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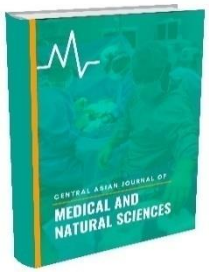

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Prevalence, Virulence, And Anibiotic Sensitivity Pattern of *Staphylococcus Xylosus* Isolated From Pregnant and Non-Pregnant Women with Symptomatic Genital Tract Infection: A Comparison Study

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Abstract: One hundred and seventy four urine specimens were collected from UTI patients women (64 pregnant and 110 non-pregnant) aged 18-45 years, presented with symptomatic genital tract infection, visiting Alkarama teaching hospitals and out patient in wasit for the period from 1.9.2009 to 1.6.2010.

Ten Staphylococcus xylosus were isolated (5.7%): Eight (12.5%) from pregnant and 2 (1.8%) from non-pregnant women. Statistically, there is no significant difference regarding S. xylosus occurence among pregnant and non-pregnant. In both groups of patients,Out of 61 Staphylocci isolates, 13 isolates were diagnosed as Co agulasepositive +ve, 48 isolates as Coagulase Negative Staphylococci CONS, 10 of which were identified as S.xylosus. This identification was confirmed by biochemical tests, Antibiogram and Api staph.

The ability of S. xylosus isolates produce urease, protease, lipase , haemolysin as well as capsule formation, were investigated. Results showed resemblance of all S. xylosus isolates in producing these enzymes in exception of urease production, since 10 isolates were urease positive while 3 isolates were urease negative.

As for antibiogram, all S. xylosus isolates were susceptible to Ciprofloxacine while 80% of them were resistant to erythromycin.

Two isolates of S. xylosus were elected, on the basis of their multidrug resistant, S. xylosus S7 urease positive and S. xylosus S112 urease nagetive in order to testify their ability to establish UTIIi in mice. Aas one group was injected via intraurethral catheter with S. xylosus S7, another group was injected with S. xylosus S12.

Introduction

Staphylococci are important pathogenic organisms involved in many of human infection particularly in bactereamia or septicemia , causing a large variety of infections worldwide (CNS-I1-3). The largest populations of human staphylococci are usually found in regions of the skin and mucous membranes surrounding openings to the body surface (165, 172). The most important species from the viewpoint of human medicine is *S. xylosus* (CNS-K4). Coagulase-negative staphylococci (CNS) are normal flora components of various parts of the skin and of the respiratory and gastrointestinal system mucosa of man; they may also appear in animals and foodstuff (CNS-L1-16, 25, CNS-G6, CNS-H5). There are presently 41 recognized taxons, designated coagulase-negative staphylococci. References show 21 CNS types representing etiological agents in human disease (CNS-L1-18). Although long considered non-pathogens as the components of normal human skin and mucosa, lately they turned into significant etiological agents causing nosocomial infections, mainly in link with the presence of foreign bodies in the human organism (CNS-L1).

Results of vaginal flora cultures for aerobic bacteria were: *Staphylococcus* spp., and coliforms were mostly present in a scanty numbers (CNS-D5, 23,24).

On the contrary, by S. saprophyticus perpetuating a common urinary tract infections are no longer or do not focus on the S.xylosus nurse bacteria to humans, particularly urinary tract infections due to lack of studies in this area (Tselenis - Kotsowilis et al; 1982; Novacova et al., 2005; Medscape, 2004), as they cause infections UTI by less than 1% of these studies

Explained that the bacteria causing the inflammation of the trough college acute in young women aged 26 years without a previous infection of the College and there were symptoms accompany the injury before the diagnosis of a month, including high temperature and lasted for a week and the pain of a college, with difficulty in urination

Pyuria were observed at the microscopic examination of normal in the three samples was then repeated to ensure infection of the implant and get diagnosed and pure colonies of bacteria. In this study, acid recovery coking wall and then promise Kjsm antigen for the investigation of antibodies formed against him in the serum after five days of diagnosis, has been obtained for specific antibodies of the TA's Pal S. xylosus in the serum of patients and this result with pea-diagnosis confirmed that these bacteria are working

Cause of infection in patients with trough college and being fast with these a Leads us to the early heat was the first attack of the disease and is associated with a bacterial food poisoning caused by S. xylosus, taking into account the possibility that strains of S. xylosus strains are positive for pentose mutant of the S. saprophyticus causing the most common infections of the urinary system. In another study proved the existence of S. xylosus in the female causing urinary tract infections UTI (Rupp et al., 1992).

