

# Risk Factors for Group B Streptococcal Colonization: Potential for Different Transmission Systems by Capsular Type

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**PURPOSE:** Group B *Streptococcus* (GBS) is a common inhabitant of the bowel and vaginal flora, with known transmission routes including sexual contact and vertical transmission from mother to infant. Food-borne transmission is also possible, as GBS is a known fish and bovine pathogen. We conducted a prospective cohort study in order to identify risk factors for acquisition.

**METHODS:** We identified risk factors for GBS acquisition among college women ( $n = 129$ ) and men ( $n = 128$ ) followed at 3-week intervals for 3 months.

**RESULTS:** A doubling in sex acts significantly increased incidence of GBS capsular type V by 80% (95% confidence interval [CI]: 1.19, 2.58), and other non-Ia or -Ib types combined by 40% (95% CI: 1.00, 2.06; incidence of capsular type Ia (odds ratio [OR] = 1.2; 95% CI: 0.71, 1.88;  $p = 0.57$ ) and Ib (OR = 1.5, 95% CI: 0.75, 2.86;  $p = 0.27$ ) were elevated, although not significantly. After adjustment for sexual activity and sexual history, gender, and eating venue, fish consumption increased risk of acquiring capsular types Ia and Ib combined 7.3 fold (95% CI: 2.34, 19.50), but not of acquiring other capsular types. Beef and milk were not associated with GBS incidence.

**CONCLUSIONS:** Different GBS capsular types may have different transmission routes. *Ann Epidemiol* 2007;17:854–862. © 2007 Elsevier Inc. All rights reserved.

**KEY WORDS:** Group B *Streptococcus*, Fish, Sexual Behavior, Epidemiology, Capsular Type, Transmission.

## INTRODUCTION

Group B *Streptococcus* (GBS) is a common inhabitant of the bowel and vaginal flora, with known transmission routes including sexual contact and vertical transmission from mother to infant. Colonization usually does not lead to disease except in vulnerable populations (1), such as the elderly and newborns. Evidence that GBS is a sexually transmitted infection (STI) includes its association with recent sexual activity, younger (adult) age, more than one sex partner in the past 30 days (2) and the fact that it is found more frequently among patients attending STI clinics than other types of clinics (3–5). In addition, colonization rates are much lower among children aged 3 to 10 years (4%) (6) and among adults who have never engaged in sexual activity (17% in women, 13% in men) (7) than among sexually active adults (38% in women, 24% in men) (7). However, GBS colonization has not been associated with increased lifetime numbers of sex partners or previous history of other

STIs, traditional STI risk factors (5, 8). Within-couple transmission has been clearly demonstrated in both pregnant (9, 10) and nonpregnant (11) cohorts. By contrast, transmission of GBS following casual contact is low, as shown in studies of college roommates (7, 12).

As GBS is a common bowel inhabitant and a known bovine and fish pathogen, it is possible that GBS is transmitted through food or by the fecal-oral route. GBS was originally isolated as the causative agent of mastitis in cows and is a pathogen of both wild and captive fish (13, 14). Furthermore, a comparison of the whole cell protein and physiological patterns of GBS serotype Ib isolated from fish and humans were highly similar, suggesting a common ancestor (13). We previously identified associations of GBS colonization with eating certain foods, but the results were inconsistent (7, 8). However, these studies were cross-sectional, so it is impossible to discern if the foods led to acquiring GBS or maintaining existing GBS carriage.

To investigate risk factors for GBS colonization, we conducted a prospective longitudinal cohort study among male and female college students living in a single dormitory.

## METHODS

### Study Protocol

As described previously (7, 12), 738 students who lived in a first-year dormitory at the University of Michigan between January and February 2001 were invited to participate via an

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advertisement in their dormitory mailbox. We randomly sampled dormitory floors and invited all inhabitants on the selected floors to participate ( $n = 738$ ). Overall, 63% (216 male and 246 female students) consented and completed the enrollment protocol. Following informed consent, participants completed a self-administered questionnaire and provided a throat and mouth (cheek) culture, self-collected initial-void urine, anal orifice, and vaginal specimens. For anal orifice specimens, the swab did not pass the anal sphincter. Nonresponders were sent up to 3 e-mail reminders and contacted by telephone. All participants positive for GBS and a random sample of those negative at enrollment were invited to return for four additional visits at 3, 6, 9 and 12 weeks after enrollment. At each follow-up visit additional specimens were collected and participants completed a self-administered questionnaire. The study protocol was approved by the Institutional Review Board at the University of Michigan.

