Comparisons Between Direct Microscopic and Cultural Methods for Recognition of *Corynebacterium vaginale* in Women with Vaginitis

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The frequency with which clue cells could be detected in Gram-stained vaginal smears and/or cervical Papanicolaou (Pap) smears was compared with the frequency of *Corynebacterium vaginale* (*Haemophilus vaginalis*) isolation in a group of 236 female patients, of whom 221 had vaginitis. Vaginal clue cells were found most often in women from whom *C. vaginale* was isolated ($P = 0.00006$) whereas, conversely, clue cells in cervical Pap smears were reported more frequently in women with negative cultures for this organism ($P = 0.006$). *C. vaginale* isolations were made more frequently from women with both vaginal and cervical clue cells reported ($P = 0.000088$). However, the combined false positive-false negative vaginal clue cell rate in the patients studied was 36.5%. Neither the detection of vaginal clue cells nor the isolation of *C. vaginale* was significantly affected by whether or not patients had trichomoniasis ($P = 0.25$). *Trichomonas vaginalis* detection in cervical Pap smears and vaginal isolation were related ($P = 0.00005$), whereas the same relationship was not significant for fungi ($P = > 0.05$).

Direct microscopic examinations of genital exudates provide rapid and simple means for laboratory identification of some sexually transmissible infections. These include trichomoniasis and candidiasis primarily in women and gonococcal urethritis in males. Techniques most often used for the above are the saline and 10% KOH wet mounts and the Gram stain (3, 8). The Papanicolaou (Pap)-stained smear taken to evaluate cervical tissues has also been used to detect infections. Thin et al. (15) found that the cervical Pap smear was as reliable as wet mounts or cultures in detecting *Trichomonas vaginalis* infection, but the Pap smear was unacceptable in detecting fungus infections.

Several lines of evidence support the probable role of *Corynebacterium vaginale* (*Haemophilus vaginalis*) in causing some forms of vaginitis (1, 2, 5, 13). Microscopic methods to examine vaginal exudates for clue cells are commonly used to detect *C. vaginale*. Clue cells are squamous epithelial cells stippled with masses of gram-negative pleomorphic coccobacilli (4). When compared to isolation of *C. vaginale*, Dunkelberg (4) found the Gram stain to be 87.2% accurate and the wet mount 95% accurate. Lewis and O'Brien (10) reported a combined cervical-vaginal Pap smear to be 87.4% accurate in detecting *C. vaginale*. In previous

studies in our venereal disease clinics, we found the Gram stain to be the best method for demonstrating clue cells (3, 14).

The objective of this study was to determine the reliability and possible clinical significance of the demonstration of clue cells in cervical Pap smears or Gram-stained vaginal secretions as compared with the isolation of *C. vaginale* from the vagina. For purposes of comparison, we decided to also attempt to confirm the work of Thin et al. (15) mentioned above. It was also of interest to include in the study women with active trichomoniasis, because clue cells have been reported to be difficult to detect in such patients (9).

MATERIALS AND METHODS

Specimen collections. Cervical and vaginal specimens were obtained by introducing a Sani-Spec disposable vaginal speculum (Burnett Instrument Co., Lawrence, Kans.). Where the cervix was coated with vaginal discharge material, swabs (all calcium alginate) were used to remove excess material from the cervix. One swab was then inserted into the endocervical canal and inoculated directly onto Thayer-Martin medium to isolate gonococci. A second swab and an Ayer spatula were used to obtain a cervical Pap smear. A third swab was used to collect vaginal discharge to prepare 10% KOH and saline wet mounts and Gram stain. A fourth swab was inserted into vaginal fluid and placed into Trichosel broth (Baltimore Biological Laboratory, Cockeys-

ville, Md. [BBL]) to isolate *T. vaginalis*. A fifth swab was used to collect vaginal fluid to inoculate starch agar (14) for isolating *C. vaginale* and fungi (3).

Cervical Pap smears were not taken on every patient cultured unless the patient was making her first visit to the clinic, was returning for examination and a yearly Pap smear was indicated, or was recalled because of some pathological findings of a recently taken Pap smear. Other patients were excluded from analysis if they were receiving antibiotics for a previously diagnosed genital tract or other infection at the time they visited the clinic during the study period.

Culture methods. All women were routinely cultured for *C. vaginale*, *Neisseria gonorrhoeae*, *T. vaginalis*, and fungi. Starch agar (14) was used to isolate fungi and *C. vaginale*. *C. vaginale* was identified by demonstrating catalase-negative, starch-fermenting, gram-negative to gram-variable pleomorphic bacilli on starch agar, which were further identified using the fermentation methods of Dunkelberg et al. (7). Trichosel broth (BBL) was used to isolate *T. vaginalis*. Gonococci were identified by their growth on Thayer-Martin medium coupled with demonstration of an oxidase reaction, Gram stain, and typical morphology. All media were incubated at 37°C for 72 h in 5 to 10% CO₂. Methods for identification of fungi were previously described (3).