Symptoms of vaginitis are common and account for about 10 million office visits a year [1]. Three major types of infections that affect the vaginal habitat are vulvovaginal candiadiasis, trichomoniasis and bacterial vaginosis [1, 2, 3]. Another type of vaginitis is described 'aerobic vaginitis' which is caused by aerobic normal vaginal flora. Aerobic Vaginitis is a term proposed to describe purulent vaginal discharge with predominance of abnormal aerobic flora [4, 5], and its characteristics are different from those of bacterial vaginosis and elicit an important host response and genital complaints are those of a real vaginitis (red inflammation, yellow discharge, vaginal dyspareunia) [2, 4, 6, 7]. Group B streptococci (GBS), *Escherichia coli*, *Staphylococcus aureus* and *Trichomonas. vaginalis* are frequently cultured in case of aerobic vaginitis [2, 6] which were three to five times more frequent in aerobic vaginitis than in the normal flora [8].

S. xylosus is part of the normal human flora. The anterior nares is the most frequent site of human colonization, although the skin, vagina, axilla, perineum, and oropharynx may also be colonized [9, 10]. There is some evidence suggesting that most women harbor S. xylosus in the vagina but that colonization is only transient [11]. Vaginitis may be an early and prominent feature of toxic shock syndrome [1, 9]. As it is clear from the above literatures.

Materials and Methods

Patients

This study included pregnant and non-pregnant women (aged 18 to 45 years) with symptomatic genital tract infection, attending Obstetrics and Gynecology Clinics in Al-Kut/Wassit Province/Iraq and were, therefore, mainly symptomatic and self referred.

Specimen Collection and Processing

Microbiologically, infection was evaluated by culture only without microscopic examination. High vaginal swabs were collected during May 2008 to March 2010, by the Gynecologist (11) and streaked immediately after collection on eosine methylene blue agar (EMB) (Himedia) and on blood agar (Oxford) plates. The plates were incubated at 37°C for 24-48 hours at ambient air. Only those samples that gave significant growth were considered as infection.

Identification of the Isolates

All isolates (Gram-positive and Gram-negative aerobic bacteria) were diagnosed biochemically (12, 13).

Antibiotic Sensitivity Test

It was carried out using agar diffusion method (13). The results were interpreted according to CLSI (14) instructions.

Statistical analysis

The results were analyzed statistically using Chi-square (15). A P value below 0.05 was considered to indicate statistical significance.

Results and Discussion

Culture Results

Significant growth of aerobic bacteria was obtained from 127 women (55 pregnant and 72 non-pregnant) aged 18-45 years, presented with symptomatic genital tract infection. Since most of the causative agents of aerobic vaginitis are part of normal vaginal flora, so that, only those samples that gave heavy growth were considered as infection, whereas samples with scanty growth were neglected. Cook *et al.*(16) and Lawson (17) reported that vaginitis-associated isolates represented the predominant vaginal flora present concurrent with symptoms. Larsen and Monif (18) reviewed that for disease to occur, exogenous or endogenous bacteria that possess pathogenic prerequisites must attain replicative dominance. The microbial load for a given organism appears to influence the relative risk of symptomatic infection. For endogenous bacteria of the female genital tract, the microbiological environment may affect the bacterial expression of virulence factors. Theoretically, if a virulence factor is constitutive, the number of organisms present will determine the amount of the virulence factor available to promote infection.

Aerobic Bacterial Isolates

The frequencies of isolation of aerobic Gram-positive and Gram-negative bacteria were summarized in Table 1.

Table 1: Frequency of Gram-positive and Gram-negative aerobic bacteria isolated from pregnant and non-pregnant women with symptomatic genital tract infection.

Patients	No. (%) of isolates			Mixed infection
	Gram-positive bacteria (Single infection)	Gram-negative bacteria (Single infection)	Significance of difference: χ^2 (P<0.05)	
Pregnant (n) = 55	28 (50.9)	16 (29)	Significant	13 (23.6)
Non-pregnant (n) = 72	36 (50)	25 (34.7)	Not significant	11 (15.2)
Total = 127	64 (50.3)	41 (32.2)	Significant	24 (18.8)

In both patient groups, Gram-positive bacteria were more common (50.3%) than Gram- negative bacteria (32.2%). These results were consistent with others. Atallah (19) found that 46.9% of the microorganisms isolated from the deep vagina were gram-positive. Also Abdul-Rahman *et al.* (20) ,when they studied the causative agents of pelvic inflammatory disease in women, reported that 71.43% of the isolates were gram-positive cocci. In Pakistan, Mumtaz *et al*. (8) demonstrated that in non-pregnant women, 63.8% of cases were caused by gram-positive cocci, and 31.5% were caused by gram-negative enteric rods. Khan and Khan (21) reported that Gram-positive organisms were more common (71%) in vaginal infections than Gram-negative organisms (29%).