Of the 149 men and 150 women invited to participate in the follow-up study, 257 of the 299 (86%) completed the first follow-up; 82%, 80%, and 79% completed the second, third, and fourth follow-ups, respectively. Follow-up rates were virtually the same by gender. Participants were compensated for participation, with a bonus for completing all 4 follow-ups.

### GBS Isolation

Specimens were self-collected as described previously (7) using the Culture Swab Plus Collection System (Baltimore Biological Labs; Sparks, MD) with Amies transport medium. Following collection, rectal and vaginal specimens were inoculated in selective broth media containing gentamicin and nalidixic acid, incubated overnight at 37°C with CO<sub>2</sub>, subcultured onto trypticase soy agar with 5% sheep blood, and incubated overnight. Urine specimens were subcultured directly to trypticase soy agar. Suspect isolates were confirmed serologically as described in previous studies (8, 11).

### Pulsed-field Gel Electrophoresis

We used pulsed-field gel electrophoresis (PFGE) to determine whether participants who had GBS colonized in multiple sites carried identical GBS strains and to distinguish between continuous carriage and new infection with a different strain. PFGE methods have been described previously (8, 11).

### Capsular Typing

We classified isolates into capsular types Ia, Ib, and II to VIII by using DNA dot blot hybridization, as previously described (15). For a subset of isolates, however, DNA hybridization was performed by using an alternative anti-fluorescein-AP

antibody, according to the manufacturer's protocol (Roche Diagnostics, Penzberg, Germany), as the reagents used previously had been discontinued. Nontypeable isolates were probed for the presence of the GBS 16S RNA gene to verify that chromosomal DNA was present on the membrane when it was initially probed with the capsular-specific gene probes. DNA extraction for two isolates was found to be inadequate for capsular typing after repeated attempts and they were excluded from the analysis. When more than one isolate from a single individual had the same PFGE pattern, we assumed the isolates had the same serotype (16). Thus for each person, only one randomly selected isolate from each observed PFGE band pattern was capsular typed. Serotype distributions of this collection have been presented previously (17).

### Data Analysis

We estimated the 3-week incidence of GBS, by capsular type, for each putative risk factor and tested for statistical significance using the chi-square test for dichotomous predictors and the Mantel-Haenszel chi-square test for trend for ordinal predictors. We estimated the incidence using GBS at any colonization site. If an infected individual became colonized with a second capsular type, he or she was counted as an incident case for the second capsular type. The number of incident cases for capsular type Ib was small ( $n = 5$ ); because the associations of risk factors with capsular type Ib were very similar to those observed for capsular type Ia ( $n = 14$ ), we present the capsular-specific risk factors with capsular types Ia and Ib combined. There were 21 incident cases of capsular type V and 19 incident cases of all other capsular types (not Ia, Ib, or V) combined.

We stratified by all variables where we observed a strong association with GBS incidence (risk ratio  $>2.0$ ) and checked for confounding and effect modification. To simultaneously assess the effects of multiple variables, we fit a series of logistic regression models predicting the 3-week incidence of each GBS capsular type, and all capsular types combined. Initially we included all variables significant in the univariate analyses and then added other variables one at a time. Nonsignificant variables were deleted, except for those retained for interpretation purposes. Since an individual can be colonized with more than one capsular type, all individuals without a particular capsular type were considered at risk. In addition, if an individual cleared a capsular type, they were considered at risk to acquire it again. We had 4 individuals who cleared infection and became reinfected. To adjust for the effects of repeated measures, we repeated the final regression models included in the manuscript using generalized estimating equations. Because these gave essentially the same results as those not adjusted using generalized

estimating equations, only the unadjusted models are presented.

As a follow-up to the previous models, we further investigated food choices, which are not necessarily independent. To determine the food(s) most associated with GBS incidence overall and by capsular type, we fit a series of logistic regression models including all foods measured, as well as variables significant in the previous analysis. We selected the variables most predictive of GBS incidence using the method of “all possible regressions” (18), which considers all possible subset models and displays the best models at each step, first considering a single covariate, then considering two covariates, etc. From these listings of comparator models, the most predictive variables can be identified for further testing in more targeted models.

Because the frequencies of the various sexual activities (vaginal intercourse, anal intercourse, and passive and active oral sex) were highly correlated and we observed an increase in GBS incidence with increasing frequency of each individual type of sexual activity, we created a summary variable of sexual activity: the maximum frequency among all types of sex acts reported in the interval. As the distribution of the maximum frequency of sex acts was highly skewed, we transformed this variable using log base 2, so the odds ratio is interpreted as the ratio of the odds of GBS incidence for a doubling in the number of sex acts. For example, an odds ratio of 1.5 corresponds to a 50% increase in the odds of GBS incidence with 4 versus 2 sex acts. All analyses were conducted using SAS 9.1 (19).

