International Scholars Journals

African Journal of Nursing and Midwifery ISSN 2198-4638 Vol. 8 (2), pp. 001-005, February, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Impacts of virulence factors of *Streptococcus mutans* isolates on the pathogenesis of acute vaginitis

Mohammed Sh. Jebur

Institute of Medical Technology, Foundation of Technical Education, Baghdad, Iraq.
E-mail: d_mohamed_1959@yahoo.com.

Accepted 09 November, 2019

***Streptococcus mutans*, known to be an etiologic agent of dental caries, also causes infective endocarditis. Adherence of these bacteria results from the interaction of adhesions that form part of their structure with salivary components (or other body's fluids), specifically those that compose the acquired pellicle. In the present study, acute vaginitis samples (vaginal swabs) were investigated from pregnant women patients who were admitted to many hospitals and maternal care centres in Baghdad city of Iraq. Ages of the women ranged from 20 to 30 years (only married women), while all samples were collected during March to July 2009. One hundred and fifty vaginal swabs were collected and cultured on blood agar plate, nutrient agar plates, chocolate agar plates and MacConkey agar plates, then incubated aerobically and anaerobically for 24 h at 37°C. Bacterial diagnosis were done according to macroscopic, microscopic and biochemical tests. The results of recent study showed that the prevalence of bacterial acute vaginitis of pregnant women indicated 18 (12%) as positive cases, while no growth was seen in the rest vaginal swabs (132, 88%). Results of bacterial isolation and identification showed that out of 18 isolates, only 8 (44.4%) isolates were diagnosed as *S. mutans* while other isolates were distributed to *Staphylococcus epidermidis* (8, 44.4%) isolates, *Staphylococcus aureus* (6, 33.3%) isolates, and 5 (27.7%) isolates each for *Streptococcus agalactiae, Streptococcus salivaris* and *Proteus* spp. Results of blood hemolysis of *S. mutans* isolates as virulence factors showed that only two isolates (25%) have the ability to produce hemolycin enzyme, and noticed that four isolates (50%) of *S.. mutans* were had the ability to produce extracellular protease enzyme on Mg media. Results of antibiotics profile resistance of *S. mutans* isolates showed that all isolates (100%) were resistant to ampicillin and amoxcillin, whereas seven isolates had also shown resistance in a lesser degree (87.5%) to tetracycline and gentamycin and to cefotaxime (75%) and ciprofloxacin and to erythromycin (62.5%) while all isolates showed high sensitivity (100%) to amoxiclave, doxicyclin and ampiclox antibiotics. *S. mutans* isolates showed moderate prevalence in the distribution of pathogenic microorganism of acute vaginitis. These isolates had 25% ability of blood hemolysis, and 50% ability to produce protease enzyme. *S. mutans* vaginal isolates appeared highly (100%) sensitive to amoxiclave, doxicyclin and ampiclox antibiotics, but it had 100% resistance to synthetic penicillin (ampicillin and amoxcillin) and there were different susceptibility to the rest antibiotics used in the study.**

Key words: *Streptococcus mutans*, virulence factors, antibiotics susceptibility.

INTRODUCTION

Vaginitis is usually characterized by a vaginal discharge or vulvar itching and irritation; a vaginal odor may be present. The three common diseases associated with vaginal infection include trichomoniasis (15 to 20%), bacterial vaginosis (40 to 45%), and vulvovaginal candidiasis (20 to 25%) or, not infrequently, a combination. Other causes of vaginal discharge or irritation include mucopurulent cervicitis caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, or herpes simplex virus, atrophic vaginitis, allergic or irritant reactions (spermicides, deodorants, minipadadhesive), vulvar vestibulitis, lichen simplex chronicus and lichen sclerosis (especially pruritis) and foreign bodies (Nester et al., 1998; Wilson, 2004).

Table 1. Antibiotic discs with its potency.

