ORIGINAL ARTICLE

Clinical and microbiological features of *Haemophilus influenzae* vulvovaginitis in young girls

R A Cox, M P E Slack

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See end of article for authors' affiliations

Correspondence to: Dr Cox, The Old Rectory, Ashley, Market Harborough, Leicestershire LE16 8HF, UK; michael.gallant@ukgateway.net

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Aims: To define the clinical and microbiological features of vulvovaginitis in prepubertal girls whose genital swabs yielded *Haemophilus influenzae*.
Methods: Laboratory based study and retrospective collection of clinical data from the requesting doctors.
Results: Thirty eight isolates of non-capsulate *Haemophilus influenzae* and one of *H parainfluenzae* were isolated from 32 girls aged 18 months to 11 years. No other pathogens, such as β haemolytic streptococci or yeasts, were present with *H influenzae*. The most common biotype was biotype II, comprising 57% of the 26 isolates biotyped. Six children had more than one episode of vulvovaginitis caused by *H influenzae* and a total of 14 children had recurrent vaginal symptoms.
Conclusion: Children who have *H influenzae* vulvovaginitis are at risk of recurrent symptoms. Biotype II is the one most commonly associated with this condition.

Vaginal discharge or vaginal irritation in young, prepubertal girls is a common problem in general practice. Because few hospitals provide specialist paediatric gynaecological outpatient services, these children are managed mainly in primary care. Although poor perineal hygiene, threadworms, foreign bodies, and other non-microbial causes may be implicated, some cases are caused by bacterial infection.[1 2] The most common organism identified in several studies has been group A β haemolytic streptococcus.[2–5] Non-capsulate *Haemophilus influenzae*, although much less well known, has been the second most frequent cause in several studies, and was the most common cause in a large study from Liverpool. Sexually transmitted infections such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are important causes of vulvovaginitis following child sexual abuse and screening for these infections should be undertaken in all children evaluated for possible sexual abuse.[6]

> "Vaginal discharge or vaginal irritation in young, prepubertal girls is a common problem in general practice"

We describe a three year study, undertaken in the laboratory of a district general hospital, to determine whether there were any particular clinical features that characterised this condition and to identify the biotypes and antibiograms of the isolates.

PATIENTS AND METHODS

Microbiological methods

Vulval and vaginal secretions were obtained from girls who complained of vaginal irritation or discharge and attended their general practitioners, paediatric outpatient clinics, or a paediatric gynaecologist. Swabs were placed in Amies transport medium with charcoal (Sterilin, Stone, Staffordshire, UK). They were sent via the laboratory transport system (ambient temperature, but same day delivery). Specimens from the paediatric gynaecology clinic were supported by a "chain of evidence"—a record of handling specimens from the time of collection to issue of the final report.[7] They were cultured on the following media: chocolate (heated blood) agar (for *H influenzae*) incubated in 5% CO_2 for 24 hours, blood agar (7% horse blood) incubated anaerobically for 24 hours (for streptococci), selective medium for *N gonorrhoeae* (brain heart infusion agar with lysed horse blood and supplemented with vancomycin, colistin sulfate, nystatin, trimethoprim), and Saboraud's agar (for candida). A wet preparation was also examined by direct microscopy for trichomonas.

Characteristic colonies were identified as *H influenzae* by standard methods.[8] Antibiotic susceptibility was tested by the disc diffusion method, using isosensitest agar with 5% horse blood supplemented with 0.2% NAD solution. The following were tested: amoxicillin, co-amoxiclav, trimethoprim, clarithromycin, and cefuroxime.

Isolates were preserved on beads in a cryopreservative (Protect STC; Technical Service Consultant, Heywood, Lances, UK) at −70°C and sent in batches to the Public Health Laboratory Services Haemophilus Reference Unit, Oxford, for capsular typing and biotyping.

Typing techniques

The strains were serotyped by slide agglutination,[9] using a polyvalent antiserum containing antisera to *H influenzae* types a–f (Difco Laboratories; supplied by Becton Dickinson, Cowley, Oxford, UK) and using monospecific antisera for types a, b, c, d, e, and f (Murex; supplied by Biostat, Stockport, Cheshire, UK). The results of the slide agglutination tests were validated by amplification of haemophilus specific DNA in a polymerase chain reaction.[10]

Strains that did not type by this method were classified as non-capsulate *H influenzae*.[11] The strains were biotyped using the methods devised by Kilian.[12]

Patients

Isolates of *H influenzae*, cultured from vulval and vaginal swabs from girls aged 14 years and under, were collected over a three year period between 1997 and 2000. At the end of each year, a letter was sent to every doctor whose patient's specimen had yielded *H influenzae*, requesting information on the patient's treatment (antibiotic course and duration) and clinical outcome.

