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Investigation of an Outbreak of *Moraxella* Conjunctivitis at a Navajo Boarding School

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In 1986, an outbreak of *Moraxella* follicular conjunctivitis occurred in girls attending a Navajo boarding school in New Mexico. We diagnosed 19 cases of culture-proven, and 21 of clinical conjunctivitis based on isolation of *Moraxella* from conjunctival cultures and the occurrence of symptoms significantly associated with positive culture. Sharing eye makeup was significantly associated with *Moraxella*-positive conjunctivitis (odds ratio [OR] = 7.2, P = .004) and showed a trend toward significance in those with clinical conjunctivitis (OR = 2.9, P = .09). Eyeliner and eye shadow were implicated (OR = 4.1, P < .05). We cultured samples of 13 students' makeup; one third of the eyeliners were positive for *Moraxella*. Nasal carriage of *Moraxella* was found in 35 (44%) of the 79 female boarders and in 20 (21%) of 97 Navajo patients at two nearby clinics. In a prospective evaluation of the effect of patient education and rifampin therapy on the occurrence of conjunctivitis during an 11-month follow-up period, both types of intervention were successful in significantly reducing the rate of conjunctivitis when compared with that in a control group.

MORAXELLA LACUNATA has been recognized as a cause of conjunctivitis since 1896.[1] Although commonly isolated from patients with conjunctival infection in the early decades of this century,[2] it presently is infrequently isolated from patients with conjunctivitis.[3-6] *Moraxella* causes two types of conjunctival infections: acute angular conjunctivitis characterized by a purulent discharge and maceration of the skin at the canthi[7] and chronic follicular conjunctivitis. Dawson[8] and Fedukowicz amd Stenson[9] reported that chronic follicular conjunctivitis caused by *Moraxella* is most common in females in the southwestern United States, with outbreaks being associated with the use or sharing of mascara. However, they gave no epidemiologic data to support these observations. During investigations of epidemic follicular conjunctivitis in Denmark[10] and New Mexico (unpublished observations, George Schmid, M.D., Centers for Disease Control), *Moraxella* was isolated from teenage girls, but risk factors for infection were not elucidated.

In November 1986, *Moraxella* was isolated from conjunctival swabs of several female students at a Bureau of Indian Affairs boarding school at Dzilth-Na-O-Dith-Hle (Dzilth), New Mexico. The Centers for Disease Control were informed of the potential outbreak, and in conjunction with the Indian Health Service an investigation was initiated. During this investigation, we documented risk factors for infection, studied nasal carriage of *Moraxella*, and prospectively evaluated the success of antimicrobial therapy and patient education in reducing the incidence of infection.

Subjects and Methods

The dormitory at the Dzilth-Na-O-Dith-Hle boarding school houses students from kindergarten through high school. Students are segregated by sex and age; junior high and high school girls are in separate wings of a single dormitory and share a single bathroom. A school for kindergarten through junior high is adjacent to the dormitory and includes boarding students from Dzilth and Huerfano—a sim-

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ilar dormitory 20 miles away—and day students from the surrounding community. High school students attend Bloomfield High School, a public school that includes both Indian and non-Indian students. The sole source of health care for all residents at the dormitories is an Indian Health Service clinic at Dzilth, one block from the school.

Conjunctivitis Study

To determine the extent of the outbreak and to identify risk factors for conjunctivitis, we interviewed, performed ophthalmologic examinations, and cultured conjunctival specimens of all junior high and high school girls attending Dzilth. We conducted similar investigations of samples of the boarding students and day students of both sexes from Dzilth, Bloomfield High School, and Huerfano dormitory who were selected from a roster of all students using a random number table. Family contacts of culture-positive students were studied based on their accessibility. The interview included questions concerning demographic characteristics, antecedent ocular injury or medication, makeup use and sharing, hygiene at the dormitory, household size, hygiene and sanitation at home, socioeconomic status, animal exposure, and symptoms of conjunctivitis. All ophthalmologic examinations were performed by a single investigator (B.S.) who was masked to the results of the questionnaire.