For Gram-positive bacteria, most of the isolates were staphylococci. Statistically, the difference is not significant regarding the occurrence of *S. aureus* which was isolated from 25.4% and 33.3% of pregnant and non-pregnant women, respectively (Table 2). Whereas the difference is significant (P<0.05) regarding the occurrence of coagulase-negative staphylococci (CNS) which were isolated from 50.9% of pregnant and 19.4% of non-pregnant patients. This result is comparable to those obtained by others. In pregnant, *S. aureus* was isolated from 14.5% and 20.5% of patients (22,23, respectively). In non-pregnant, Mumtaz *et al*. (8) found that *S. aureus* was the most prevalent organism (46%).

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Table 2: Identity and frequency of aerobic Gram-positive bacteria isolated from pregnant and non-pregnant women with symptomatic genital tract infection.

P-CNS isolates

Series	Isolates	Identification	Hemolysis	urease	protease	lipase	slime
1	P60	*S. xylosus*	no	-	-	+	+
2	P72	*S. xylosus*	no	-	-	+	+
3	P79	*S. xylosus*	no	-	-	+	+
4	P86a	*S. xylosus*	no	-	-	-	+
5	P99	*S. xylosus*	β	-	-	+	+
6	P101c	*S. xylosus*	α	-	-	+	+
7	P107+	*S. xylosus*	no	+	+	+	+
8	P111	*S. xylosus*	no	-	+	+	+
TOTAL	8	Positive no. (5.7%)	25%	12.5%	25%	87.5%	100%

NP-CNS isolates

Series	Isolates	Identification	Hemolysis	urease	protease	lipase	slime
1	NP26	*S. xylosus*	B + α	+	+	+	+
2	NP114	*S. xylosus*	no	+	-	+	-
Total	2	Positive no. (1.8%)	50%	100%	50%	100%	50%

CNS: coagulase-negative streptococci.

Commonly isolated Gram-positive bacteria were Staphylococci. Staphylococci (*S. xylosus* CNS) represent part of normal vaginal flora (24, 25). Most individuals who develop *S. xylosus* infections do so with their own colonizing strains. However,. Demba *et al.* (23); Reid and Bruce (27) ; and Schlivert *et al*.(28) demonstrated that the vaginal mucosa of 20.5-23% of the females is colonized by *S. aureus*, in whom it predisposes them to toxic shock syndrome. Shivadas (3) and Kasper *et al.* (26) demonstrated that other causes of vaginal discharge or vaginitis is the ulcerative vaginitis associated with staphylococcal toxic shock syndrome, so that *S aureus* should be promptly identified in vaginal fluid by Gram's stain and by culture. In vaginal cultures most researchers considered CNS as contaminants (23, 29). In this work, there is a question about the pathogenic potential (as opportunistic pathogens) of these agents as they were isolated as heavy growth from 54.5% of pregnant and 29.1% of non-pregnant women. Demba *et al.* (23) found that the vaginal flora cultures for aerobic bacteria were *Staphylococcus* spp., and coliforms which were mostly present in a scanty numbers. da Cunha *et al*. (30) concluded that these microorganisms should not be ignored or classified as mere contaminants. Rosenstein *et al*. (24) found that the vaginal flora is not static but can convert from a normal state to a grossly abnormal state and back again. Zunin *et al*. (31) reported that comparisons among groups (healthy and patients) shows little differences in the microbial population between healthy women and patients with genital tract infections. Otto (32) and Longauerova (33) reviewed that CNS may participate, as commensal flora, in the development of infections only when external barriers (e.g. skin) were damaged due to wounds, inoculation, or implantation of foreign bodies. Most importantly the results that were obtained by Rosenstein *et al.* (34) who reported that the initial disturbance in the vagina causes certain bacteria, such as CNS, to appear first, in large numbers, followed by *Bifidobacterium* spp. However, it is then unclear whether rapid multiplication of these organisms provides an environment conductive to the multiplication of other bacterial spp.

Antibiotic sensitivity pattern of the isolates

The detailed results of the percentage sensitivity of the common isolates against the various antibiotics are presented in Table 2. For all isolates, the most effective antibiotic was imipenem whereas there were high resistance (sensitivity < 80%) to all other groups of this study included antibiotics which

present the traditionally used agents in the world and in our country. This

required a serious work to lessen and control this resistance's spread by

depending on clinical laboratory-based results to describe the appropriate treatment instead of empirical description of treatment. Khan and Khan (21) showed that infections pertaining genitalia if not treated or ignored could debilitate the patient and could become a source of infection for the neonates especially in case of women belonging to the childbearing age.