RESULTS

During the study period, the microbiology laboratory examined 1016 vulval and vaginal swabs from 814 girls aged 14 years or less. Thirty eight isolates of *H influenzae* and one of

Table 1 Biotypes of *Haemophilus*

Type	Number	Percentage
H influenzae (n=25)		
I	3	11.5
II	15	57
III	2	7.6
IV	1	3.8
V	4	15
H parainfluenzae II	1	
Not typed	13	

Table 2 Antibiotic resistance in *Haemophilus influenzae* and *H parainfluenzae* isolated from children with prepubertal vulvovaginitis

Antibiotic	Number of isolates resistant (n=39)	Percentage
Amoxicillin	4	10
Co-amoxiclav	0	0
Trimethoprim	7	18
Clarithromycin	2	5
Cefuroxime	0	0

H parainfluenzae were obtained from 32 children. All were non-capsulate. The ages ranged from 1.5 to 11 years, with a median of 4 years and mean of 4.9 years. Five children had two episodes of haemophilus vulvovaginitis and one had three. Of the 26 isolates that were biotyped, 15 were biotype II (14 *H influenzae*, one *H parainfluenzae)*. Table 1 shows the distribution of biotypes.

Four isolates from four girls were resistant to amoxicillin and β lactamase positive (table 2). Three of these girls suffered from recurrent *H influenzae* vulvovaginitis. For two children the first isolate was sensitive to amoxicillin, but the second was resistant. Both had received amoxicillin as treatment for their initial infections. Two children had amoxicillin resistant *H influenzae* isolated from a single episode of vulvovaginitis. It is not know whether they had recently received amoxicillin as empirical treatment for some other infection.

In this small group, amoxicillin resistance was not apparently associated with biotype because two isolates were not typed, one was biotype II, and one was biotype V.

Seven isolates were resistant to trimethoprim. Six isolates from five children were resistant to trimethoprim only and one was resistant to both amoxicillin and trimethoprim. Four of the isolates were biotype II, one was biotype V, one biotype I, and one was not typed.

There were two clarithromycin resistant isolates: the single *H parainfluenzae* isolate, which was biotype II, and one *H influenzae* isolate that was not typed.

None of the strains was resistant to cefuroxime.

No other recognised pathogens such as group A streptococci or candida were present together with *H influenzae*. (*Neisseria gonorrhoeae* was not isolated from a prepubertal child during the three year study.) No threadworms were detected on the swabs (no perianal samples were received from these children). Eight isolates were accompanied by mixed anaerobic flora. Five of these isolates were biotyped. Four were biotype II and one was biotype V.

All the children investigated had presented with vaginal discharge and/or itching. Twenty five children were investigated and managed by their general practitioners. Six were seen by a consultant paediatric gynaecologist and one by a consultant paediatrician. These children were managed jointly by a specialist and their general practitioner. Treatment information was available for 29 of the 32 children. They received a variety of antibiotics (table 3). Fifteen children received a single course of antibiotic, but for 11, treatment was either changed or a repeat course of the same antibiotic was given. *Candida* spp were not isolated from the five post-treatment swabs received.

Table 3 Antibiotic treatment of children with *Haemophilus influenzae* vulvovaginitis

Antibiotic	Number of treatment courses
Amoxicillin	19
Co-amoxiclav	9
Trimethoprim	6
Clarithromycin/erythromycin	2
Miconazole	6
Metronidazole	2

Five children had more than one episode of *H influenzae* vulvovaginitis during the three year study period. In addition, an 8 year old child, who was referred to the paediatric gynaecologist from a general practitioner outside the laboratory's area, was said to have had a previous episode of *H influenzae* vulvovaginitis. Two children with recurrent infection had different biotypes on each occasion (indicating reinfection, rather than relapse) and one had the same biotype with the same antibiotic sensitivity pattern (indicating failure to eradicate the original infecting organism). For two children, biotyping results were not available for all the isolates.

A further eight children had recurrent episodes of vulvovaginitis, but in only one case was a recognised pathogen isolated. This was a 6 year old girl who had a group A streptococcal vaginitis nine months after her *H influenzae* infection. Thus, almost half of the children studied had recurrent symptoms.

DISCUSSION

There are many conditions that can give rise to vaginal irritation and discharge in prepubertal girls. These range from the physiological—leucorrhoea of the newborn and menarche—to the pathological, including congenital malformations, tumours, endocrine abnormalities, and skin diseases.

Viral, bacterial, and parasitic infections are common causes of juvenile vulvovaginitis. The vagina of the prepubertal girl lacks the oestrogenic influence present in the sexually mature woman and has a different vaginal flora, pH, and cellular structure. This results in a different spectrum of both pathogenic and commensal flora.