We obtained bacterial cultures by swabbing the tarsal plate and the lower palpebral conjunctiva of the eye that had the most signs of conjunctivitis or, if neither eye looked infected, the right eye. We cultured swab specimens directly on heart infusion agar or blood agar under CO_2 at 35 C. We examined cultures at 24 and 48 hours and *Moraxella* was presumptively identified by colony morphologic studies, positive oxidase reaction, and appearance with Gram stain.[11] We sent a sample of positive isolates to the microbiology laboratory of the New Mexico Health and Environment Department and to the Centers for Disease Control for confirmation. We also sent to the state laboratory viral cultures and chlamydia smears for fluorescent antibody testing from a sample of persons who, on examination, had signs of acute ocular infection. We reviewed the Dzilth clinic charts of persons enrolled in the study for episodes of conjunctivitis during the preceding year. Because of variations in the data included in patient charts, we did not attempt to evaluate symptoms or signs retrospectively.

Carriage Study

We obtained anterior nasal specimens for culture from all students enrolled in the conjunctivitis study. We also cultured the conjunctiva and nares of Navajo patients attending the clinic at Dzilth and a private clinic in Farmington, New Mexico, approximately 50 miles from Dzilth. A short version of the conjunctivitis questionnaire was administered to all clinic patients from whom specimens were obtained. Swabs were plated and processed as for the conjunctivitis study.

Treatment Study

We prospectively evaluated the effect of patient education and systemic antibiotic therapy in decreasing the rate of conjunctivitis in female boarding students. Consent for therapy was obtained in loco parentis from school officials, and this intervention was approved by the clinic staff and a member of the community health board. We divided high-risk students into three groups by dormitory. Group 1 (Dzilth high school students) received 600 mg of rifampin orally twice a day for two days and participants were instructed to avoid risk factors identified in the conjunctivitis study. Group 2 (Huerfano dormitory residents) received instructions on avoiding risk factors. Group 3 (Dzilth junior high school students) was informed of hypothesized risk factors during the course of the study but received no special instruction after its completion. For all students taking rifampin, bilateral conjunctival and nasal specimens were taken before and four days after therapy. Over the next 11 months, all clinic visits for conjunctivitis by students in the three study groups were recorded on clinical report forms, and conjunctival and nasal specimens were obtained. Topical therapy was administered at the discretion of the clinic physician.

Laboratory methods—The Centers for Disease Control bacterial reference laboratory confirmed and identified the species as *M. lacunata* based on the following characteristics: no hemolysis occurred on rabbit blood agar; oxidase, catalase, and nitrate reduction tests were positive; Loeffler blood serum slants were digested; acid was not produced, oxidatively or fermentatively, from carbohydrates; urea was not hydrolyzed; the cultures did not grow on MacConkey agar; the cellular fatty acid profiles were consistent with the profile of the American Type Culture Collection strain 11748 of *M. lacunata*.

Statistical methods—Univariate analysis of potential risk factors was done using the chi-squared or Fisher exact test for dichotomous variables and the Wilcoxon rank-sum test for continuous variables. All tests were two-tailed. Multivariate models, incorporating potential risk factors, were evaluated by unconditional logistic regression.

Results

Conjunctivitis Study

Of 143 students from Dzilth Junior High School and Bloomfield High School enrolled in this study, 19 (13%) had conjunctival cultures that were positive for *Moraxella*. All 19 were girls and 18 were boarding students, for an infection rate of 26% in female boarders compared to 3% in female day students (odds ratio [OR] = 11.5, P = .007) (Table 1). No male students had positive cultures. Three conjunctival cultures were positive for other bacteria in symptomatic patients, and all nine viral cultures and eight chlamydia fluorescent antibody smears were negative. Since *Moraxella* conjunctivitis was clustered in female boarding students, the remainder of the analyses in the conjunctivitis study include only members of this group.