## RESULTS

Most of the 257 participants were white (86% of women/85% of men) and 19 years of age; all were residents of a single freshman dormitory. Most had engaged in sexual activity (84% of women/70% of men), defined as vaginal, oral, or anal intercourse, with a median age at first sex of 16 years for women and 17 years for men. In the baseline survey (7), 34% of the women and 20% of the men were colonized with GBS at one or more sites. As previously reported, the overall 3-week incidence of GBS was 11.3% (95% confidence interval [CI]: 7.4–15.2) among women and 8.8% (95% CI: 5.8–11.8) among men; the most common capsular types were Ia and V (12). The 3-week incidence of type Ia was 2.3% for women and 2.4% for men; type Ib, 1.6% for women and 0.9% for men; type V, 4.7% for women and 3.5% for men (12). Since an individual can be colonized with more than one capsular type, all individuals without a particular capsular type were considered at risk. In addition, if an individual cleared a capsular type, they were considered at risk to acquire it again.

## Risk of Acquiring GBS by Sexual Activity

Engaging in any sexual activity (defined as vaginal, oral, or anal intercourse) during the previous 3 weeks was significantly associated with an increased incidence of GBS overall and of all capsular types with the exception of types Ia and Ib, although the trend was in the same direction (Table 1). A significantly increased incidence of GBS with increasing frequency of vaginal intercourse, frequency of active and passive oral intercourse, the maximum frequency of all types of sex acts measured, number of sex partners in the past 3 weeks, and number of sex partners in the past 12 months was observed overall and for capsular types excluding Ia, Ib or V. (The numbers of students engaging in anal intercourse were too small to provide stable estimates.) Use of a condom was not associated with incidence, nor was sex with a new partner.

For an infection that is transmitted via sexual contact, we can indirectly measure risk of contact with an infected individual by inquiring about initiating a new sexual relationship. Because there is a transmission probability associated with each sex act, more sex acts with an infected partner increases risk. As the duration of GBS carriage is short, colonization among partnerships of longer duration may have already cleared from both partners; thus we anticipated observing a higher risk of GBS acquisition with a new than with an existing partner. Further exploration of the apparent lack of association between acquiring a new partner and GBS risk revealed that frequency of sexual activity with a new sex partner in the previous interval was low. Indeed, no individuals reporting new sex partners in the previous interval reported a frequency of vaginal intercourse more than one to two times per week (data not shown). However, for existing partners, the 3-week GBS incidence rose with increasing frequency of the maximum of sex acts measured (Fig. 1). For new partnerships, the opposite trend was observed, although the confidence intervals are very wide. The highest incidence among those with a new partner occurred among those who reported no sex. Unfortunately, we did not measure other forms of intimate contact, such as kissing, that may lead to transmission, and there was no association with frequency of hands on partners' genitals ( $p = 0.40$ ; data not shown).

## Risk of Acquiring GBS by Eating Selected Foods, Eating Locations, and Hand Washing

There was no increase in the 3-week GBS incidence by capsular type or overall with the consumption of pork, beef, chicken or eggs or milk (Table 2). However, we observed a trend of greater incidence of capsular types Ia and Ib with eating fish more frequently and for all capsular types combined. There was also a trend of eating yogurt and incidence of capsular types Ia and Ib but no other capsular type;

**TABLE 1.** Incidence of group B *Streptococcus* by capsular type/3 weeks/100 persons, by selected sexual behaviors in previous 3 weeks\*