Other methods. Pap smears were read by a private pathology laboratory on a contract basis. Individuals reading Gram stains and other slide preparations had no knowledge of Pap smear results or culture results. Individuals who examined culture media were not aware of the results of wet mount and Gram stain results. Pap smear reports considered positive for *C. vaginale* were reported as: "organism resembling *C. vaginale*" or "clue cells present."

Clinical definitions and treatment. Reference to the term "treatment" of *C. vaginale* infection used in the text denotes the oral administration of 500 mg of ampicillin four times per day for 7 days, a total of 14 g. In the symptomatic patient considered to have *C. vaginale* infection, there is usually a characteristic

homogenous, moderately thin gray-white to white discharge, occasionally frothy and adherent to the vaginal walls (13). Whereas the presence of vaginal clue cells in most cases was used as immediate laboratory confirmation of infection, patients were treated who were negative for vaginal clue cells when physicians considered the discharge to be characteristic for *C. vaginale* in the absence of trichomoniasis or candidiasis. Nonspecific vaginitis was defined as a non-characteristic, atypical vaginal discharge with failure to identify *T. vaginalis*, fungi, or *C. vaginale* by microscopic or cultural methods.

RESULTS

From March through August, 1976, clinical examinations and various cultures were taken on approximately 1,000 women attending three county venereal disease clinics. The average monthly isolation rate of *N. gonorrhoeae* in these patients was 20%; for *Candida albicans* or other yeasts, 23%; and for *T. vaginalis*, 18%. The average monthly isolation rate of *C. vaginale* was 30% and ranged from 19 to 45%. Complete clinical and laboratory data, including cervical Pap smears, were obtained on 236 women of whom 221 had signs or symptoms of vaginitis. *C. vaginale* was isolated from 118 women (Table 1). Vaginal clue cells were reported significantly more often in women from whom *C. vaginale* was isolated (52 of 118) than in patients whose cultures were negative for this organism (21 of 118, $P = 0.00006$). This relationship was reversed for clue-positive cervical Pap smears; i.e., there were more reported for women with negative cultures for *C. vaginale* (50 of 118) than for those from whom the organism was isolated (30 of 118, $P = 0.006$). The organism was isolated from 17 of 118 women with clue cells reported in both cervical Pap smears and vaginal Gram stains. Only one patient in 118 from whom *C. vaginale* was not

TABLE 1. Relationship between detection of clue cells in vaginal and cervical smears and isolation of *C. vaginale*

Source	Smear results	No. of patients ^a			χ^2	P value
		Culture +	Culture -	Total		
Vaginal (Gram stain)	Clue +	52	21	73	19.06	0.00006
	Clue -	66	97	163		
Cervical (Pap smear)	Clue +	30	50	80	7.56	0.006
	Clue -	88	68	156		
Vaginal and cervical	Clue +	17	1	18	15.4	0.000088
	Clue -	101	117	218		

^a Of the 236 women cultured, 221 were found to have a discharge or other signs and symptoms of vaginitis. *C. vaginale* was isolated from 118 women and not from an additional 118 patients. There were seven patients without vaginitis in the culture-positive group and eight in the culture-negative group.

isolated was reported to have cervical Pap and vaginal clue cells ($P = 0.00088$).

Fifty-two of the 236 patients had active trichomoniasis (Table 2). The positive correlation between detection of vaginal clue cells and *C. vaginale* isolation was not significantly affected by whether women had or did not have trichomonas infection ($P = 0.25$).

T. vaginalis (Table 3) was reported in cervical smears of 52 women from whom the organism was isolated ($P = 0.00005$). Fungi, all being *C. albicans*, were isolated from 35 women but were reported present in only one Pap smear ($P = >0.05$).

Among the 30 women who were Pap smear and *C. vaginale* culture positive (Table 4, group A), 3 (10%) were normal and 16 (53%) were treated for *C. vaginale* infection. Treatment (14 g of ampicillin) was based upon combined clinical evaluation and microscopic and cultural data. However, 17 of the 30 patients had vaginal clue cells reported, and 15 of the 16 treated patients were in the positive vaginal clue cell category. Eleven (22%) of the 50 women with negative *C. vaginale* cultures (Table 4, group B) but positive cervical Pap smears were found to be normal. Four of the 50 patients were treated for *C. vaginale* vaginitis. One of the latter patients, who also had vaginal clue cells reported, was found to have a discharge, an inflamed cervix, and complained of pruritus. She did not return to the clinic for follow-up examination.

Excluding Pap smear data, 64 of 118 women with *C. vaginale* isolated were treated for infection by this organism, and 24 of the treated patients had vaginal clue cells. Of the 118 women with negative culture, 15 of 21 patients treated for *C. vaginale* vaginitis had vaginal clue cells reported.

DISCUSSION

If vaginal clue cells are seen in Gram stain smears, the probability is that *C. vaginale* will also be isolated from the vagina. The majority of the patients were symptomatic, but only 52

TABLE 4. Diagnosis and treatment of patients with cervical Pap smears reported positive for *C. vaginale*^a

Infection agent diagnosed	Group A (Pap +, culture +, 30 patients)	Group B (Pap +, culture -, 50 patients)
Normal (no vaginitis)	3	11
Fungus (<i>C. albicans</i>)	4	11
<i>C. vaginale</i>	16	4
<i>N. gonorrhoeae</i>	10	14
<i>T. vaginalis</i>	1	8
Herpetic lesions	1	1
Nonspecific vaginitis	2	3
Scabies	0	1

^a The numbers of patients treated total more than 30 and 50 patients, respectively, in each column, to reflect treatment of mixed infections. In group A, 10 of 30 patients were treated for mixed infections, whereas in group B, 8 patients were treated for mixed infection.