Symptoms associated with positive conjunctival culture included a history of conjunctival redness, pain, and adherent eyelids in the morning (Table 2). For most students it was not possible to determine the duration of symptoms. Signs of conjunctivitis, other than follicles on the tarsal plate or lower palpebral conjunctiva, were rarely present.

TABLE 1
RESULTS OF CONJUNCTIVAL CULTURES IN JUNIOR HIGH AND HIGH SCHOOL STUDENTS AT DZILTH-NA-O-DITH-HLE SCHOOL*

TYPE OF STUDENT	TOTAL NO.	*MORAXELLA* POSITIVE NO.	(%)
Boarding students	92	18	(20)
Female	70	18	(26)
Male	22	0	(0)
Day students	51	1	(2)
Female	32	1	(3)
Male	19	0	(0)

*Female vs male: OR = undefined, P = .008; female boarding vs female day: OR = 10.7, P = .007.

TABLE 2
SYMPTOMS AND SIGNS OF CONJUNCTIVITIS IN FEMALE BOARDING STUDENTS

	NO. (%) CULTURE POSITIVE (N = 18)	NO. (%) CULTURE NEGATIVE (N = 45)	P VALUE
Symptoms			
Painful eyes	12 (67%)	17 (38%)	.04
History of redness	11 (61%)	19 (42%)	.18
Itching eyes	11 (61%)	21 (47%)	.30
Adherent eyelids in the morning	10 (56%)	14 (31%)	.07
Discharge	6 (33%)	14 (31%)	.86
Crusting on eyelashes	4 (22%)	11 (24%)	.85
Signs			
Tarsal plate follicles	14 (78%)	22 (49%)	.04
Lower conjunctival follicles	15 (83%)	28 (62%)	.10
Palpebral conjunctival injection	2 (11%)	9 (20%)	.49
Bulbar conjunctival injection	1 (6%)	6 (13%)	.66
Discharge	0	0	

A combination of two of the three symptoms of redness, pain, and adherent eyelids was associated with positive *Moraxella* cultures. Two or more of these symptoms were present in 14 (74%) of the culture-positive group compared to 16 (36%) of 45 of the boarding girls with negative cultures (OR = 4.7, P = .008). Although tarsal plate follicles were more common in those with a positive culture, they were also associated with makeup use in culture-negative girls and thus of no use in defining *Moraxella* infection.

We defined culture-proven cases as illness in students with conjunctival culture positive for *Moraxella* and at least two of the three previously mentioned symptoms. We defined girls with positive cultures and without this symptom complex as carriers and did not include them in the analysis of risk factors for conjunctivitis. We defined as clinical cases illness in those with the associated symptom complex and negative culture. Of the 21 clinical cases, 19 occurred in girls (OR = 5.6, P = .01), and 16 of the girls were boarding students (OR = 3.9, P = .03).

We analyzed potential risk factors for *Moraxella*-positive and clinical conjunctivitis by comparing the case groups with 33 female boarding student controls who had negative cultures and fewer than two of the three symptoms: four had one symptom and 34 had no symptoms (Figure). While makeup use in general and use of specific types of makeup were not significant risk factors, sharing eye makeup (OR = 7.2, P = .004) and sharing with an infected student (OR = 15.0, P = .002) were significantly associated with conjunctival infection (Table 3). Sharing eyeliner and eye shadow were implicated (both types of makeup: OR = 4.1, P =.05). When we incorporated eyeliner, eye shadow, and mascara in a multivariate model, sharing both eyeliner and eye shadow were significant, independent risk factors, and sharing mascara was unrelated to infection.

We cultured available samples of makeup from several of the girls included in the study: four (31%) of 13 eyeliner pencils were culture positive. Three of five samples from girls with positive conjunctival cultures were positive, and one eyeliner pencil remained culture positive for three days after being taken from the infected student. There were no positive cultures in three types each of mascara and eye shadow cultured, although the small sample size renders the difference between types of makeup inconclusive.

