***Streptococcus agalactiae*: a vaginal pathogen?**

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The significance of *Streptococcus agalactiae* as an aetiological agent in vaginitis was evaluated. A total of 6226 samples from women who presented with vaginal symptoms was examined. The presence of >10 leucocytes/high-power field (h.p.f.) was taken to be the criterion of active infection. *S. agalactiae* was isolated from 10.1% of these samples. The isolation rates of other common pathogens such as *Candida* spp., *Gardnerella vaginalis* and *Trichomonas* spp. were 54.1%, 27.2% and 4.2%, respectively, in the same group of patients. In contrast, the isolation rates of these micro-organisms in the group of patients who had no infection (<10 leucocytes/h.p.f.) were 4.2%, 38.3%, 33% and 0.5%, respectively. In the majority of samples from which *S. agalactiae* was isolated, it was the sole pathogen isolated (83%) and its presence was associated with an inflammatory response in 80% of patients. Furthermore, the relative risk of vaginal infection with *S. agalactiae* (2.38) in patients with purulent vaginal discharge was greater than that of *Candida* spp. infection (1.41) and lower than that of *Trichomonas* spp. infection (8.32). These data suggest that *S. agalactiae* in symptomatic women with microscopic evidence of inflammation should be considered a causative agent of vaginitis.

Introduction

Streptococcus agalactiae (β -haemolytic streptococcus group B) is known to cause various infections in adults, but clinical interest in these bacteria mainly relates to their ability to cause serious neonatal illness, especially meningitis and sepsis. Although neonates born by caesarian section have presented with *S. agalactiae* infection, indicating ascending transmission of the micro-organism from the vagina of their mothers, in the majority of cases the neonate acquires the infection during labour through contact with the vaginal secretions of the mother colonised by *S. agalactiae* [1–6]. It is well known that *S. agalactiae* colonises the female genital tract but it is unclear if this bacterium can cause true infection of the vagina.

In this context, the frequency of *S. agalactiae* isolations in vaginal secretions of women with symptoms that included vaginal discharge, itching and a burning sensation was examined.

Materials and methods

A total of 6226 vaginal specimens taken from that

number of women who presented to the Gynaecology Department of the Hellenic Air Force Hospital complaining about vaginal discomfort, in the years 1992–1994 was examined. Their age range was 17–45 years. All specimens were obtained in a uniform manner by swabbing the posterior vagina, through a colposcope to enable a better view of the area. Swabs for microscopy were placed in 1 ml of normal saline and swabs for culture in Stuart's transport medium. Processing of specimens was performed within 2 h. Two specimens were collected from each patient, one for culture and the other for direct microscopy of a wet preparation followed by Gram's stain. Both stained and unstained preparations were examined for pus cells, epithelial cells, 'clue cells' and micro-organisms, especially *Trichomonas* spp. Culture media included blood agar, chocolate agar and Sabouraud agar. Each plate was streaked in such a manner as to allow estimation of the density of growth of the micro-organisms [7]. Cultures were examined for growth of *S. agalactiae*, *Candida* spp. and *Gardnerella vaginalis*, as well as for growth in significant numbers of any other micro-organism. Streptococcal strains were identified by gram-staining, production of catalase, CAMP test, serogrouping and the API20 Strep system (API System, bioMérieux, France).

Three serotypes of *S. agalactiae* were identified (types I, II and III), by slide agglutination with latex particles

sensitised with purified rabbit immunoglobulins specific for each of the three types (Pastorex B streptococci; Diagnostics Pasteur) [8].

The degree of association of various micro-organisms in causing vaginitis was assessed by the relative risk (RR) and population attributable risk (PAR) statistical parameters [9].

Results

A total of 6226 specimens from patients who presented with vaginal discomfort was submitted for culture; 4394 (65.6%) were positive for potentially pathogenic micro-organisms. *Candida* spp. were the micro-organisms most frequently isolated (34.2%), followed by *G. vaginalis* (20.6%), *S. agalactiae* (5.7%) and *Trichomonas* spp. (3.2%).

