Changes in periurethral microflora after antimicrobial drugs

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Abstract

The periurethral flora was examined in 18 girls by use of a quantitative sampling method before, during, and three weeks after treatment with antibiotics for upper respiratory tract infections. Eight girls received amoxicillin. In five of them the anaerobic flora showed a reduction in total counts and in numbers of different species, and all eight girls got a heavy colonisation with enterobacteria during treatment. Three weeks after treatment the anaerobic and aerobic flora had reversed to the pretreatment composition. In 10 girls treated with trimethoprim-sulphamethoxazole the anaerobic flora remained unaffected and no enterobacterial overgrowth was registered during the study period. We propose that antibiotics could be one among several factors involved in the pathogenesis of urinary tract infection, by suppression of the anaerobic microflora and promotion of the colonisation with enterobacteria.

The periurethral microflora in healthy females is dominated by anaerobic bacteria with only scanty growth of aerobic Gram negative rods.[1 2] In females prone to recurrent urinary tract infections, the periurethral region is often colonised with uropathogens, even during infection free intervals.[2–4] A disturbed anaerobic microflora is also found.[5] The normal flora on various body surfaces provides a natural defence against colonisations with new potentially pathogenic bacteria, that is, 'colonisation resistance'.[6–8] Antibiotics may disturb the colonisation resistance and thereby facilitate the development of infections.

The present study is part of a project investigating factors involved in the pathogenesis of urinary tract infection, and examines the effects of antibiotics on the periurethral microflora in girls treated for upper respiratory tract infections and with no history of urinary tract infection. The aims were to investigate the effects on the predominant anaerobic flora and the concomitant effects on colonisation with Gram negative aerobic bacteria.

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Subjects and methods

SUBJECTS

Eighteen prepubertal girls (aged 3–13 years, median 4 years) were included in the study. They all attended the emergency ward for otitis media and upper respiratory tract infections, requiring antibiotic treatment. None had a history of urinary tract infection or had been treated with antibiotics during the three month period before entering the study. Informed parental consent were given in all cases.

STUDY DESIGN

Ten girls were treated with trimethoprim-sulphamethoxazole (6 mg trimethoprim and 40 mg sulphamethoxazole/kg/day) and eight girls with amoxicillin (50 mg/kg/day) orally for five to seven days. Sampling of periurethral bacteria was performed immediately before treatment, on the last day of medication, and three weeks after termination of the antibiotic course.

METHODS

The aerobic and anaerobic periurethral flora were examined by a quantitative sampling method.[1] A 5 ml plastic syringe filled with sterile 12% gelatin (Difco), was cut at the needle end of the syringe and the gelatin part was pressed against the urethral orfice. A 2 mm slice of gelatin with the bacteria bearing surface (about 1 cm^2) was cut off and dissolved in 1·8 ml prereduced anaerobic buffer solution. All samples were transported to the laboratory in an anaerobic jar (GasPac System, Becton and Dickinson), and further handled within 60 minutes after collection.

To cultivate the aerobic and anaerobic flora the gelatin was dissolved and serial dilutions (1/10, 1/100, 1/1000) were made. Portions (0·1 ml) of the gelatin sample and from each dilution step were spread onto blood agar plates and incubated in an anaerobic jar at 37°C for five

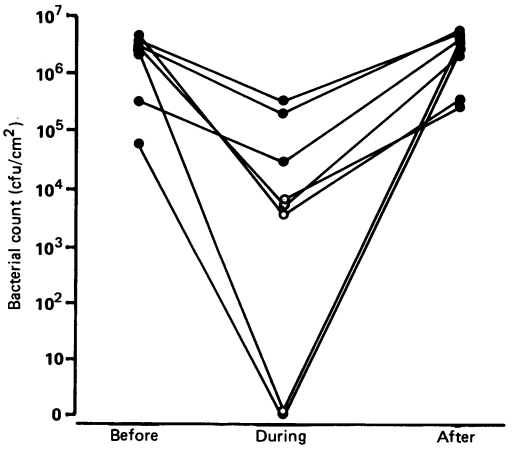

Figure 1 The periurethral anaerobic microflora in eight girls before, during, and three weeks after treatment with amoxicillin (○=growth of ≤2 species).

days. A parallel cultivation for estimation of aerobic bacteria was performed on cysteine-lactose-electrolyte deficient and hematin agar plates incubated aerobically at 37°C for 18 hours.