In clinical cases in female boarders, risk factors were similar. Sharing makeup and sharing with an infected student were associated with conjunctivitis, although not as strongly as for culture-positive students (sharing eye makeup: OR = 2.9, P = .09; sharing with an infected student: OR = 5.8, P = .04; sharing eyeliner: OR = 4.1, P = .04). By univariate analysis, additional risk factors included sharing makeup with family members at home (OR = 4.1, P = .03) and sharing towels (OR = 10.8, P = .002).

TABLE 3
RISK FACTORS FOR CULTURE-POSITIVE *MORAXELLA* CONJUNCTIVITIS IN FEMALE BOARDING STUDENTS

RISK FACTOR	NO. (%) POSITIVE: CULTURE-POSITIVE STUDENTS (N = 13)	NO. (%) POSITIVE: CONTROLS STUDENTS (N = 38)	ODDS RATIO
Eye makeup use	11 (85%)	27 (71%)	2.0
Eyeliner	11 (85%)	25 (66%)	2.9
Eye shadow	9 (69%)	23 (61%)	1.5
Mascara	6 (46%)	21 (55%)	0.7
Eye makeup sharing	10 (77%)	12 (32%)	7.2*
Sharing with an infected student	6 (46%)	2 (5%)	15.0*
Eyeliner sharing	5 (38%)	5 (13%)	4.1*
Eye shadow sharing	5 (38%)	5 (13%)	4.1*
Mascara sharing	5 (38%)	9 (24%)	2.0

*P < .05.

Unrelated to case or control status were previous ocular injury, socioeconomic status measured by the presence of electricity and the number of rooms at home, the presence of running water at home, and animal exposure. Only one (3%) of 30 family contacts of culture-positive students had a positive conjunctival culture.

Of 60 female boarding students whose charts were available for review, 48 (80%) had been to the clinic at least once during the preceding year with symptomatic conjunctivitis; 72 cases had been diagnosed in this population, repre-

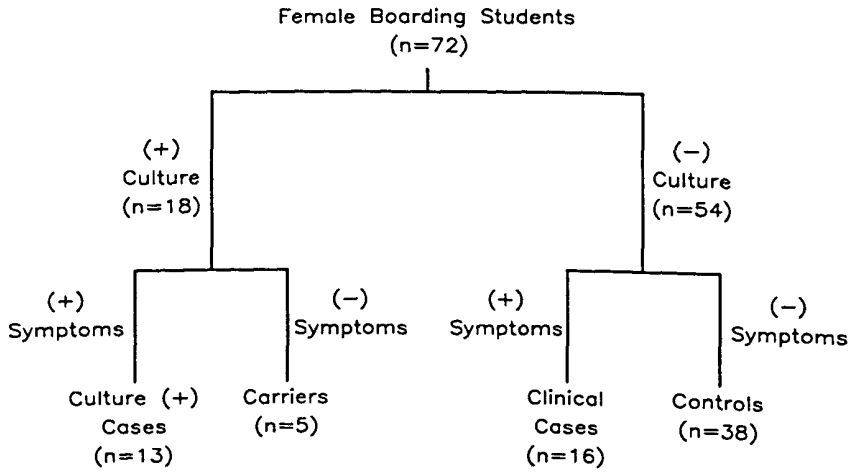

Figure (Schwartz and associates). Classification of female boarding students based on results of conjunctival cultures and symptoms.

senting a mean of 1.6 clinic visits for conjunctivitis per patient. All students in both the culture-positive and clinical case groups had been seen for conjunctivitis compared with 25 (65%) of 38 controls (OR = undefined, P = .01). Day students had been seen significantly less frequently than boarders (OR = 21.3, P < .00001), and boys less often than girls (OR = 20, P = .00002).