TABLE 2. Detection of clue cells and isolation of *C. vaginale* in women with and without trichomoniasis^a

Patient group	Vaginal smear	No. of patients			χ^2	P value
		<i>C. vaginale</i>		Total		
		Culture +	Culture -			
With trichomoniasis	Clue +	11	6	17	7.35	0.0067
	Clue -	9	26	35		
Without trichomoniasis	Clue +	41	15	56	12.87	0.0003
	Clue -	57	71	128		

^a The relationship between clue cells and *C. vaginale* isolation in patients with and without trichomoniasis was $\chi^2 = 4.54$, $P = 0.25$.

TABLE 3. Relationship between detection of *T. vaginalis* and fungi in cervical Pap smears and isolation from vaginal secretions

Organism	Smear result	No. of patients			χ ²	P value
		Culture +	Culture -	Total		
<i>T. vaginalis</i>	Pap +	17	4	21	46.58	0.00005
	Pap -	35	180	215		
Fungi	Pap +	1	4	5	0.12	Not significant
	Pap -	34	195	231		

(44%) of 118 *C. vaginale*-positive patients were reported to have vaginal clue cells, and 21 (17%) of the 118 *C. vaginale* culture-negative patients had vaginal clue cells. In evaluations of this type, a false positive result denotes microscopic detection of an organism in a specimen not recovered by culture, whereas a false negative result denotes failure to detect an organism microscopically but with positive isolation (4, 6, 10). When such criteria are applied here, the combined false positive and false negative vaginal clue cell-culture relationship is considerable: 36.5% [(56% + 17%)/2]. These results, derived mostly from symptomatic patients, differ sharply from those of Dunkelberg et al. (6), who found clue cells and isolated *C. vaginale* in 10.7% of 550 healthy asymptomatic women with a 3.5% total combined false positive-false negative clue cell-culture relationship. A vaginal-cervical swab was used to prepare Gram stain smears and streak plates. However, Akerlund and Mardh (1) reported that the frequency of cervical smears containing clue cells did not vary with the presence or absence of *C. vaginale* isolated from women with vaginitis. Dunkelberg (4) attributed most false positive clue cells to confusion resulting from separating *C. vaginale* from other diphtheroids. This explanation, coupled with the possible overgrowth of contaminants on starch plates, nonviable organisms, or presence of strict anaerobic strains of *C. vaginale* (11), would further explain the discrepancy. These possibilities of explaining false positive vaginal clue cells have not been fully evaluated and represent valid criticisms of this study. We assume false positive vaginal clue cells were largely due to misinterpretation by the microscopist.

Most patients with clue cells reported in cervical Pap smears were treated for *C. vaginale* vaginitis because they had vaginal clue cells. This latter information was immediately available to physicians examining patients. The larger number of patients who were culture negative but Pap smear positive could obviously be the result of culturing the vagina only, and not the cervix, for *C. vaginale*. Distinctions have not always been made (2, 7, 8, 10) as to the source of *C. vaginale* from cervical, posterior vaginal fornix, or other vaginal sites. *C. vaginale* was reported isolated from the cervix of 8 of 100 pre hysterectomy patients (12). Malone et al. (11) described *C. vaginale* strains from the cervix and uterus. Cervical and combined cervical-posterior vaginal fornix *C. vaginale* isolation rates of 31% reported by Akerlund and Mardh (1) and Dunkelberg et al. (8) are nearly identical to our average monthly isolation rate for *C. vaginale* (30%). Akerlund

and Mardh (1) did not confirm the accuracy of the cervical Pap smear in detecting *C. vaginale* as was reported by Lewis and O'Brien (10). In women with discharges, the cervix is likely to be contaminated with vaginal secretions. Therefore, it seems unlikely that the larger number of women who were Pap smear positive but culture negative can be explained by the fact that the cervix was not cultured.

Microorganisms may not be seen microscopically in urine, blood, sputum, or other tissues, whereas cultural isolation can be highly significant in supporting a diagnosis of infection. We conclude that *C. vaginale* can be isolated from the cervix and/or vagina of infected and noninfected patients. When infection exists, the focus is primarily the vaginal tract. The cervical Pap smear and vaginal Gram stain are neither specific nor sensitive enough to be used in the diagnosis of *C. vaginale* infection. The positive correlation between the detection of *T. vaginalis* in cervical Pap smears and vaginal isolation, and the lack of correlation between detection of fungi in cervical Pap smears and vaginal isolation, are in agreement with the work of Thin et al. (15).

Active trichomoniasis did not significantly affect detection of vaginal clue cells or isolation of *C. vaginale*. Additional evaluation of the clinical significance of isolating *C. vaginale* and detecting clue cells on a routine basis is in progress.

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