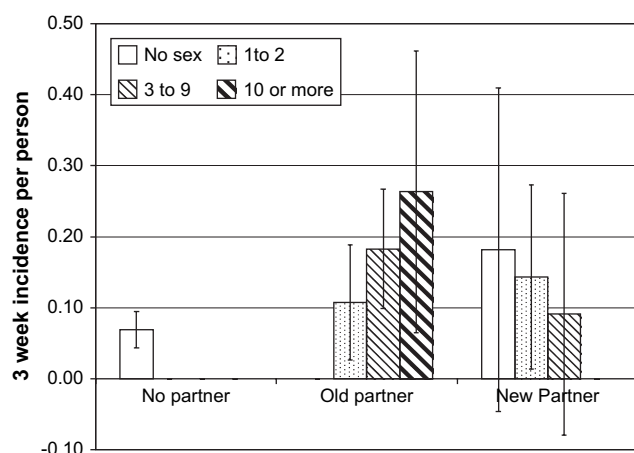
Characteristic	Person intervals	% Capsular type Ia or Ib	% Capsular type V	% Non-Ia, Ib, or V capsular types	% All capsular types combined
Gender					
Male	340	2.9	3.2	2.7	8.8
Female	256	3.5	3.9	3.9	11.3
Sex in last 3 wk					
No	377	2.9	2.1	1.9	6.9
Yes	216	3.7	6.0	5.6	15.3
$\chi^2$ p Value		0.60	<b>0.01</b>	<b>0.01</b>	<b>0.001</b>
Frequency of vaginal intercourse previous 3 wk					
None	447	2.9	2.7	1.8	7.4
1 to 2	51	7.8	2.0	5.9	15.7
3 to 9	76	1.3	7.9	7.9	17.1
$\geq 10$	17	5.9	5.9	11.8	23.5
Test for trend <sup>†</sup>		0.75	0.10	<b>0.001</b>	<b>0.002</b>
Frequency of active oral sex in past 3 wk					
None	439	3.6	2.3	2.5	8.4
1 to 2	93	1.1	8.6	4.3	14.0
3 to 9	55	1.8	5.5	5.5	12.7
$\geq 10$	4	25.0	0	25.0	50.0
Test for trend <sup>†</sup>		0.39	0.23	<b>0.009</b>	<b>0.005</b>
Frequency of passive oral sex in past 3 wk					
None	435	3.5	2.1	2.3	7.8
1 to 2	88	1.1	11.4	4.6	17.1
3 to 9	61	3.3	3.3	6.6	13.1
$\geq 10$	6	16.7	0	16.7	33.3
Test for trend <sup>†</sup>		0.27	0.48	<b>0.007</b>	<b>0.008</b>
Maximum frequency of all sex acts in past 3 wk					
None	395	2.8	2.5	1.7	7.1
1 to 2	84	4.8	3.6	3.6	7.1
3 to 9	93	2.2	7.5	7.5	17.2
$\geq 10$	20	10.0	5.0	10.0	25.0
Test for trend <sup>†</sup>		0.17	0.12	<b>0.003</b>	<b>0.0004</b>
No. of sex partners in past 3 wk					
None	378	2.9	2.4	1.9	7.1
1	191	3.7	6.3	5.2	15.2
2	11	9.1	0	9.1	18.2
$\geq 3$	12	0	0	8.3	8.3
Test for trend <sup>†</sup>		0.71	0.27	<b>0.01</b>	<b>0.02</b>
No. of sex partners in past 12 mo					
None	191	2.1	1.6	1.6	5.2
1	219	3.2	4.6	3.2	11.0
2	85	2.4	5.9	2.4	10.6
$\geq 3$	100	6.0	3.0	7.0	16.0
Test for trend <sup>†</sup>		0.12	0.32	<b>0.03</b>	<b>0.005</b>
Used a condom in past 3 wk					
No	499	3.4	2.8	2.8	9.0
Yes	94	2.1	7.5	5.3	14.9
$\chi^2$ p Value		0.52	<b>0.03</b>	0.20	0.08
Sex with new partner in previous 3 wk?					
No sex	380	3.9	6.1	1.8	6.8
Sex with old partner	164	5.8	6.1	2.1	15.9
Sex with new partner	52	3.9	3.7	2.9	13.5
$\chi^2$ p Value		0.60	<b>0.03</b>	<b>0.05</b>	<b>0.005</b>

\*Participants followed in 3-week intervals. Study population: 129 female and 138 male college students living in a single dormitory. Bolded text indicates statistically significant values.

<sup>†</sup>Value of p for the Mantel-Haenszel chi-square test for trend.

cheese and ice cream showed no trends with any capsular type. There was little association with eating fresh or canned fruit, but eating raw vegetables was associated with an

increased incidence of all capsular types except for capsular type V; we also observed a trend of increasing incidence of capsular type Ia and Ib with eating cooked vegetables. In



**FIGURE 1.** Three-week incidence rate of group B streptococcus of any capsular type, by maximum frequency of vaginal or oral intercourse in previous 3 weeks, for those with no current partner, an existing partner, or a new sex partner in previous 3 weeks. Ticked lines indicate 95% confidence intervals. Study population: 129 female and 128 male freshmen college students living in a single dormitory.

the vast majority (99%) of the risk periods, students reported eating at least once a week in their dormitory in the previous interval; however, those that did not had almost 4 times the incidence of GBS. Those who ate at a “sit down” restaurant one or more times a week were also more likely to acquire GBS.