No	Antibiotics	Symbol	Disk-potency (µg/ml)
1	Gentamycin	GN	10
2	Cefotaxime	CTX	30
3	Tetracycline	TE	30
4	Ampicllin	AM	10
5	Erythromycin	ERY	30
6	Amoxcillin	AMX	10
7	Doxicyclin	DOX	30
8	Ciprofloxacin	CIP	30
9	Amoxiclave	AMC	15
10	Ampiclox	APX	15

Source: Oxiod company.

The vagina is a dynamic ecosystem that normally contains approximately 10^9 bacterial colony forming units per gram of vaginal fluid. The normal bacterial flora is dominated by lactobacilli, but a variety of other organisms, including some potential pathogens, are also present at lower levels. Lactic acids and other organic acids are metabolized from glycogen by the lactobacilli, maintaining the vaginal pH between 3.8 and 4.2. The acidic environment inhibits the overgrowth of bacteria and other organisms with pathogenic potential. The normal vaginal discharge is clear to white, odorless, and of high viscosity (Amelio, 2000; Rizvi et al., 2003).

One of the most common bacteriological agent to vaginitis is *Streptococcus mutans*, which is gram positive bacteria, spherical in shape that form pairs or chains during its growth; some are members of normal flora of oral cavity, intestine and vagina (Rizvi et al., 2003). Rizvi et al. (2003) also reported that the major route for early acquisition of *S. mutans* is vertical transmission from mother to child.

Virulence factors of *S. mutans* help to protect the bacterium against possible defenses and maintain its ecological niche in the sites of growth. Probable virulence factors include adhesions, acid tolerance, protease production and production of glycosyltransferase and intracellular polysaccharides (Kuramitisu, 1999).

Pathogenic *S. mutans* produce hemolycin enzyme as a virulence factors, which plays a role in blood invasion and in supplying the bacteria with their requirements of iron (Hardie, 1996). Also, antibiotic resistances of the bacteria are considered in some cases as virulence factors because of the ability of *S. mutans* to avoid the effects of these antibiotics and have another way to avoid these effects that allow the growth and multiplication in sites of infections (Murray, 1995).

The recent study was aimed at isolating and identifying *S. mutans* which are associated with vaginitis infection in women patients, and detecting the virulence factors of these isolates such as production of hemolycin enzyme, protease enzyme and its profile of antibiotics susceptibility. These factors are necessary to explain the pathogenesis of *S. mutans* which are beneficial in primary health care of pregnant women and medical quality of treatments.

MATERIALS AND METHODS

Collection of specimens

One hundred and fifty vaginal swabs were collected from women who attended the gynecologist clinic, with symptoms of acute vaginitis. These patients were admitted to many hospitals and maternal care centres in Baghdad city of Iraq. Ages of the women ranged from 20 to 30 years (only married women), while all sample were collected during March to July 2009.

All patients had not taken any antibiotics within the previous 5 to 10 days before attending the hospital. The swabs were taken by a gynecologist from vaginal fonices with use of a sterile speculum. Swabs for culture were placed in sterile tubes containing 5 ml of brain heart infusion broth, until being examined in the laboratory (Collee et al., 1996).

Fifty micro liter of broth were immediately inoculated on blood agar plates, nutrient agar plates, chocolate agar plates and MacConkey agar plates, and then incubated aerobically and anaerobically for 24 h (Baron et al., 1994). All media used for isolation and diagnosis were taken from Difeco company and prepared according to the recommendation of the company.

Bacterial culture

This study prepared a media (after primary culturing) of nutrient agar supplemented by sodium azide with a concentration of 1:16000 to inhibit gram negative bacteria and crystal violate with a concentration of 1:500000 to inhibit *Staphylococci* that became enriched with the selective media for *Streptococcal* growth (Marie et al., 1985).

Bacterial diagnosis

All procedures were done according to Marie et al. (1985), Collee et al. (1996) and Piret et al. (1983) through:

1. Macroscopic examination of single colony which was taken from pure bacterial culture that depended morphology, appearance, texture, and other phenotypic nature.
2. Microscopic examination of selected colony which investigated after gram stain to observe specific features of the isolated bacteria. c. Biochemical tests: Specific biochemical tests (oxidase, catalase, coagulase, vancomycin susceptibility and motility test) were done to have the final identification.

