Pili on *Gardnerella vaginalis* studied by electronmicroscopy

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Summary. Fourteen recently isolated strains and two laboratory strains of *Gardnerella vaginalis* were examined by electronmicroscopy for the presence of pili. All strains isolated recently from both men and women were heavily pilated. In contrast only a few pili were seen on organisms of the two laboratory strains, with many of the organisms having no pili. The importance of multiple subculture in this loss was supported by the observation that the degree of pilation of one freshly isolated strain decreased on repeated subculture. Other findings suggested that this was probably due to gradual loss of pili and not to selection of organisms that were non-pilate originally.

Introduction

Gardnerella vaginalis is a gram-negative coccobacillus which is found in the lower genital tract, particularly of women but also of men. The number of organisms of this bacterium isolated from the vagina of women with bacterial vaginosis is greatly increased in comparison with the number from healthy women (Spiegel *et al.*, 1980, 1983) although their role in the pathogenesis of the disease is unclear.

In a previous study of *G.vaginalis* by electronmicroscopy, with negative staining (Johnson and Davies, 1984), some of the organisms were found to possess narrow pili. Three of the eight strains examined, however, appeared non-pilate. Moreover, in preparations of the five pilate strains, many non-pilate organisms were also seen. The relatively sparse pilation observed in that study may have been due to each of the strains having had multiple passages, albeit an unknown number, in medium in the laboratory. The current study, therefore, was undertaken to examine the expression of pili by *G.vaginalis* organisms that had been subcultured less than ten times after isolation and to determine to what extent passage in medium diminished pilation.

Materials and methods

Bacteria

Sixteen strains of *G.vaginalis* were studied (table). Four strains had been isolated in this laboratory from the

vaginas of patients diagnosed as having bacterial vaginosis, six strains were from the vaginas of women who were 9–10 weeks pregnant and undergoing chorionic villous sampling, and four strains were from the urethras of men with non-gonococcal urethritis. Two strains (67·1 and 40·1) that had been examined in the earlier study (Johnson and Davies, 1984) were re-examined.

All strains were isolated on human blood-tween 80 (HBT) agar (Totten *et al.*, 1982) and were subcultured on Columbia blood agar (Difco). They were identified as *G.vaginalis* as described previously (Taylor and Phillips, 1983).

Negative staining

Bacteria were grown on Columbia blood agar at 37°C in an atmosphere of CO₂ 5% in air for one or two days. Electronmicroscope grids were prepared according to the method of Henrichsen and Blom (1975), as modified previously (Johnson and Davies, 1984), and were examined with a Philips EM 300 electronmicroscope at 60 kV. A minimum of 40 organisms of each strain was examined and the degree of pilation of each organism was assessed subjectively by one observer (YLB) as follows: ++ = pili radiating from > 25% of the periphery; + = pili radiating from 1–25% of the periphery; – = no pili seen. The diameter of the pili was measured from the electronmicrographs with an eyepiece graticule.

Results

All 14 recently isolated strains of *G.vaginalis* possessed pili (table). The extent of pilation differed among these strains, although no difference in expression of pili was seen between strains isolated from men and women. Organisms without any pili were not seen in 12 of the recently isolated strains

Table. Degree of pilation of 16 strains of *G.vaginalis*

Strain	Patient category*	Percentage of bacteria with indicated degree of pilation†		
		++	+	-
352	BV	82	18	0
6916	BV	73	23	3
762	BV	53	47	0
194 P ₄ ‡	BV	78	22	0
194 P ₂₁ ‡	BV	80	20	0
194 P ₄₀ ‡	BV	26	74	0
314	CVS	94	6	0
908	CVS	91	9	0
121	CVS	73	27	0
815	CVS	59	41	0
403	CVS	48	52	0
214	CVS	33	75	3
69	NGU	88	12	0
153	NGU	86	14	0
169	NGU	59	41	0
72	NGU	39	61	0
67·1	PS	7	57	36
40·1	PS	0	57	43

* BV = bacterial vaginosis; CVS = chorionic villous sampling; NGU = non-gonococcal urethritis; PS = previous study.

† See "Materials and methods".

‡ P₄, P₂₁, P₄₀ = strain 194 subcultured 4, 21 and 40 times, respectively, since isolation.

and the other two strains had a small proportion only of non-pilate organisms. Two of the strains (40·1 and 67·1) had had multiple subcultures and when studied previously (Johnson and Davies, 1984) were considered to be non-pilate. On re-examination, similar results were obtained in that a large proportion of non-pilate organisms was seen. A likely explanation for the difference between the expression of pili in freshly isolated and laboratory strains of *G.vaginalis* could be loss of pili as a result of repeated subculture. This possibility was tested by repeatedly subculturing one of the freshly isolated strains (194) of *G.vaginalis*. The results presented in the table show that there was an increase in the proportion of sparsely pilate organisms, although none of them lost their pili completely. Five colonies of one of the least pilate strains (67·1) were cloned and cultures on agar medium were prepared for each of them. None of these cultures comprised organisms all of which were non-pilate.

A comparison of *G.vaginalis* organisms grown for 24 h and 48 h and growth on human blood agar compared to that on horse blood agar did not show any difference in expression of pili. The diameter of the pili on organisms of all sixteen strains of *G.vaginalis* fell within the size range (3·0–7·5 nm) reported previously (Johnson and Davies, 1984).

Discussion

The finding that the organisms of *G.vaginalis* strains isolated recently were heavily pilate is in contrast to the results obtained in a previous study (Johnson and Davies, 1984) in which five of eight strains were seen to have a large proportion of non-pilate organisms and the other three strains appeared to be completely non-pilate. These were "laboratory" strains that had undergone multiple subcultures and their sparse pilation was confirmed when two of them were re-examined. It is known that some micro-organisms, for example gonococci, cease to express pili when subcultured non-selectively (Jephcott *et al.*, 1971) and in this regard it was of interest to note that the degree of pilation of one of the *G.vaginalis* strains also diminished on multiple subculture. Cloning of single colonies of one of the strains which was least pilated did not result in cultures of organisms all of which were non-pilate. This suggested that loss of pili on subculture was not due to selection of organisms that were non-pilate originally but to a gradual loss of pili.

It has been shown previously that *G.vaginalis* is able to attach to vaginal epithelial cells *in vitro* (Sobel *et al.*, 1982; Peeters and Piot, 1985) as well as to attach *in vivo* to produce the clue cells characteristic of bacterial vaginosis (Blackwell and Barlow, 1984). The observation that clinical isolates of *G.vaginalis* are highly pilate raises the question of whether the pili have a role in mediating attachment. The fact that the organisms manufacture pili suggests that they may have such a specific function. The existence of heavily and sparsely pilate strains should enable the relationship between pilation and adherence to vaginal epithelial cells to be determined and therefore help to define the role of adherence in the pathogenesis of bacterial vaginosis.

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