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Sexual activity: an important risk factor for colonization of *Streptococcus agalactiae* in mature female genitalia

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Abstract

Infection by group B streptococci or *Streptococcus agalactiae* (GBS) is an emerging disease in nonpregnant adults of all ages, particularly in elderly persons and those with significant underlying diseases. The source of infection may be due to GBS colonization in the female genitalia. The aim of this study was to determine the prevalence of GBS in genitalia among 2 participant groups; sexually active ($\geq$ 3 times/week) and non-sexually active women. Vaginal swabs were obtained from 138 sexually active women and 49 non-sexually active women for GBS isolation. Percentages of sexually active women with vaginal colonization of GBS were 34.06% which was significantly higher than non-sexually active women (10.20%). Antibiotic resistance among GBS isolates to tetracycline, clindamycin, erythromycin and ofloxacin were 96.15%, 38.46%, 34.62% and 25.0%, respectively, while all isolates were susceptible to penicillin, ampicillin, vancomycin and cefotaxime. Our research suggested that frequent sexual activity was an important risk factor for vaginal colonization of GBS; which could be the source for transmission to persons at high risk of infection, including neonates, pregnant women, and adults, especially those with frequent sexual activity.

***Keywords**: Streptococcus agalactiae, invasive GBS infection, sexual activity, vaginal colonization, risk factor*

1. Introduction

Group B *Streptococcus* (GBS), or *Streptococcus agalactiae*, is best known as the most common cause of neonatal sepsis. However, over the last 2 decades the incidence of neonatal GBS disease has decreased continuously due to successful preventive programs, but the incidence of invasive GBS infections in nonpregnant adults of all ages have reported a 2 to 4-fold increase, especially in the elderly and those with significant underlying diseases (Ballard et al., 2016; Cools et al., 2016; Farley, 2001; Verani, McGee, Schrag, & Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC), 2010; Xie, Yang, Zhao, Jia, & Wang, 2016). The most common underlying disease among nonpregnant adults with GBS infection was diabetes, followed by cancer, acute bed sores, AIDS, long-course corticotherapy, chronic renal disease, cirrhosis, and neurological vessel disorder. Several other opportunistic health issues were common in invasive GBS infections among nonpregnant adults including the following: Skin, soft tissue, and bone infections, bacteremia, urinary tract infections, pneumonia, and peritonitis. Septic arthritis, meningitis, and endocarditis were less common but associated with high morbidity and mortality (Abid, Charfeddine, & Kammoun, 2016; Li, Cheema, & Goel, 2016; van der Mee-Marquet et al., 2008). The fatality rate among adults with invasive GBS infection ranged from 3%-47%, and was markedly higher than neonates (Chaiwarith et al., 2011; Crespo-Ortiz, Castañeda-Ramirez, Recalde-Bolaños, & Vélez-Londoño, 2014; Farley, 2001; Matsubara & Yamamoto, 2009; Peirotti et al., 2002; Skoff et al., 2009; van der Mee-Marquet et al., 2008). The source of these additional infections may result from the spread of the colonizing area of GBS carriages. In most studies 10% - 30% of healthy adults were colonized with GBS in their genital and gastrointestinal tracts, while only 2% and 4% were colonized with GBS on skin and throats, respectively (Bidgani, Navidifar, Najafian, & Amin, 2016; Brimil et al., 2006; van der Mee-Marquet et al., 2008). GBS is transmitted via direct person-to-person contact, including sexual contact (Percha, Newman, & Foxman, 2011; Yamamoto, Nagasawa, Nojima, Yoshida, & Kuwabara, 1999). Several reports indicated that the sexual behavior of women was related to GBS colonization in their genital tracts.

Women who had sexual activity $\geq$ 3 times/week significantly increased the chance of finding GBS in their reproductive organs (Foxman, Gillespie, Manning, & Marrs, 2007; Meyn, Moore, Hillier, & Krohn, 2002; Newton, Butler, & Shain, 1996).

2. Objectives

The aim of this study was to determine whether sexual activity was the risk factor for GBS vaginal colonization by comparing the prevalence of GBS vaginal colonization with the average frequency of having sex $\geq$ 3 times/week and < 3 times/week during the last 3 months.

3. Materials and methods

3.1 Study design and population

This cross-sectional study included 137 female workers from 4 massage parlors who might have frequent sexual activity and 50 female students and women with other occupation during June to October 2013. After informed consent had been completed, trained research personnel asked all enrolled participants about pregnancy, age, sexual behavior and the recent used of antimicrobial agents. Criteria that excluded participants from the study were pregnancy, recent usage of antimicrobial agents or less than 20 years old.