Carriage Study

Anterior nasal cultures were positive for *Moraxella* in 35 (44%) of 79 teenage female boarding students (Table 4). Results of nasal culture were not associated with a student's having culture-confirmed or clinical conjunctivitis. We also found positive nasal cultures in 19 (24%) of 79 day students (27% in females and 20% in males) and in 20 (21%) of 97 patients in the general clinic population at Dzilth and at the clinic in Farmington (Table 4). Only two patients at Dzilth and one at Farmington were conjunctival carriers. We investigated risk factors for nasal carriage in the clinic patients using a questionnaire similar to that used in the conjunctivitis study. Nasal carriage was significantly more common in those who lived in homes without electricity (OR = 2.9, P = .05). Carriage was unrelated to makeup use or sharing, the presence of teenage students in the family (boarders or day students of either sex), age, and gender.

Treatment Study

At the beginning of the prospective treatment phase of the investigation, *Moraxella* was isolated from conjunctival cultures in 22 (27%), and nasal cultures in 37 (45%) of 82 students. The rate of infection in the three treatment groups did not differ significantly at either culture site (range for conjunctival cultures: 24% to 29%, P = .91; for nasal cultures: 39% to 52%, P = .62).

TABLE 4
RESULTS OF CONJUNCTIVAL AND NASAL CULTURES

GROUP	*MORAXELLA*-POSITIVE/TOTAL CULTURES (%)			
	CONJUNCTIVAL		NASAL	
Boarding students				
Female	19/79	(24)	35/79	(44)
Male	0/22	(0)	2/8	(25)
Day students (both sexes)	1/51	(2)	9/37	(24)
Clinic patients	3/97	(3)	20/97	(21)

TABLE 5
CLINIC VISITS FOR CONJUNCTIVITIS MEETING THE CLINICAL CASE DEFINITION BY FEMALE BOARDING STUDENTS DURING THE 11-MONTH PERIOD AFTER THE INVESTIGATION

STUDENT GROUP	TOTAL NO.	TREATMENT	CLINICAL CONJUNCTIVITIS	
			NO. OF STUDENTS	NO. OF CLINIC VISITS
Dzilth High School	38	Rifampin and education	10*	14
Huerfano dormitory	23	Education	6*	7
Dzilth Junior High School	21	—	16	20

*Compared to Dzilth Junior High School: OR = 9, P < .001.

In those who received rifampin, *Moraxella* was eradicated from the conjunctiva in ten (91%) of 11 and from the nares in all 17 carriers. During the 11-month follow-up period, ten (26%) of the 38 rifampin-treated girls had one or more episodes of conjunctivitis meeting the previously established clinical case definition. Three of these patients had *Moraxella* isolated from conjunctival swabs (Table 5). The rate of conjunctivitis in the girls from Huerfano dormitory who were educated concerning risk factors for conjunctivitis but received no systemic therapy was also 26% (six of 23), with a positive culture in one girl. In contrast, 16 (76%) of 21 controls (Dzilth junior high school girls) had one or more episodes of conjunctivitis, a proportion significantly greater than that in the other two groups (P < .001), with *Moraxella* isolated from two students (Table 5). Eight controls (38%) had *Moraxella*-positive nasal cultures at the time of their clinic visit for conjunctivitis compared with two (5%) high school students and two (9%) students from Huerfano.

Discussion

The origin of conjunctivitis is often difficult to determine. Since the conjunctiva is not a sterile site, culture results must be interpreted cautiously. Bacteria may be carried asymptomatically, while viruses, which often are not

cultured, may be responsible for disease. In this outbreak, there was strong evidence that *Moraxella* was the etiologic agent for culture-positive cases. *Moraxella* was isolated significantly more frequently from conjunctival swabs of symptomatic patients than asymptomatic persons (P = .001). The symptoms of conjunctival irritation and of a scanty discharge causing adherent eyelids in the morning in our subjects were also consistent with previous reports of the chronic follicular conjunctivitis caused by *Moraxella*,[10,12] and this combination of symptoms was significantly associated with positive culture. Although viral cultures and chlamydia fluorescent antibody smears were obtained for only a sample of students, all results of both tests were negative, and no other bacterial pathogens were isolated from the students with *Moraxella* isolates. The epidemiologic results also support the causative role of *Moraxella*, with positive cultures clustered in a clearly defined risk group and absent in other persons. Finally, eyeliner, one of the vehicles of infection identified by our investigation, was culture positive for *Moraxella* in one third of all samples and in two thirds of samples taken from infected students.