Virulence factors of *S. mutans* isolates mentioned by blood hemolycin test (using sheep blood), protease production test and antibiotics profile susceptibility

Antibiotics susceptibility was done according to disc diffusion method (Hinder, 1998) by using pure culture of four previously identified isolates. The inoculums used were prepared through 1 ml of bacterial suspension ($1.5x10^8$ CFU/ml), inoculated by streaking on Mueller-Hinton Agar; antibiotics discs (Table 1) were placed on the surface of cultures then incubated overnight at 37°C. Antibiotic zone of inhibitions were measured and compared to standard zones to determine the susceptibility of these isolates to each

Table 2. Distribution of cases and cultures.

Culture	Samples	Culture (%)	Mixed cultures	Single cultures
Positive	150	18 (12%)	15 (83%)	3 (16%)
Negative	150	132 (88%)	-	-

Table 3. Isolation and identification of bacterial isolates from 150 vaginal swabs.

Isolates	Number of isolates	Percentage
*S. mutans**	8	44.4
*S. epidermidis**	8	44.4
S. aureus	6	33.3
S. agalactiae	5	27.7
S. salivaris	5	27.7
Proteus spp.	5	27.7
S. pyogens	2	11.1
Neisseria spp.	2	11.1
Total	41	100

*Statistically significant differences P< 0.05.

antibiotic. All chemicals, reagents, and stains, were prepared and used according to scientific references as listed.

Statistical analysis

To determine the statistical significance differences of variables, SPSS program were used. Chi-square test was used to explain the significant differences between bacterial isolates and antimicrobial susceptibility of agents. Probability value less than 0.05 was considered as statistically significant (*), while P-value more than 0.05 was considered as statistically not significant. Also, the mean value and percent for the incidence of bacterial distributions was used.

RESULTS AND DISCUSSION

The results of recent study showed 18 (12%) positive cases in the prevalence of acute bacterial vaginitis of pregnant women (in different months of pregnancy). These results indicated the susceptibility of women for infection, whereas these finding might be due to the alteration in female hormones during pregnancy and menstruation periods (Wearhaug, 1987) and explain that in these two physiological conditions, the level of progesterone increased, causing vasodilatations which led to the inflammations of tissues (Wie et al., 1986).

All swabs subjected for culturing on available media showed that out of total 150 samples, only 18 (12%) had positive bacterial culture, while no growth was seen in the rest vaginal swabs (132, 88%). These results also indicate the presence of other pathogenic non bacterial agents (Table 2).

Results of culturing showed that most vaginal swabs gave mixed cultures (15, 83%); this might be attributed to the fact that vaginal media provide favorable environments for colonization of more species, aerobic, facultative anaerobic bacteria and other pathogenic non bacterial agents (Todar, 2008).

The results of bacterial isolation and identification according to macroscopic, microscopic finding and biochemical tests showed that out of 18 isolates, there were only 8 isolates diagnosed as *S. mutans*, while other isolates were distributed to *Staphylococcus epidermidis* (8 isolates). There were statistically significant differences (P<0.05) from other isolates which included *Staphylococcus aureus* (6 isolates), and 5 isolates each for *Streptococcus agalactiae, Streptococcus salivaris* and *Proteus* spp. The results also reported the presence of two isolates of facultative aerobic bacteria -*Streptococcus pyogen and* anaerobic bacteria *Neisseria* spp. (Table 3). These results were obtained from pregnant women suffering from acute vaginitis which might be considered as normal flora of the vagina (Moreillon and Que, 2004) but these patients had symptoms of pelvic pains (Lerner et al., 1977), with disappearance of *Lactobacillus* spp. bacteria which explained the opportunistic nature of these isolates (Feikin et al., 2001) and presents in coaggregation of each other which plays critical role in the ecology of bacterial growth to vaginal site.