3.2 Ethical considerations

The women recruited in the study were informed about this study and signed consent forms. The study was approved by the Ethical Committee of Research Institute of Rangsit University.

3.3 Specimen collection

Vaginal swabs were collected from participants and immediately inoculated onto Lim selective broth media; a Todd Hewitt base broth supplemented with Gram-negative bacterial inhibitors colistin (10 µg/ml) and nalidixic acid (15 µg/ml) (Fenton & Harper, 1979), then were incubated 24 hours under aerobic conditions with 5% CO_2 at 37 °C.

3.4 Isolation and identification of GBS

Following enrichment in Lim broth, swabs were subcultured on 5% blood agar plates and were incubated 24 hours under aerobic conditions with 5% CO_2 at 37 °C. Five narrow β-hemolytic colonies on blood agar plate of each specimens were selected to identify GBS by Gram's stain, catalase test, and pigment production in proteose peptone rice agar (PPRA). GBS is a Gram-positive cocci in chain, does not produce catalase enzyme and has the ability to produce unique orange/red pigmented colonies when incubated anaerobically on media containing starch products like PPRA (Merritt & Jacobs, 1978). All suspected isolates were confirmed by a positive CAMP test on sheep blood agar plates (Darling, 1975).

3.5 Antimicrobial susceptibility testing of GBS

Susceptibility tests of GBS isolates were performed by agar disk diffusion on Mueller-Hinton agar with 5% sheep blood with incubation at 35°C $\pm$ 2°C and 5% CO_2 for 24 hours under aerobic conditions according to Clinical and Laboratory Standards Institute. Antibiotic disks containing the following antimicrobial agents were used: penicillin (10 units), ampicillin (10 µg), cefotaxime (30 µg), vancomycin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), and ofloxacin (5 µg). Erythromycin-resistant phenotype was determined by D-test with disks containing erythromycin (15 µg) and clindamycin (2 µg). In addition, disk diffusion zone diameter quality control limits were determined using *Streptococcus pneumoniae* ATCC® 49691 (Cockerill & Clinical and Laboratory Standards Institute, 2013).

3.6 Statistical data

Chi-square tests were used to test associations, and a *p*-value of 0.05 or less was considered significant. Odds ratios (OR) and their 95% confidence intervals (CI 95%) were calculated to estimate the effects of factors independently associated with GBS colonization.

4. Results

4.1 Population studied

A total of 187 women was enrolled in the study, including 137 female workers from 4 massage parlors and 50 female students and women with other occupations. The questionnaires indicated 138 participants had sexual activity > 3 times/week, all 137 massage parlors worker had sex > 3 times/week (range from 4-27 times/week with the average of 13.24 times/week), while only one participant from the group of students and other occupations indicated that she had sex > 3 times/week. Furthermore, sexual activity of students and other occupations range from 0-4 times/week with the average of 0.23 times/week. The age of participants were 20-55 years old, age of massage parlors workers were 20-55 years old, while

age of students and women with other occupations were 20-36 years old.

4.2 Prevalence of GBS colonization based on risk factors

Of the 187 women enrolled, GBS was isolated from vaginal swabs of 52 participants (27.81%). Among 52 women positive for GBS, 47 of 137 were massage parlors workers (34.31%), 5 of 50 were students and women with other occupations (10.0%). Prevalence of GBS colonization among massage parlors workers was significantly higher than students and other occupations ($p = 0.001$). Women working in massage parlors had higher risk of vaginal GBS colonization than women with other occupations, with OR = 4.7, 95% CI: 1.75, 12.64, ($p = 0.0022$).

4.2.1 Sexual activity

The colonization rates of GBS according to sexual activity are shown in Table 1. Among 138 participants who had sex $\geq$ 3 times/week, the GBS prevalence was 34.06%, with the sexual activity 7.5-27 times/week. Among participants who had sex < 3 times/week, the prevalence was 10.20%, with the sexual activity between 0-1.5 times/week. Prevalence of GBS colonization among participants with sexual activity $\geq$ 3 times/week was significantly higher than participants with sexual activity < 3 times/week when analyzed by chi-square ($p = 0.001$). Risk of women who had sex more than 3 times/week to be colonized with GBS in the vagina was higher than women who had sex less than 3 times/week, with OR = 4.55, 95% CI: 1.69, 12.23, ($p = 0.0027$).