The strong association with sexual activity and previous studies (4, 5, 7–9) suggests that sexual transmission occurs. Therefore we stratified the associations for each food item by sexual activity in the past 3 weeks to identify possible modification or confounding by sexual activity. After stratification, the association with eating fish, but no other food item, remained (Fig. 2). Among those engaging in sexual activity in the previous 3 weeks, we observed an increased risk of GBS for each capsular type grouping with fish consumption. However, among those who reported no sexual activity in the previous interval, fish consumption increased the incidence only for capsular types Ia and Ib combined (incidence among fish eaters, 10.3% of 58 risk periods vs. 1.6% of 319 risk periods among non-eaters of fish; among those engaging in sexual activity, the corresponding incidences were 16.7% of 18 risk periods vs. 2.5% of 198 risk periods).

### Multivariable Analysis

We further explored these associations by fitting a series of multivariable logistic regressions, modeling the incidence of each capsular type grouping as the outcome, as well as the incidence of all capsular types combined (Table 3). Fish consumption two or times per week was associated with a 7.3-fold increase (95% CI: 2.78, 18.90) in the odds

of acquiring capsular types Ia and Ib combined, after adjusting for frequency of sex acts (the log base 2 of the maximum of frequency of vaginal sex and oral sex), number of sex partners in the previous year, frequency of eating in the dormitory, and gender. When we ran models for capsular types Ia and Ib separately, including only frequency of sex acts, gender, and fish consumption in the model, the odds ratios for fish eating remained significant for capsular type Ia (OR = 7.8; 95% CI: 2.6, 23.1) and marginally so for capsular type Ib (OR = 5.5; 95% CI: 0.9, 34.2), reflecting the small number of incidence capsular type Ib acquisitions. The frequency of sexual acts was not statistically significantly associated with acquiring either capsular type Ia (OR = 1.2; 95% CI: 0.71, 1.88;  $p = 0.57$ ) or Ib (OR = 1.5, 95% CI: 0.75, 2.86;  $p = 0.27$ ).

By contrast, there was no statistically significant association between fish consumption and acquiring capsular type V or other capsular types. The frequency of sex acts in the previous 3 weeks was, however, strongly associated with increased risk of acquiring capsular type V and all non-Ia, Ib or V capsular types, but not types Ia or Ib. Number of sex partners in the previous year was also a significant predictor of acquiring capsular types other than V, Ia, or Ib.

After adjustment for sexual activity and fish consumption, there was no longer a significant association of yogurt (OR = 1.1; 95% CI: 0.8, 1.5) and raw vegetables (OR = 1.1; 95% CI: 0.4, 2.8) with overall or capsular-specific GBS incidence (data not shown). Adjustment for sexual activity did not change the associations between other food variables and GBS incidence either overall or by capsular type. Repeating the models adjusting for repeated measures by using generalized estimating equations also did not change the observed associations.

### DISCUSSION

In a prospective cohort study of 257 healthy male and female college students living in a single college dormitory followed up at 3-week intervals for 12 weeks, we observed sexual activity, particularly frequency of vaginal or oral intercourse, to be the strongest predictor of GBS incidence for capsular type V and all types other than Ia, Ib, and V. However, even among those students who were not sexually active, the rate of GBS was surprisingly high, leading us to consider other sources of exposure. The finding of a 7.3-fold increase (95% CI: 2.78, 18.90) in the incidence of GBS capsular types Ia and Ib with fish consumption two or more times a week in the previous 3 weeks (after adjustment for frequency of sex acts during the previous 3 weeks, gender, number of sex partners in the previous year, and frequency of eating in the dormitory) led us to consider the potential of fish as a food-borne source of GBS. We found no associations



**TABLE 2.** Incidence/3 weeks/100 persons of group B *Streptococcus* by capsular type, by eating selected foods one or more times per week, eating locations, and handwashing practices in previous 3 weeks