Table 2: Percentage of sensitivity of 2 strain *S. xylosus* isolates non pregnant woman to various antibiotics.

	Isolates	ME		TE		CN		SXT		MET	
1.	NP26		R	25	S	18	S	23	S		R
2.	NP114		R	22	S	29	S	12	R		R
total	140			47		47		35			
%											

	Isolates	VA		AMC		AM		AX		B		NV		P	
1.	NP26	12	R	16	R		R	14	R		R	29			
2.	NP114	20	S	25	S	25	S	30	S	13		21		32	R
total	140	32		41		25		44		13		50		32	
%															

Table 2: Percentage of sensitivity of 8 strain *S. xylosus* isolates from pregnant woman to variouantibiotics

	Isolates	NOR		IMP		CIP		CTX		CAZ		DO	
1	P60 S. xylosus	22	S	15	R	14	R	15	R	15	R		R
2	P72 S. xylosus	19	S	35	S	20	R	20	R	14	R	15	R
1.	P79 S. xylosus	14	R		R	15	R		R		R		R
2.	P86a S. xylosus		R	21	S		R		R		R		R
3.	P99 S. xylosus	15	R	10	R	15	R		R		R		R
4.	P101c S. xylosus	12	R	20	S	20	R	12	R	11	R	8	R
5.	P107+ S. xylosus	36	S	44	S			10	R		R	27	S
6.	P111 S. xylosus			22	S	20	R		R		R	18	S
total													
%													

	Isolates	ME		TE		CN		SXT		MET	
1	P60		R		R	9	R	20	S		R
2	P72	15	R	12	R	12	R	11	R		R
3.	P79		R		R		R		R		R
4.	P86a		R		R	13	R		R		R
5.	P99		R	7	R		R	15	R		R

6.	P101c		R		R	10	R	15	R		R			
7.	P107+		R	30	S	12	R				R			
8.	P111		R	14	R		R		R		R			
total														
%														

	Isolates	VA		AMC		AM		AX		B		NV		P	
1	P60		R		R		R		R						
2	P72	9	R	13	R		R		R						
3.	P79		R		R		R		R		R	28			
4.	P86a		R		R	10	R	20	S	15		16			
5.	P99		R		R		R		R						
6.	P101c	12	R	12	R	10	R	18	S	13		24			
7.	P107+	18	S	15	R	13	R	14	R	12					
8.	P111	17	S		R		R	13	R		R	25			R
total															
%															

	Isolates	NOR		IMP		CIP		CTX		CAZ		DO	
1.	NP26 S. xylosus	13	R	47	S	15	R	13	R		R	25	S
2.	NP114 S. xylosus			44	S	31	S	14	R		R	30	S
total													
%													

For *S. aureus*, both pregnant and non-pregnant isolates, have a comparable resistance to most commonly used antibiotics, except to imipenem, vancomycin, and amoxicillin-clavulanic acid, to which the non-pregnant women's isolates were more susceptible (93.3%; 53.3%; and 24.1 %, respectively) than the pregnant women's isolates (71.4%; 21.4%; and 0%, respectively). Coagulase negative staphylococci (CNS) isolated from non-pregnant women were more susceptible than pregnant women's isolates especially to ciprofloxacin (57.1% vs. 32.1%, respectively), gentamicin (64.2% vs. 17.8, respectively), co-trrimoxazole (57.1% vs. 21.4%, respectively), doxycycline (64.2% vs. 25%, respectively), and tetracycline (64.2% vs. 10.7%, respectively). Both *S. aureus*'s and CNS's isolates had high resistance to penicillins (including methicillin) and third generation cephalosporins. In non-pregnant, Mumtaz *et al*. (8) found that the most effective antibiotics against *S. aureus* were imipenem (98.64%) and vancomycin (93.6%), while lesser activity has been noted against penicillins, tetracycline (49.3%), sulphonamides (23.6%), first generation cephalosporins (36.8%) and monobactams (19.13%).