The presence of >10 leucocytes/high power field (h.p.f.) was taken as an objective criterion for

Table 1. Presence of micro-organisms in vaginal discharge and inflammatory response

Micro-organism	Number (%) of cases with <10 leucocytes	Number (%) of cases with >10 leucocytes
<i>S. agalactiae</i>	68 (4.3)	287 (10.1)
<i>Candida</i> spp.	598 (38.3)	1534 (54.1)
<i>G. vaginalis</i>	517 (33.0)	771 (27.2)
<i>Trichomonas</i> spp.	8 (0.5)	118 (4.2)
Enterobacteriaceae	168 (10.8)	62 (2.2)
<i>Enterococcus</i> spp.	115 (7.4)	41 (1.5)
CNS	56 (3.6)	17 (0.6)
<i>S. aureus</i>	30 (2.0)	4 (0.1)
Total	1560 (100)	2834 (100)

Table 2. Degree of association of various organisms with vaginal infection

Micro-organism	Relative risk (RR)	Population attributable risk (PAR)
<i>S. agalactiae</i>	2.38	2.7
<i>Candida</i> spp.	1.41	7.18
<i>G. vaginalis</i>	0.82	2.6
<i>Trichomonas</i> spp.	8.32	1.6

infection. Thus, according to this criterion, specimens were divided into two groups, those with ≥ 10 leucocytes/h.p.f. and those with <10 leucocytes/h.p.f. group (Table 1). In the majority of cases, isolation of *S. agalactiae*, *Candida* spp. and *Trichomonas* spp. was associated with an inflammatory response. The presence of *S. agalactiae* was associated with a greater incidence of inflammatory response than that of *Candida* spp. and a lower incidence than that of *Trichomonas* spp. (Table 1). In contrast, the isolation from 4394 samples of 493 strains of micro-organisms considered as commensals, such as Enterobacteriaceae, staphylococci and enterococci, and included in the study only for statistical purposes, did not correlate with the presence of inflammatory cells (Table 1). In regard to the association of various organisms with vaginitis, the RR was 2.38, 1.41 and 8.32 for *S. agalactiae*, *Candida* spp., and *Trichomonas* spp., respectively, and the PAR was 2.7, 7.18 and 1.6, respectively (Table 2).

In 295 (83%) of 355 cases, *S. agalactiae* was isolated as the sole pathogen. There were 60 cases (17%) of mixed infections, in which *S. agalactiae* was isolated along with *Candida* spp., *G. vaginalis* or *Trichomonas* spp. (Table 3).

The serotype distribution of *S. agalactiae* isolates is shown in Table 4. Serotypes I and III were more commonly associated with an inflammatory response.

Discussion

Until now, *S. agalactiae* has been regarded as a normal component of the vaginal flora, associated with premature birth, amnionitis, neonatal meningitis and sepsis, but recent studies have concluded that invasive infections caused by *S. agalactiae* are not uncommon and that they pose a major problem not only in pregnant women and neonates but also in non-pregnant adults, especially the elderly and patients with chronic diseases [1–6].

According to current evidence, the gastrointestinal tract is the primary colonisation site. This is supported

Table 3. Mixed vaginal infections associated with *S. agalactiae*

Micro-organisms	Number of specimens			Total
	with leucocytes (/h.p.f.)			
	10–20	20–50	>50	
<i>S. agalactiae</i>	136	92	6	234
<i>S. agalactiae</i> + <i>Candida</i> spp.	15	9	4	28
<i>S. agalactiae</i> + <i>G. vaginalis</i>	5	5	–	10
<i>S. agalactiae</i> + <i>Trichomonas</i> spp.	2	8	1	11
<i>S. agalactiae</i> + <i>Trichomonas</i> spp. + <i>Candida</i> spp.	–	1	1	2
<i>S. agalactiae</i> + <i>Trichomonas</i> spp. + <i>G. vaginalis</i>	–	1	–	1
Total	158	116	12	286