Food	Person intervals	% Capsular type Ia or Ib	% Capsular type V	% Non-Ia, -Ib, or -V capsular types	% All capsular types combined
Pork					
None	322	3.7	3.1	2.8	9.6
1 time/wk	148	2.7	3.4	4.1	10.1
2-3 times/wk	101	3.0	5.9	4.0	12.9
≥4 times/wk	16	0.0	0.0	0.0	0.0
Test for trend <sup>†</sup>		0.43	0.47	0.75	0.87
Beef					
None	119	5.0	2.5	5.0	12.6
1 time/wk	141	3.6	5.0	2.1	10.6
2-3 times/wk	213	2.4	3.8	2.8	8.9
≥4 times/wk	118	2.5	2.5	3.4	8.5
Test for trend <sup>†</sup>		0.37	0.78	0.68	0.33
Chicken					
None	46	6.5	2.2	2.2	10.9
1 time/wk	47	0.0	4.3	0.0	4.3
2-3 times/wk	255	2.4	5.1	5.5	12.9
≥4 times/wk	247	4.1	2.0	1.6	7.7
Test for trend <sup>†</sup>		0.73	0.35	0.53	0.46
Fish					
None	299	2.0	2.0	3.3	7.4
1 time/wk	212	1.9	5.7	3.3	10.9
2-3 times/wk	72	9.7	2.8	2.8	15.3
≥4 times/wk	4	50.0	25.0	0.0	75.0
Test for trend <sup>†</sup>		0.0001	0.07	0.77	0.001
Eggs					
None	156	3.2	2.6	1.9	7.7
1 time/wk	188	1.6	6.9	3.7	12.2
2-3 times/wk	170	5.3	0.6	4.1	10.0
≥4 times/wk	76	1.3	3.9	2.6	7.9
Test for trend <sup>†</sup>		0.83	0.54	0.73	0.95
Milk					
None	41	12.2	2.4	7.3	22.0
1 time/wk	51	2.0	3.9	3.9	9.8
2-3 times/wk	132	2.3	3.0	0.0	5.3
4-6 times/wk	110	0.9	2.7	3.6	7.3
Daily	261	3.5	4.2	3.8	11.5
Test for trend <sup>†</sup>		0.14	0.55	0.99	0.63
Yogurt					
None	321	2.8	3.1	4.1	10.0
1 time/wk	136	1.5	4.4	2.9	8.8
2-3 times/wk	73	5.5	5.5	0.0	11.0
≥4 times/wk	65	6.2	1.5	3.1	10.8
Test for trend <sup>†</sup>		0.05	0.91	0.22	0.73
Ice cream					
None	113	3.5	3.5	0.9	8.0
1 time/wk	162	0.6	4.3	6.2	11.1
2-3 times/wk	202	4.0	3.5	2.0	9.5
≥4 times/wk	119	5.0	2.5	3.4	10.9
Test for trend <sup>†</sup>		0.22	0.77	0.96	0.61
Cheese					
None	15	13.3	0.0	13.3	26.7
1 time/wk	27	0.0	18.4	3.7	22.2
2-3 times/wk	200	2.5	2.0	2.0	6.5
≥4 times/wk	353	3.4	3.4	3.4	10.2
Test for trend <sup>†</sup>		0.51	0.39	0.76	0.74

(Continued)

TABLE 2. (Continued)

Food	Person intervals	% Capsular type Ia or Ib	% Capsular type V	% Non-Ia, -Ib, or -V capsular types	% All capsular types combined
Fresh fruit					
None	35	5.7	0.0	2.9	8.6
1 time/wk	125	0.8	4.0	3.2	8.0
2–3 times/wk	239	3.8	2.5	2.9	9.2
≥4 times/wk	196	3.6	5.1	3.6	12.2
Test for trend <sup>†</sup>		0.27	0.23	0.81	0.13
Canned fruit					
None	237	1.3	3.4	3.0	7.6
1 time/wk	129	4.7	3.9	4.7	13.2
2–3 times/wk	109	6.4	3.7	1.8	11.9
≥4 times /wk	120	2.5	3.3	3.3	9.2
Test for trend <sup>†</sup>		0.36	0.92	0.98	0.54
Raw vegetables					
None	125	2.4	5.6	0.0	8.0
1 time/wk	122	2.5	1.6	2.5	6.7
2–3 times/wk	170	1.2	0.6	5.3	7.1
≥4 times/wk	178	6.2	6.2	3.9	16.3
Test for trend <sup>†</sup>		<b>0.03</b>	0.62	<b>0.06</b>	<b>0.007</b>
Cooked vegetables					
None	56	3.6	5.4	1.8	10.7
1 time/wk	111	0.0	5.4	3.6	9.0
2–3 times/wk	232	2.6	2.2	3.5	8.2
≥4 times/wk	196	5.6	3.6	3.1	12.2
Test for trend <sup>†</sup>		<b>0.03</b>	0.84	0.87	0.26
Ate at dormitory					
No	6	16.7	0.0	16.7	33.3
Yes	587	3.1	3.2	3.1	9.4
$\chi^2$ p Value		0.06	0.65	0.06	<b>0.05</b>
Ate at another dormitory					
No	245	2.5	2.5	3.3	8.2
Yes	330	3.9	2.4	2.7	9.1
$\chi^2$ p Value		0.32	0.98	0.71	0.70
Ate at fast food restaurant					
No	146	2.7	2.1	0.7	5.5
Yes	445	3.4	3.2	4.0	10.6
$\chi^2$ p Value		0.71	0.49	<b>0.05</b>	0.07
Ate at sit-down restaurant					
No	114	2.6	0.9	0.9	4.4
Yes	467	3.4	3.9	3.9	11.1
$\chi^2$ p Value		0.67	0.11	0.11	<b>0.03</b>
Frequency of hand washing					
<3 times/d	75	2.7	6.7	2.7	12.0
3–5 times/d	361	3.9	3.3	3.3	10.5
≥6 times/d	150	1.3	2.7	3.3	7.3
Test for trend <sup>*</sup>		0.36	0.18	0.96	0.22

