
Occasionally (more than once a week)	17 (22)	
Never	6 (8)	
Consumption of spicy food		0.390
Occasionally	57 (74)	
Often	17 (22)	
Rarely	3 (4)	
Sleep after midnight		0.141
Rarely	0 (0)	
Often (>3 times a week)	56 (73)	
Occasionally (2-3 times a week)	21 (27)	

Table 4 (cont)

Variable	Frequency ^a n (%)	p-value ^b
<i>Demodex</i> species detected ^c		0.014
<i>D. brevis</i>	32 (42)	
<i>D. folliculorum</i>	52 (68)	
<i>D. brevis</i> infection according to sex (N = 32)		0.926
Male	14 (44)	
Female	18 (56)	
<i>D. folliculorum</i> infection according to sex (N = 52)		0.236
Male	26 (50)	
Female	26 (50)	

Note: All statistical analyses were conducted using the chi-square test.

^aN = 77 unless indicated otherwise; ^bSignificant when $p \leq 0.05$; ^cMixed infection were detected in some participants.

N/A: not applicable

cheeks, back, buttocks, nipples, and penis (Enginyurt *et al*, 2015). We determined a total positive detection rate of 31% in 500 college students in Zhengzhou, higher than those of other reports (Biernat *et al*, 2018; Aktaş Karabay and Aksu Çerman, 2020; Ye *et al*, 2022). However, other studies reported even higher (40-50%) positive rates of detection (Wesolowska *et al*, 2014). In our investigation and

those of others (Wang *et al*, 2017; Zhao *et al*, 2017; Li *et al*, 2018; Wang *et al*, 2020), *Demodex* spp detected was mainly *D. folliculorum*. The higher detection rate of *D. folliculorum* compared to *D. brevis* may be attributed to their distinct biological characteristics, with the former inhabiting hair follicles and the latter residing in sebaceous glands (Elston *et al*, 2014).

Although the number of female

participants were higher than males, percent *Demodex* infection is not statistically different. The positive detection rate of ethnic minority students was lower than that of Han students, but meaningful statistical analysis could not be conducted due to the small number of non-Han participants. Further studies using a larger cohort of minority ethnic subjects are needed to confirm our observations.

Other factors investigated, *viz* frequencies of facial cleanser usage, bedding and room cleaning, spicy food consumption, and late-night sleeping, did not demonstrate any statistically significant impact on *Demodex* infection. A facial cleanser commonly used to eliminate *Demodex* infection is a 5-50% tea tree oil solution (Murphy *et al*, 2018); however, a lower concentration of tea tree oil is unlikely to have any therapeutic benefits (Hammer, 2015). Zhao *et al* (2011) earlier reported that hygiene habits have no significant relationship with *Demodex* infection.

Analyzing the influence of skin types on *Demodex* infection, we

found a positive detection rate of oily skin or a combination of oily and dry skin was higher than that of dry skin among participants who washed their faces with warm water before undergoing testing; however, the results of students who washed their faces with cool water are not statistically significant. It may be due to the oil on skin surface is better removed by washing with warm than cool water, thereby making it easier to adhere *Demodex* to the cellophane tape for subsequent analysis. The higher *Demodex* infection rate from oily than dry skin may be due to the lipid nutrients in oily skin (Rather and Hassan, 2014).

In conclusion, the positive detection rate of *Demodex* from faces of college students in Zhengzhou was 31%, with *D. folliculorum* being predominant (80%) compared to *D. brevis* (35%). Washing face with warm rather than cool water facilitated the detection of *Demodex* employing the cellophane tape collection method and detection under a light microscope. Among a variety

of possible contributing factors, only skin type was associated with *Demodex* prevalence, with oily skin being more susceptible to *Demodex* infection than dry or a combination of oily and dry skin. The survey research has enhanced college students' awareness of skin issues and their understanding of *Demodex* mites, and provided a simple self-testing method for *Demodex* detection.

ACKNOWLEDGEMENTS

The study was supported by grants from the Henan Province Undergraduate University Curriculum Ideological and Political Project of the education department of Henan Province, China's Ministry of Education's Industry Education Cooperation Collaborative Education Project (no. 220900413021914) and the Medical Science and Technology project of Henan Province (no. LHGJ20200372).

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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