Table 4. Distribution of *S. agalactiae* serotypes (%)

Serotype	Number with leucocytes (/h.p.f.)	
	<10 (n = 68)	>10 (n = 287)
I	37	36
II	37	16
III	17	34
NT	5	15

NT, not typable.

by the relatively high isolation rates of *S. agalactiae* from cultures of the anal region, compared to the genital region [3]. An estimated 40% of pregnant women carry the organism throughout their pregnancy, but transient vaginal colonisation may be more frequent [3]. In a survey in Israel on pre-term Israeli and Arabic women, the colonisation rates were 5.4% and 1.6% respectively [10]. A similar study in Germany revealed a 3.8% rate [11]. In three Italian hospitals, 7.5% of mothers and 4.9% of babies were found to be colonised [12]. Higher rates of colonisation were reported in England (28%) [13] and in the USA (20%) [14].

The role of *S. agalactiae* as an aetiological agent of vaginitis has not yet been established. Studies dealing with the organism as an infectious agent in bacterial vaginitis are limited either in the manner of reporting numbers of positive isolates or in epidemiological considerations concerning colonisation of the genital or gastrointestinal tract [15, 16]. Vaginitis is a common gynaecological disease, characterised by objective and subjective signs of infection. The presence of leucocytes, combined with clinical symptoms, may be considered an objective criterion of infection. The exception to this is bacterial vaginosis where the absence of an inflammatory response may be due to the production of succinate, an inhibitor of chemotaxis, by *Bacteroides urealyticus* and *Prevotella bivia* [17].

This study evaluated the possible pathogenic role of *S. agalactiae* based on the criterion of the presence of >10 leucocytes/h.p.f. in the vaginal secretions to denote infection of the vagina and not only on the presence of clinical symptoms. *S. agalactiae* was isolated from 10.1% of the specimens with >10 leucocytes/h.p.f. and from 4.3% of the specimens with <10 leucocytes/h.p.f. We strongly believe that *S. agalactiae* is as pathogenic as the other established pathogens and should be considered a vaginal pathogen for the following reasons. Firstly, the recovery rate of *S. agalactiae* in the leucocyte-positive group was much higher than in the leucocyte-negative group. The association between *S. agalactiae* and symptomatology was at least as high as that of *Candida* spp. and slightly lower than that of *Trichomonas* spp. Secondly, in the majority of specimens from which *S. agalactiae* was isolated (83%) it was the sole pathogen. There-

fore, it is reasonable to consider it as the cause of the infection.

Differences in the prevalence of *S. agalactiae* internationally may be attributed to different methods of specimen collection, transport media and isolation methods. In the present study chocolate and blood agar were used exclusively as culture media. Use of selective solid culture media such as the Columbia colistin-nalidixic acid (CNA) agar and enrichment broth (Todd-Hewitt supplemented with gentamicin 8 µg/ml) may have yielded higher isolation rates [18].

Irrespective of the patient's age, the distribution of isolates was not divided evenly among the three major serotypes (I, II, and III), as has been reported for asymptotically colonised patients [6]. Serotypes I and III were present more than twice as often as serotype II in the leucocyte-positive group.

Although no resistance to penicillin was observed, treatment failures have been reported when penicillin was used to eradicate group B streptococci from the genital tract of women [19]. This has brought about the use of erythromycin in empirical antimicrobial treatment. In a previous study, 9% of strains were found to be resistant to erythromycin [20]. Such strains have also been detected in other studies but with a lower frequency [21–24]. This resistance to erythromycin may be related its extensive use in the empirical treatment of female lower genital tract infections [20]. Susceptibility testing for *S. agalactiae* is not usually performed as the species remains susceptible to penicillins. However, high rates of penicillin treatment failures as well as the resistance rates to erythromycin suggest that the antibiotic susceptibility trends of these pathogens should be monitored carefully.

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