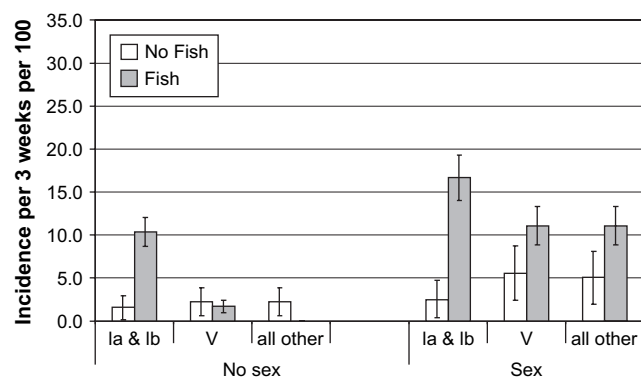
\*Participants followed in 3-week intervals. Study population: 129 female and 138 male college students living in a single dormitory. Bolded text indicates statistically significant values.

<sup>†</sup>Value of *p* for the Mantel-Haenszel chi-square test for trend.

with other food items, including beef, milk, and milk products, which in theory also might be a source of GBS, perhaps because milk from cows with clinical mastitis is not sold. We also found no significant association with handwashing practices, although there was a trend toward decreasing incidence with increased frequency of handwashing for capsular type V and all capsular types combined.

We are aware of no other reports of GBS risk factors by capsular type or of an association between GBS acquisition

and fish consumption. Although the results require confirmation, transmission of GBS via fish is biologically plausible, as GBS serotype Ib is a known fish pathogen (13), and when strains colonizing fish were compared with human strains, they had identical whole protein and physiologic patterns (20). GBS of unknown serotypes also colonize fish (13), which may explain why we observed an association between fish consumption and capsular type Ia. Should this result be confirmed, the findings have public health



**FIGURE 2.** Three-week incidence rate of group B streptococcus by capsular type, fish consumption, and sexual activity in previous 3 weeks. Ticked lines indicate 95% confidence intervals. Study population: 129 female and 128 male freshmen college students living in a single dormitory.

implications for the diet of individuals at high risk of GBS disease (e.g., pregnant women and adults with underlying chronic conditions).

GBS serotype Ib has been isolated from a wide variety of fish, both farm-raised and wild, including tilapia, mullet, and

bluefish (13). However, we found no reports describing colonization rates among apparently healthy fish; although farm-raised fish are vaccinated against GBS disease, this may not affect colonization. We estimate that if the association were causal in our sample, 86% of all persons colonized with GBS capsular type Ia or Ib who ate fish could attribute their colonization to eating fish. Given that serotypes Ia and Ib cause approximately 20% of all neonatal disease and adult disease (21–23), if serotype Ia is also found in fish, fish consumption may account for as much as 17% (i.e., 86% of 20%) of the cases of GBS disease among neonates and adults. The extent to which food preparation influences risk of acquisition of GBS has yet to be determined, as we had no information regarding how the fish was prepared. It seems likely that eating fish raw would pose a greater risk than cooked. We did note an increase in GBS acquisition among students who reported eating in “sit-down” restaurants, although we did not inquire about the food or the restaurant type. Whether GBS is found among commercially sold fish and survives normal cooking temperatures is unknown.