4.2.2 Age

The colonization rates of GBS according to age are also showed in Table 1. Among 91 participants between 20-25 years old, 16 were GBS carriers and rate of GBS colonization was 17.58%. Of the 16 GBS carriages, 12 were massage parlors workers and 4 were students and women with other occupations. While among 96 participants older than 25 years old, 36 were GBS carriers, rate of GBS colonization was 37.50%. Of the 36 GBS carriers, 34 were massage parlors workers and only 1 was a student. Prevalence of GBS colonization among participants older than 25 years old were significantly higher than participants between 20-25 years old, when analyzed by chi-square ($p = 0.002$). Women older than 25 years old were significantly more likely to be colonized with GBS in their vaginas than women between 20-25 years old, with OR = 2.81, 95% CI: 1.43, 5.55, ($p = 0.0029$).

Table 1 Association of GBS colonization with 2 participant groups, sexual activity and age

Characteristics	GBS colonization		No GBS colonization		χ^2	Odds ratios (OR)		
	No.	%	No.	%	*p*-value	OR	95%CI	p-value
No. of GBS carriage among 2 participants					0.001	4.7	1.75- 12.64	0.0022
Massage parlors workers (n = 137)	47	34.31	90	65.69				
Students/other occupations (n = 50)	5	10.00	45	90.00				
No. of GBS carriage according to sexual activity					0.001	4.5	1.69- 12.23	0.0027
$\geq$ 3 times/week (n = 138)	47	34.06	91	65.94				
(Range of sexual activity)	(7.5-27)		(4-20)					
< 3 times/week (n = 49)	5	10.20	44	89.80				
(Range of sexual activity)	(0-1.5)		(0-1.5)					
No. of GBS carriage according to age					0.002	2.81	1.43-5.55	0.0029
>25 (n = 96)	36	37.50	60	62.50				
20-25 (n = 91)	16	17.58	75	82.42				

4.3 Antimicrobial susceptibility testing of GBS isolates

The antimicrobial susceptibilities of 52 GBS isolates are shown in the Table 2. All strains were susceptible to penicillin, ampicillin, cefotaxime and vancomycin. Most isolates were resistant to tetracycline (96.15%), with 3.85% showing intermediate resistance. Resistant rates to clindamycin, erythromycin, ofloxacin and chloramphenicol of GBS isolates were 38.5%, 34.6%, 25% and 17.3% respectively. Among GBS isolates, 3 isolates (5.77%) had increased clindamycin resistance upon induction with erythromycin as determined by the D test.

When susceptibility test results were compared among the 2 participant groups all 5 isolates from students and women with other occupations were susceptible to penicillin,

ampicillin, cefotaxime, vancomycin, ofloxacin, chloramphenicol and clindamycin. Of 5 GBS isolates from students and women with other occupations, 4 were resistant to tetracycline (80%) and another isolate was intermediately resistant to tetracycline, while none was resistant to erythromycin and only one isolate was intermediately resistant to erythromycin. All isolates from massage parlors workers were susceptible only to penicillin, ampicillin, cefotaxime and vancomycin. The resistant rates of GBS isolates from massage parlors workers to tetracycline, clindamycin, erythromycin, ofloxacin and chloramphenicol of GBS isolates were 97.87%, 42.55%, 38.30%, 27.66% and 19.14% respectively; with 6.38% having inducible clindamycin resistance.

Table 2 Antimicrobial susceptibility testing of GBS isolates

Antimicrobial agent	Number of GBS Isolates (%)								
	From Massage parlors workers (n= 47)			From Students/other occupations (n = 5)			Total (n=52)		
	S	I	R	S	I	R	S	I	R
Penicillin	47 (100)	0 (0.00)	0 (0.00)	5 (100)	0 (0.00)	0 (0.00)	52 (100)	0 (0.00)	0 (0.00)
Ampicillin	47 (100)	0 (0.00)	0 (0.00)	5 (100)	0 (0.00)	0 (0.00)	52 (100)	0 (0.00)	0 (0.00)
Cefotaxime	47 (100)	0 (0.00)	0 (0.00)	5 (100)	0 (0.00)	0 (0.00)	52 (100)	0 (0.00)	0 (0.00)
Vancomycin	47 (100)	0 (0.00)	0 (0.00)	5 (100)	0 (0.00)	0 (0.00)	52 (100)	0 (0.00)	0 (0.00)
Erythromycin	20 (42.55)	9 (19.15)	18 (38.30)	4 (80.00)	1 (20.00)	0 (0.00)	24 (46.15)	10 (19.23)	18 (34.62)
Tetracycline	0 (0.00)	1 (2.13)	46 (97.87)	0 (0.00)	1 (20.00)	4 (80.00)	0 (0.00)	2 (3.80)	50 (96.20)
Ofloxacin	34 (72.34)	0 (0.00)	13 (27.66)	5 (100)	0 (0.00)	0 (0.00)	39 (75.00)	0 (0.00)	13 (25.00)
Chloramphenicol	38 (80.85)	0 (0.00)	9 (19.15)	5 (100)	0 (0.00)	0 (0.00)	43 (82.69)	0 (0.00)	9 (17.31)
Clindamycin	24 (51.06)	3 (6.38)	20 (42.55)	5 (100)	0 (0.00)	0 (0.00)	29 (55.77)	3 (5.77)	20 (38.46)