To quantitate and identify the micro-organisms total counts and counts of different organisms based on colony morphology were registered. The three predominant colony types growing on the anaerobic media were selected and one colony from each of them was isolated and incubated anaerobically and aerobically to test air tolerance. Further identification of each anaerobic isolate was performed by Gram staining, gas-liquid chromatography, and biochemical tests.[9] Total aerobic bacterial counts were estimated and all isolates were Gram stained and identified by standard laboratory procedures.[10]

The study was approved by the ethics committee, Södersjukhuset, Stockholm.

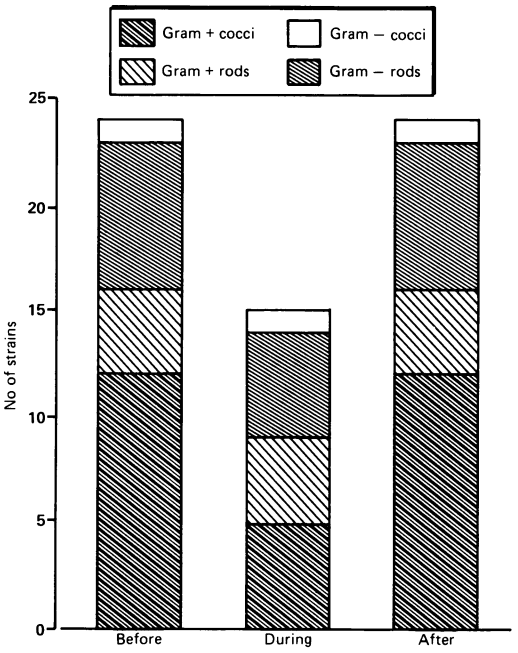

Figure 2 Gram staining properties of anaerobic bacteria in periurethral samples obtained before, during, and three weeks after treatment with amoxicillin.

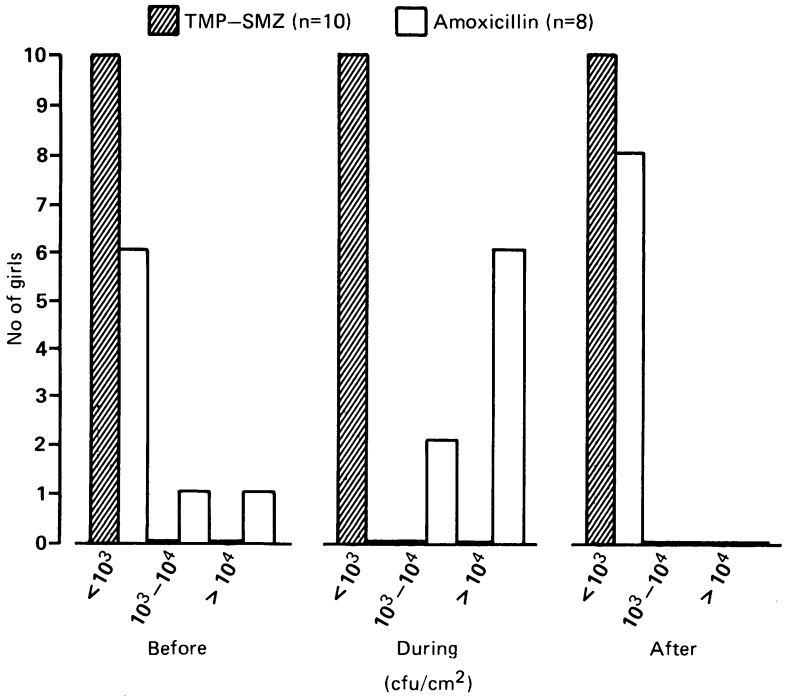

Figure 3 The aerobic, Gram negative periurethral flora before, during, and three weeks after treatment with amoxicillin and trimethoprim-sulphamethoxazole (TMP-SMZ).

Results

TREATMENT WITH AMOXICILLIN

Total counts of periurethral anaerobic bacteria/cm^2 in samples obtained before, during, and three weeks after treatment with amoxicillin is shown in fig 1. Before treatment all eight girls showed total bacterial counts ranging between 10^4-10^7 colony forming units (cfu)/cm^2. Five to seven different types of micro-organisms were registered in each sample. During antibiotic treatment total bacterial counts were reduced >3 log cycles in five of eight girls. In these five girls, a reduced number of colony types was also found. In three samples only two different species were recovered, and in the remaining two no anaerobes could be isolated. However, the proportions of different types of micro-organisms remained unaffected when grouped according to Gram staining properties (fig 2). Three weeks after treatment, total counts and numbers of different micro-organisms were similar to that before start of treatment (fig 1). Colonisation with enterobacteria during amoxicillin treatment is shown in fig 3. Before treatment two of eight girls harboured moderate numbers of enterobacteria. During treatment a dense growth with enterobacteria was registered in all eight girls (six *Escherichia coli* strains and two klebsiella strains). Three weeks after termination of treatment no enterobacteria could be recovered from any of the eight girls.