The origin of the clinical cases is more difficult to determine. By definition, the symptoms experienced by these students were similar to those experienced by the *Moraxella*-positive group. The absence of other identified causes and the occurrence of a clinically similar disease in the same risk group with many of the same risk factors in an outbreak of *Moraxella* make it the most likely agent. Several factors may explain the absence of a positive culture in these students. During the initial investigation, swabs were taken from only one eye, and if cultures were not 100% sensitive or disease was not bilateral, *Moraxella* could have been missed. During the prospective phase of the investigation we obtained cultures from both eyes of students in the three treatment groups; cultures were positive bilaterally in 84% and unilaterally in 16%, indicating that in some clinical cases a positive culture may have been missed. Additionally, many of the girls in the clinical case group had received topical antibiotic therapy for conjunctivitis during the two months before the investigation, potentially resulting in negative cultures at the time of the study.

Our epidemiologic investigation implicated sharing eyeliner and eye shadow as significant risk factors for conjunctivitis. Previous reports, while mentioning sharing makeup as a risk factor, have emphasized makeup use—especially mascara use—as a causative factor.[8,9] Analysis of our data uncontrolled for boarding status showed eye makeup use to be a significant risk factor for culture-positive disease (OR = 4.4, P = .05), but since both rates of disease and of makeup use depend on boarding status, when we controlled for this variable in a multivariate model including only boarding students, makeup use was no longer significantly associated with disease. The power of the study to detect a significant association with makeup use, however, was limited by the high rate of use in both study and control groups.

We evaluated the contribution of using and sharing each type of makeup to the risk of conjunctivitis and found that while sharing either eyeliner or eye shadow was independently associated with infection, mascara use and sharing did not contribute to the risk of conjunctivitis. The epidemiologic association of eyeliner with *Moraxella* conjunctivitis was further supported by the positive eyeliner cultures, and many girls were noted to be wearing eyeliner between the eyelashes and conjunctiva. Before concluding that some types of makeup are risk-free, however, other studies are needed to corroborate these results.

There have been few previous studies of the rate of *Moraxella* nasal carriage. Van Bijsterveld[2] reported a 12.9% carriage rate in adults in the Netherlands. A study of New York City alcoholics by Baum, Fedukowicz, and Jordan[13] found 35% nasal carriage. They attributed this higher rate to poor sanitation and poor nutrition. Other studies have associated *Moraxella* carriage with pyridoxine deficiency.[14,15] The rate of carriage in the girls at the dormitory in our study was significantly greater than that reported in either of the two previous studies, and the rate in the Navajo clinic patients was greater than the rate found in the Netherlands. In the community study, nasal carriage was more common in those without electricity at home; this may be a surrogate for households with poorer sanitation. We did not assess pyridoxine levels and no study participant was clinically malnourished.

Results of the prospective treatment phase of the investigation suggest that patient education, with or without a short course of rifampin, effectively reduced the rate of clinical conjunctivitis. However, because of the small number of students in each group, the lack of

random assignment to treatment groups, the potentially greater access to therapy for the group at Dzilth than those at Huerfano, and the lower isolation rate for *Moraxella* during the follow-up period, further study should be done to evaluate the necessity of systemic therapy.

Additionally, the success of educating students not to share makeup in decreasing the rate of conjunctivitis lends further support to the role of makeup sharing as the major risk factor responsible for the high rate of conjunctivitis in this population. The failure of case-by-case therapy with topical antibiotics in preventing recurrences and transmission of conjunctivitis in this setting may be the result of poor compliance with therapy, reinfection from sharing infected makeup, or self-reinoculation from nasal *Moraxella*. Our results suggest that in epidemics of disease, patient education and oral rifampin treatment may be necessary to eradicate *Moraxella* and interrupt the transmission of infection.

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