Results of blood hemolysis of *S. mutans* isolates as virulence factors showed that only two isolates (25%) have the ability to produce hemolycin enzyme (Table 4), which explain the flexibility of such bacteria to have different mechanisms for acquisition of iron from its environments (Litwin and Calderwood, 1993). While there is another mechanism for iron acquisition to produce siderophores which chelate iron with very high affinity, transferring of lactoferrin to mobilize iron for bacterial use was completely effective (Neilands, 1995). Also, the results showed that all isolates (100%) of *S. mutans* have the ability to produce siderophores (Table 4); this ability was tested by siderophore synthesis grown on Mg media containing dipyridyl (Abdul and Abdul, 2005).

Furthermore, it is known that bacteria which are able to produce hemolycin have no ability to produce siderophore, so, this explains the results of recent study (high siderophore production with low hemolycin production), and such bacteria need only one mechanism for obtaining iron (Al-Saeed, 1997).

As shown in Table 4, the results noticed that four isolates (50%) of *S. mutans* have the ability to produce extracellular protease enzyme on Mg media. When this enzyme reaches vaginal mucosa, it might often encounter

Table 4. Results of virulence factors of 8 *S. mutans* isolates.

No of isolates	Hemolysin enzyme	Extra cellular protease	Antibiotics susceptibility
1	+	+	+/-
2	-	-	+/-
3	-	-	+/-
4	-	+	+/-
5	-	+	+/-
6	+	-	+/-
7	-	+	+/-
8	-	-	+/-

+/-: Different susceptibility to different antibiotics.

Table 5. Antibiotics susceptibility of *S. mutans* isolates.

No. of isolates	Antibiotics									
	GN	CTX	TE	AP	ERY	AMX	DOX	CIP	AMC	APX
1	S	R	R	R	R	R	S	R	S	S
2	R	R	R	R	R	R	S	R	S	S
3	R	R	R	R	R	R	S	R	S	S
4	R	S	S	R	S	R	S	S	S	S
5	R	S	S	R	S	R	S	S	S	S
6	R	S	R	R	R	R	S	S	S	S
7	R	R	R	R	R	R	S	R	S	S
8	R	R	R	R	S	R	S	R	S	S

R: Resistant isolates; S: sensitive isolates.

the vaginal fluid IgA, then, it could inhibit their adherence and attachment to the vaginal tissues, which causes vaginitis and play central role for local and humoral immune response (Cheng et al., 2002). Protease production from causative agent could contribute directly to pathogenesis of vaginitis by degrading the host defense protein such as immunoglobulin and complement and or cleave to other streptococcal surface protein (Labibe et al., 2009).

Results of antibiotics profile susceptibility of *S. mutans* isolates showed that all isolates were resistant (100%) to ampicillin and amoxcillin, whereas seven isolates had also shown resistance in a lesser degree (87.5%) to tetracycline and gentamycin, cefotaxime (75%), ciprofloxacin and to erythromycin(62.5% each) (Table 5).

But also, all isolates showed high sensitivity (100%) to amoxiclave, ampiclox and doxicyclin antibiotics. The results of wide range susceptibility of *S. mutans* to different antibiotics which were used in this study (from 100% resistance to 100% sensitivity) may be obtained due to recurrent or random usage of antibiotics in treatment of vaginitis (Sharat, 2004); also, there was sensitivity of some isolates to other antibiotics not widely used in the treatment of vaginitis (Culebras, et al., 2002).

The increase observed in the resistance of antibiotics may be due to abuse of antibiotics which leads to transfer of resistance through genetic factors such as plasmid and transposons, or by the change observed in the wall permeability (Ferretti and Ward, 1988), which indicate the virulence factors of *S. mutans.*

Conclusion

The study concluded that *S. mutans* isolates showed moderate prevalence in the distribution of pathogenic microorganism of acute vaginitis. These isolates were having 25% ability of blood hemolysis, and 50% ability to produce protease enzyme. *S. mutans* vaginal isolates appeared highly sensitive (100%) to amoxiclave, doxicyclin and ampiclox antibiotics, but it had 100% resistance to synthetic penicillin (ampicillin and amoxcillin) and there were different susceptibility to the rest antibiotics used in the study.

The study recommended that the laboratory diagnosis of *S. mutans* was very necessary to avoid its virulence effects by administration of suitable antibiotic, which have highly clinical effectiveness.