TREATMENT WITH TRIMETHOPRIM-SULPHAMETHOXAZOLE

In the group treated with trimethoprim-sulphamethoxazole total counts of anaerobic bacteria before treatment ranged between 10^4–10^7 cfu/cm^2 (fig 4). During and after treatment no major changes were registered in total bacterial counts or numbers of different micro-organisms. All 10 girls were free from aerobic enterobacterial colonisation throughout the study period (fig 3).

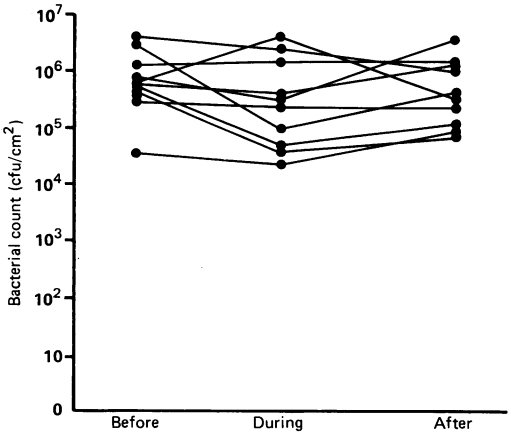

Figure 4 The periurethral anaerobic microflora in 10 girls before, during, and three weeks after treatment with trimethoprim-sulphamethoxazole.

Discussion

The present study showed that treatment with amoxicillin reduced the normal periurethral anaerobic flora and promoted colonisation with Gram negative enterobacteria, whereas trimethoprim-sulphamethoxazole did not influence the normal periurethral flora. Colonisation of the periurethral region is a first step in ascending urinary tract infection.[2 3] The basic defect responsible for this lowered colonisation resistance against uropathogens is still unknown. Among host factors an abnormal anaerobic microflora may be involved, and consequently, antibiotics might interact in the development of urinary tract infection by suppression of the normal microflora. Clinical support for this hypothesis is the finding that children with pyelonephritis had been treated with antibiotics for respiratory tract infections before onset of urinary tract infection significantly more often than a control group.[11]

There are only few previous studies reporting on the effect of antibiotics on the periurethral flora. In monkeys local flushing with amoxicillin facilitated colonisation with uropathogenic *E coli* originating from the intestinal flora.[12 13] Two different studies on women treated with amoxicillin for urinary tract infection showed periurethral colonisation with resistant enterobacteria and suppression of the indigenous lactobacillus population, respectively.[14 15]

The present study shows a heavy colonisation with enterobacteria coinciding with a suppressed anaerobic periurethral flora during amoxicillin treatment, agreeing well with these previous studies. Our findings are also consistent with studies of other microbiotas where eradication of the normal floras by antibiotics was rapidly followed by overgrowth with potential pathogens.[16–19] We have recently shown that anaerobic periurethral bacteria in healthy girls were highly susceptible in vitro to several commonly used antibiotics, including ampicillin, whereas a majority of strains were relatively resistant to trimethoprim-sulphamethoxazole.[20] Our in vivo data, with reduction of the anaerobic flora during amoxicillin treatment and an unaffected microflora during trimethoprim-sulphamethoxazole treatment, accord well with these previous in vitro data. Apparently, the impact on the anaerobic flora varies with different antibiotics.

This study thus supports the concept that a disturbed periurethral anaerobic microflora is associated with a pathological colonisation with enterobacteria, which may be a factor of importance in pathogenesis of urinary tract infection. However, it is still a matter of question what part preceding treatment with antibiotics plays for the emergence of urinary tract infection, as the disturbances of the periurethral flora caused by antibiotics in this study differ from findings in individuals prone to urinary tract infection in certain important respects. The periurethral flora rapidly became normal in these healthy girls after withdrawal of antibiotic treatment, indicating a basic inherent stability of the periurethral ecosystem. In individuals prone to urinary tract infection on the other hand a more persistent pathological colonisation is present, eventually leading to an ascending infection. In our healthy individuals there was a reduction in total counts of anaerobic bacteria, but the proportion of different types of micro-organisms did not change during antibiotic treatment. This contrasts with previous studies on girls prone to urinary tract infection, which showed a disturbed composition of the anaerobic flora.[4] The basic defect explaining the persistent enterobacterial periurethral colonisation in females prone to urinary tract infection thus remains unknown.

Hypothetically, in a population with a lowered resistance against urinary tract infection, the adverse effect of antibiotics on the periurethral flora could be a triggering factor in the development of ascending urinary tract infection.

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