This high rate of staphylococcal resistance to most traditionally available antibiotics, makes treatment of such infections very difficult especially if we note that these organisms represented one of the most prevalent causative agents. Most of this study's patients, as explained by the Gynecologists, had recurrent infection and although did have treatment, there were no symptom relief. Schmidt and Hensel (42) reviewed that treatment of *Staphylococcus* infections becomes increasingly difficult, since resistance to a growing number of antibiotics has been observed in clinical isolates. The remarkable

ability of both *S. aureus* and CNS to acquire antibiotic resistance limits therapy options and consequently may increase patient morbidity and mortality (42,43). Forbes *et al.* (13) stated that although a broad spectrum of agents may b e used for therapy, most staphylococci are capable of acquiring and using one or more of the resistance mechanisms. Increasing resistance against antibiotics in staphylococci is an enormous problem for the public health system and one of primary reasons for the in-depth investigation of staphylococcal pathogenicity and resistance factors (32). For a long time, penicillins have been a main stay for the management of a variety of staphylococcal infections but the organism has gradually acquired resistance towards them. In most cases of staphylococci, resistance to penicillin is attributable to β-lactamase production (21,44). Also these β-lactamase producers are resistant to all β-lactamas, penicillins, cephalosporins,carbapenemes, and penemes (44). The high resistance to ampicillin could possibly be due to the prevalence of self-medication and misuse of ampicillin, usually without prescription, with the resultant emergence of resistance gene pools, following the selection of resistant bacterial strains (45). Schmidt and Hensel (42) reviewed that a major problem in the treatment of *S. aureus* infections is the presence of resistance to multiple antibiotics. Resistance to methicillin in methicillin resistant *S. aureus* (MRSA) strains is usually accompanied by resistance to variety of other β-lactam antibiotics. The very recent alarming finding that high level resistance against vancomycin has been transferred from enterococci to *S. aureus* , very likely means that we will face highly vancomycin-resistant CNS in the very near future. Multiple resistance is also not uncommon (32).

In both patient groups, the most effective chemotherapeutic agent against *E. coli* isolates was imipenem (sensitivity 100%). Most of them were highly resistant especially to penicillins and third generation cephalosporins (cefotaxime and ceftazidime). For other agents, *E. coli* isolated from pregnant and non-pregnant had comparable resistance except to ciprofloxacin (41.6 vs. 84.6%, respectively), gentamicin (38.4 vs. 77.7%, respectively), and tetracycline (15.3 vs. 48.1%, respectively). Mumtaz *et al*. (8) found that the most effective chemotherapeutic agents against gram-negative rods (*E. coli*) were imipenem (96.0%) and piperacillin/tazobactam (92.1%) whereas the least active antimicrobials were those beloniging to the groups of penicillins, tetracycline, and sulfonamides. Gillespie and Hawkey (46) showed that susceptibility of *E. coli* strains to amoxicillin has decreased over recent years owing to the presence of TEM-1 and TEM-2 β-lactamase. The effectiveness of cotrimoxazole and trimethoprim has been reduced by frequent carriage on plasmids and integrons of resistance genes. Because resistance is high, a fluoroquinolone or nitrofurantoin should be considered for empirical treatment. Significant increase in the incidence of strains resistant to ampicillin and ciprofloxacin was reported.This sensitivity of Gram-negative isolates to imipenem and their high resistance to penicillins and third generation cephalosporins can be due to the possession of extended spectrum β-lactamases (ESBL). The ESBL enzymes are capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation cephalosporins, penicillins, and aztreonam but have no detectable activity against cephamycins and imipenem. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family, most commonly in *Klebsiella* spp., followed by *E. coli* (47,48). Chaudhary and Aggarwal (47) and Sharma *et al*. (48) reviewed that ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic options.

The reason for the spread of this high rate of resistance among our isolates may be explained, as we thought, by the fact that the causative agents of aerobic vaginitis are part of the host normal flora (opportunistic pathogens), this means that these organisms are continuously exposed to antibiotics used for treating different infections and acquiring resistance to these antibiotics especially if their use is random and sometimes without physician description. Gillespie and Hawkey (46) demonstrated that the resistance among enteric bacteria, including *E. coli*, has increased markedly over the past 50 years since of the widespread use of antibiotics Mumtaz *et al*. (8) found that there were very few antibiotics

among the conventially available aminoglycosides, third generation cephalosporins, penicillins, quinolones, sulfonamides, and tetracyclines, possessing good sensitivity (> 80%) against any one of the common aerobic vaginal pathogens. Neu (44) and Karami *et al.* (49) repotred that unnecessary use of antibiotics in humans may be more hazardous than misuse of antibiotics in animal husbandry, because the former targets strains with capacities to persist in the human microbiota and also to cause clinical disease in humans. Also Sharma *et al*. (48) observed a high rate of ESBL production by *E. coli* which may be due to the selective pressure imposed by extensive use of antimicrobials.

Conclusion

From this study results, it can be concluded that the most common aerobic bacterial causative agents of vaginitis in our patients, were staphylococci (*S. aureus* and CNS) and *E. coli* and that most of these isolates were resistant to traditionally used antibiotics.

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