Despite the supporting evidence and biological plausibility, to our knowledge this is the first report of an association

**TABLE 3.** Risk factors for acquiring group B *Streptococcus* by capsular type: Logistic regression model predicting incidence of a capsular type or capsular type grouping, using all other data as reference

Risk factor	Beta	Standard error	OR (95% CI)	p Value
<b>Capsular type Ia or Ib</b>				
Log frequency of sex acts*	0.172	0.220	1.2 (0.77, 1.83)	0.43
No. of sex partners previous year	0.079	0.149	1.1 (0.81, 1.45)	0.59
Eats fish $\geq 2$ times/wk	1.982	0.489	<b>7.3 (2.78, 18.90)</b>	<b>&lt;0.001</b>
Frequency of eating in dormitory	−0.220	0.277	0.80 (0.47, 1.38)	0.43
Male gender	−0.100	0.509	0.90 (0.33, 2.45)	0.84
<b>Capsular type V</b>				
Log frequency of sex acts*	0.560	0.198	<b>1.8 (1.19, 2.58)</b>	<b>0.001</b>
No. of sex partners previous year	−0.244	0.202	0.8 (0.53, 1.16)	0.23
Eats fish $\geq 2$ times/wk	0.538	0.659	1.7 (0.47, 6.23)	0.41
Frequency of eating in dormitory	0.786	0.520	2.2 (0.79, 6.07)	0.13
Male gender	−0.313	0.495	0.73 (0.38, 1.93)	0.53
<b>All other capsular types</b>				
Log frequency of sex acts*	0.362	0.184	<b>1.4 (1.00, 2.06)</b>	<b>0.05</b>
No. of sex partners previous year	0.328	0.117	<b>1.4 (1.10, 1.75)</b>	<b>0.005</b>
Eats fish $\geq 2$ times/wk	−0.134	0.786	0.88 (0.19, 4.08)	0.87
Frequency of eating in dormitory	−0.504	0.253	<b>0.6 (0.37, 0.99)</b>	<b>0.05</b>
Male gender	−0.024	0.508	0.9 (0.36, 2.64)	0.96
<b>All capsular types, combined</b>				
Log frequency of sex acts*	0.383	0.119	<b>1.5 (1.16, 1.85)</b>	<b>0.001</b>
No. of sex partners previous year	0.142	0.082	1.2 (0.98, 1.36)	0.08
Eats fish $\geq 2$ times/wk	1.113	0.354	<b>3.0 (1.51, 6.10)</b>	<b>0.002</b>
Frequency of eating in dormitory	−0.136	0.183	0.9 (0.61, 1.25)	0.46
Male gender	−0.085	0.301	0.9 (0.51, 1.66)	0.78

Risk periods calculated assuming no infection with multiple capsular types, so there is a slight underestimate. Study population: 129 female and 138 male college students living in a single dormitory. Bolded text indicates statistically significant values.

CI = Confidence interval; OR = odds ratio.

\*Log base 2 of maximum (frequency of oral sex, vaginal sex), interpreted as the ratio of the odds of GBS incidence for a doubling in the number of sex acts. For example, an OR of 1.7 is interpreted as a 70% increase in odds of GBS with a doubling in the number of sex acts.



between eating fish and acquiring GBS; thus the findings should be viewed with caution until replicated in other studies. Given that we tested the association with 13 food items, in addition to many other variables, there is always the possibility that this finding is an alpha error.

Acquisition of capsular types other than Ia and Ib was strongly associated with frequency of sexual intercourse during the previous 3 weeks. This finding is consistent with a Pittsburgh study, which followed 1089 women and found sexual activity in the 5 days prior to culture and frequent sexual intercourse in the previous 4 months predicted vaginal acquisition of GBS, although the authors did not report associations by capsular type (5). Acquisition of types Ia and Ib appears to be essentially independent of sexual activity or any effect was too small to detect given our sample size. Although GBS has been strongly associated with sexual activity, it is unclear what type(s) of sexual activity confer the highest risk. Unfortunately, the frequencies of sexual behaviors measured in our study were highly correlated, so we can provide no further insight. The lack of a protective effect with condom use was surprising. However, as GBS is a common bowel inhabitant and can be transmitted by the fecal-oral route, it may be that oral sex is more important for transmission than vaginal intercourse. Because we studied sites in addition to the vagina, any locally protective effect of condoms may have been dwarfed by transmission through other routes. Alternatively, normal condom use may be insufficient to protect against GBS transmission.

Although we observed strong effects, our association was with GBS colonization, not disease, and the study was conducted among healthy young college students. Pregnant women or adults with underlying chronic conditions may be more or less susceptible to colonization following exposure either from eating fish or via sexual intercourse, which would modify the observed risk of colonization. Further studies confirming that GBS found in fish can colonize humans and cause human disease are required.

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