S = Susceptible; I = Intermediate; R = Resistant

5. Discussion

Currently, GBS is one of the most common pathogens of invasive diseases in nonpregnant women and men (Ballard et al., 2016; Cools et al., 2016; High, Edwards, & Baker, 2005). The source of infection for adults is unknown, however, GBS can colonize various body sites, including gastrointestinal and genital tracts of men and women. Among nonpregnant adults, transmission occurs via direct contact, with some studies suggesting that sexual transmission occurs (Bliss et al., 2002; Hill, 1990; Percha et al., 2011; Yamamoto et al., 1999).

The results of this study showed that sexual activity during the previous 3 months was associated with vaginal GBS colonization in nonpregnant women. Women who had frequent sexual activity ($\geq$ 3 times/week) had significantly higher risks of vaginal GBS colonization (34.06%) than less sexually active women (< 3 times/week) (10.02%), (OR = 4.55, 95% CI: 1.69, 12.23). Meyn et al. also found that women who had multiple sex partners (>2) or frequent sexual activity more than 3 times/week had significantly higher risks of vaginal GBS colonization than women with less or no sexual activity during the past 4 months (Meyn et al., 2002). Similar to previous reports, sexual contact appears important for GBS transmission. Frequent sexual activity was the strongest predictor of GBS colonization for both men and women. Among sexually active adults, colonization rates were 38% in women and 24% in men, while adults who have never engaged in sexual activity the rates were 17% in women and 13% in men (Manning et al., 2004). The limitation of our studies was the lack of information about the number of sexual partners among both groups in our study. However, during the interview process massage parlors workers indicated that they had more than 2 sexual partners.

In healthy, nonpregnant adults, GBS is only an occasional pathogen associated with urinary tract

infection, pneumonia, bacteremia and soft tissue infection, whereas in pregnant women, it is the second most common cause of urinary tract infection and a frequently isolated bacterium in cases of amnionitis, endometritis and postpartum wound infections. With early therapy, most GBS patients have a good outcome. But in nonpregnant adults with underlying diseases or elderly adults, fatal outcome is not uncommon despite appropriate treatment (Farley, 2001).

Our study showed that women in massage parlors had higher sexual activity, which increased the risk for having sexually transmitted diseases, including HIV infection which resulted in weakened immune system. Fatal outcome of GBS infection in HIV patients had been reported (Chaiwarith et al., 2011). Massage parlors workers not only had high risk for GBS infection, they could also be the source of GBS among men who attended the parlors. If those men were elderly with underlying diseases, they also had high risk for GBS infection, and could be the source of GBS among their spouses. Yamamoto et al. (1999) reported high possibility of sexual transmission of GBS between spouses. There were no significant differences of the rate of GBS detection between the spouses. Moreover, 91.2% of the couples had the same serotypes of GBS (Yamamoto et al., 1999).

Our isolates were susceptible to ampicillin, penicillin, cefotaxime and vancomycin, which were the drugs of choice for treatment of GBS infections or decolonization of female genitalia. But the isolates had high resistance rates to tetracycline, clindamycin and erythromycin. Among clindamycin resistance isolates, 3 showed inducible clindamycin resistance. Increasing resistance rates to tetracycline, clindamycin or erythromycin among GBS isolates have been reported (Borchardt et al., 2006; Castor et al., 2008; Malek-Jafarian, Hosseini, & Ahmadi, 2015). This suggested that before using tetracycline, clindamycin and erythromycin for treatment of GBS infection or prophylaxis to prevent GBS disease, susceptibility test results should be required.

6. Conclusion

Our research suggested that frequent sexual activity was an important risk factor for vaginal colonization of GBS; this could be the source for transmission to persons at high risk of infection, including neonates, pregnant women, adults with underlying medical conditions, and elderly persons.

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