REFERENCES

Amelio C (2000). Vaginitis: Finding the cause prevents treatment failure.

Clin. J. Med., 67:634-646.
Cheng Q, Stafslien D, Purushoth SS, Cleary P (2002). The group B Streptococci C5a peptidase is both a specific protease and an invasive infection and immunity, 70(5): 2408-2413.
Collee JG, Fraser AG, Marmian BP, Simon SA (1996). Mackin and McCartney. Practical Medical Microbiology. 14th Ed. The Churchill Livingston. Inc. USA, pp. 131-149
Collee JG, Miles RS, Watt B (1996). Practical Medical Microbiology. Churchill. Livingstone, London, pp. 131-149
Culebras E, Rodriguez-Azial I, Redondo M, Picazo JJ (2002). Macrolide and Tetracycline resistance and molecular relationship of clinical strains of Streptococcus agalactiae. Antimicrob. Agents Chemother., 46(5): 1574-1576.
Feikin DR, Thorsen P, Zywichi S, Arpi M, Westergaard JD, Schuchat A (2001). Association between colonization with group B Streptococci during pregnancy and delivery among Danish women. Am. J Obstet. Gynaecol., 184: 427-433.
Ferretti JJ, Ward M (1988). Susceptibility of Strep. Mutans to antimicrobial agents. Antimicrob. Agents Chemother., 10(2): 274-276.
Hardie J (1996). Oral Streptococci in: Bergey's Manual of systemic bacteriology. Sheath PHA. Mair N S, Sharpe E, Holt JG, eds Williams and Wilkins: Baltimor, Maryland, 2: 1059-1063.
Hinder J (1998). Antimicrobial susceptibility testing in: Essential procedures for clinical microbiology press, Washington, pp. 207-248.
Kuramitisu H (1999). Virulence factors of Strep. mutans role of molecular genetics. Crit. Rev. Oral Biol. Med., 4: 159-176.
Labibe RS, Calvanico NJ, Tomasi TB (2009). Studies on extracellular proteases of Strep. Sanguis . Biochem. Biophys. Acta. Cited by Al-Rubiae FM, Bacteriological study on Strep. Mutans associated with dental caries and periodontal diseases in Hilla city. MSC. Thesis. Collage of Medicine, University of Banylon, 526: 547-559.
Lerner PK, Gopalakrishna E, Wolinsky MD, Tan JS (1977). Group B Streptococci (Strep. Agalactiae) bacteremia in adults. Medicine, 56: 457-473.
Litwin CM, Calderwood SB (1993). Role of iron in regulation of virulence Genes. Clin. Microbiol. Rev., 6: 137-149.
Marie B, Coyl D, Josephino A, Marello P, Smith B (1995). Aerobic Bacteria (section three). Manual of clinical microbiology 4th ed. American Society for microbiology. Washington, DC.
Moreillon P, Que YA (2004). Infective endocarditis. Lancet, 363: 139-149.
Murray PR (1995). Manual of clinical microbiology. Sixth ed. Editors by Baron E J, Pfaller M A, Tenover FC and Yolken R H. Asm Res Washington, pp. 15-18,
Neilands JB (1995). Siderophores: Structure and function of microbial iron transport compounds, J. Biol. Chemist., 270(45): 26723-26726.
Piret J, Millet J, Demain A (1983). Production of intracellular protease during sporulation of Bacillus brevis Eur. J. Appl. Microb. Biotechnol., 17: 227-230
Rizvi TH, Fatima H, Sayeed S, Ali SS (2003). Vaginal infection and birth weight. Pakistan J. Med. Res., 42(1): 1-6.
Sharat K (2004). Group B Streptococcus infection. Medicine. 16(3): 1425.
Todar K (2008). Microbial World (Microbe and dental disease). University of Wisconsin – Madison, pp. 375-390.
Wie S, Yang S, Barmes D (1986). Needs and implementation of preventive dentistry in China. J. Community Dent. Oral Epidemiol., 16: 194-198.
Wilson J (2004). Managing recurrent bacterial vaginosis. Sex Transm. Infect., 80: 8-11.