> "Nearly half (14 of 32) of the children in this small study had a recurrence of symptoms"

Several studies of infectious causes of juvenile vulvovaginitis have been published, either comparing symptomatic subjects with age matched controls,[13–15] or attempting to identify putative pathogens in symptomatic children.[1–3 5] No specific infective cause was identified in most of the children. When no specific pathogen is isolated, the condition is often referred to as "non-specific vaginitis". It has been suggested that inflammation of the vulva and lower vagina may be caused by a mixture of faecal flora resulting from poor perineal hygiene.[1 2]

Most of these studies found that group A β haemolytic streptococci were the most common cause of juvenile vulvovaginitis, followed by *H influenzae.* In a large study from Liverpool, *H influenzae* was a more common cause of this complaint than β haemolytic streptococci.[1]

Haemophilus influenzae is an important bacterial pathogen in children. Since the introduction of the Hib vaccine in the UK in 1992, there has been renewed interest in disease caused by non-capsulate strains of *H influenzae*.[16 17] They are part of the normal flora of the nasopharynx, but only occasionally form part of the vaginal flora in prepubertal girls.[13–15] They are common causes of otitis media, pneumonia, and sinusitis.[18] They cause a rare, but often fatal neonatal sepsis syndrome, associated with preterm birth.[19]

The association between *H influenzae* and prepubertal vulvovaginitis was first highlighted by MacFarlane in 1987.[20] Because most young girls with vaginal discharge are seen by their general practitioners, there have been few studies of the specific characteristics of this infection. In addition, *H influenzae* is fastidious in its growth requirements and laboratories may not isolate it unless they include appropriate culture medium for genital swabs received for young girls.[21]

Although our study was incomplete in that only 26 of 39 isolates were available for biotyping, all were non-capsulate and biotype II was the most common strain identified. It was present in over half of the episodes studied. MacFarlane reported that most strains isolated in his study were capsulate, but qualified this by indicating that the slide agglutination test he used for capsule typing may have been unreliable. MacFarlane also found that biotype II was the most common biotype associated with juvenile vulvovaginitis and accounted for two thirds of the isolates tested.[20]

Nearly half (14 of 32) of the children in this small study had a recurrence of symptoms. Six children had more than one episode of *H influenzae* vulvovaginitis during the period of observation (had the group been followed for longer this figure would have been even higher). Biotyping indicated that at least some of these were reinfections because different biotypes were identified on different occasions. Whether relapse or reinfection is a feature of particular strains of *H influenzae* is unknown. We were unable to implicate a particular biotype in those children who had more than one infection.

"Advice on hygiene and behaviour may be an important strategy to prevent recurrences"

Clinicians used a variety of antibiotics (some inappropriate) to treat the children's symptoms. The small number of children in our study makes it difficult to draw firm conclusions about the efficacy of treatment, but children treated with inappropriate antibiotics (metronidazole and miconazole) seemed to fare no worse than children treated with more appropriate ones (amoxicillin, co-amoxiclav, and trimethoprim).

Vulvovaginitis caused by group A β haemolytic streptococci is thought to result from digital transmission from the nasopharynx to the vagina.[22] Because non-capsulate *H influenzae* is also a commensal organism in the nasopharynx, it is possible that it is also transferred to the vagina in this way. Advice on hygiene and behaviour may be an important strategy to prevent recurrences.

Further studies on recurrent infections, seasonality and concurrent infection elsewhere in the body (similar to those undertaken with group A β haemolytic streptococci[22]) are needed.

There have been several studies of non-capsulate *H influenzae* that have attempted to correlate biotype with virulence, site of disease, and antibiogram.[23–27] Long *et al* studied nearly 500 isolates of *H influenzae* from children with invasive *H influenzae* disease and compared these with isolates from children with *H influenzae* respiratory disease and from well children.[24] Invasive disease was predominantly associated with serotypable strains, which were usually biotype I. Respiratory isolates from children with *H influenzae* respiratory infection and acute otitis media were frequently non-serotypable, biotype I strains. Respiratory isolates from well children were non-serotypable; a minority (8%) were biotype I.

The findings of Long *et al* regarding middle ear isolates differed from those of De Maria *et al*, who found that biotype II was the most common biotype isolated from this site.[25]

Others have tried to establish whether certain biotypes are more likely than others to produce β lactamase. Ampicillin resistance was less frequent among biotype I isolates than other biotypes in the study of Long *et al*. Watson and colleagues found that half of the biotype V strains that they examined produced β lactamase.[26] However, Holmes *et al* reported no correlation between β lactamase production and biotype.[27]

In conclusion, although many children with *H influenzae* vulvovaginitis responded well to treatment, a substantial minority (14 of 32) had recurrent symptoms, some associated with repeat *H influenzae* infection. Antibiotic resistance was not a problem in the clinical management of children, but amoxicillin resistance was seen in two children after treatment. Biotype II was the most common biotype causing infection at this site.

Take home messages

- *Haemophilus influenzae* is a common cause of vulvovaginitis in prepubertal girls
- Girls with *H influenzae* vulvovaginitis are at risk of recurrent symptoms
- Biotype II is the most common biotype associated with infection at this site
- Antibiotic resistance was not a problem in the clinical management of children, but amoxicillin resistance was seen in two children after treatment

Authors' affiliations
R A Cox, Department of Microbiology, Kettering General Hospital NHS Trust, Rothwell Road, Kettering, Northants NN16 8UZ, UK
M P E Slack, PHLS Haemophilus Reference Unit, Department of Microbiology, Level 6/7, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

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H Holzel, P van Diest