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A theoretical and experimental analysis of bacterial growth in the bladder

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Summary

A mathematical model of human micturition dynamics and bacterial growth predicts the population growth rate required for a bladder infection to become established in the absence of adhesin-mediated surface growth. *Escherichia coli* strains isolated from the urinary tract have significantly higher *in vitro* growth rates in urine than strains isolated from the intestinal flora. The results suggest that, for *E. coli* isolated from the urinary tract, adhesin-mediated surface growth may not be required for infections to become established and persist. The growth-rate differences observed between urinary tract and intestinal isolates suggests that the ability to survive and efficiently utilize the resources available in urine is an important adaptation for *E. coli* inhabiting the urinary tract.

Introduction

Escherichia coli is the species responsible for most bacterial urinary tract infections in humans (Svanborg-Eden, 1978). Although the commensal intestinal flora have been implicated as the primary reservoir of infection (Bollgren and Winberg, 1976; Caugant, 1983; Turck *et al.*, 1962; Vosti *et al.*, 1964), certain properties are observed among urinary tract strains of *E. coli* that are not prevalent among intestinal isolates. Urinary tract isolates have higher frequencies of O and K antigens, haemolysin production, aerobactin release, serum resistance and adhesins (Arthur *et al.*, 1989; Dootson *et al.*, 1973; Glynn *et al.*, 1971; Kaijser *et al.*, 1977; Orskov *et al.*, 1971; Plos *et al.*, 1989). Of these properties, adhesins are thought to be critical, the possession of which enables *E. coli* to colonize the urinary tract (Arthur *et al.*, 1989; Plos *et al.*, 1989; Svanborg-Eden, 1978). There are several different adhesin proteins expressed in *E. coli*, such as those encoded by the *pap* (pyelonephritis-associated pili), *prs* (pap-related sequence), *pilC*, and *afal* operons (Arthur *et al.*, 1989; Kallenius *et al.*, 1982; Labigne-Roussell and Falkow, 1988; O'Hanley *et al.*, 1985; Plos *et al.*, 1989).

The potential role of bladder hydrodynamics on the elimination of bacteria from the bladder has long been recognized (Boen and Sylwester, 1965; Cox and Hinman, 1961; Dugdale, 1969; Hinman, 1968; Hinman and Cox, 1966; Mackintosh *et al.*, 1975a,b; O'Grady and Cattell, 1966). It has been suggested that without adhesin-mediated surface growth *E. coli* could not overcome the losses due to micturition, and hence could not establish populations in the urinary tract (Arthur *et al.*, 1989; Reid and Sobel, 1987; Svanborg-Eden, 1978). Implicit in this hypothesis is the suggestion that the growth rate of *E. coli* in urine is insufficient to overcome these losses.

The purpose of this study was to determine if *E. coli* is able to colonize the human bladder in the absence of adhesion. First, we present a mathematical model of human micturition dynamics and bacterial growth. The model represents a 'worst-case scenario'; it assumes that there is no surface growth. The model predicts the growth rate required for the establishment and maintenance of bacterial populations in the bladder in the absence of adhesion. We then determine the *in vitro* growth rates, in urine, of *E. coli* isolated from the intestinal flora and urinary tract infections.

Results

A model of bacterial growth in the bladder

The model predicts the fate of a small number of bacteria that have succeeded in reaching the bladder. We assume that the bladder fills at a constant rate λ (ml h^{-1}). Micturition occurs when the amount of urine in the bladder reaches a fixed volume, V_x (ml). Following micturition there is a residual amount of urine remaining in the bladder, V_n (ml). Thus the volume of urine in the bladder at time t through the period between micturitions is

$$V(t) = V_n + \lambda t.$$

We assume that the bacteria in the urine exist as randomly distributed planktonic cells; there is no surface growth. We also assume that the bacterial population

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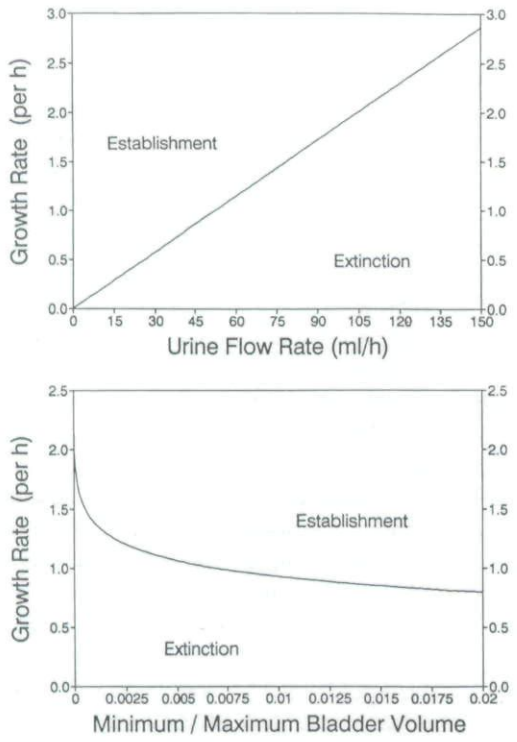

Fig. 1. The growth rate extinction–establishment boundary for bacterial populations as a function of human micturition dynamics. If the bacterial growth rate lies below the boundary the bacteria will fail to establish; if above, the population will establish and be maintained.
A. Effect of the rate of urine production (λ) on the boundary. Residual bladder volume (V_n) was 1 ml and maximum bladder volume (V_x) was 300 ml.
B. Effect of the ratio of residual:maximum bladder volume on the boundary. The flow rate (λ) was 60 ml h^{-1}.

grows at a constant rate ψ (h^{-1}). The validity of this assumption in the context of the model is demonstrated in a later section of this paper. In the time between micturitions, the number of bacteria in the bladder is given by

$$N(t) = N_0 \exp(\psi t),$$

where N_0 is the number of bacteria present in the residual urine. These two equations specify our model, and with these we can determine the condition for establishment. That is, given the dynamics of urine flow through the bladder, we can ask what growth rate the bacterial population must exhibit in order to establish and persist in the bladder, assuming no adhesion.

The interval T (h) between micturition events is

$$T = (V_x - V_n)/\lambda.$$

Let N_k be the number of bacteria remaining in the bladder after the kth micturition. Then

$$N_{k+1} = (V_n/V_x)N_k \exp(\psi T).$$

The bacterial population is growing if, and only if, $N_{k+1} > N_k$, that is when

$$\exp(\psi T) > V_x/V_n, \text{ or } \psi > \ln(V_x/V_n)/T.$$

The establishment condition states that the number of bacteria produced between micturition events must exceed the number that are lost due to micturition. The number of bacteria produced is a function of their growth rate and the time available for growth to occur. The duration of the growth period is determined by the rate of urine production and the amount of urine that accumulates in the bladder between micturitions.

Figure 1 presents growth rate (ψ) extinction-establishment boundaries as a function of flow rate (λ), and maximum (V_x) and residual (V_n) bladder volumes. If the bacterial population has a growth rate which lies above a boundary it will establish a population. When the population growth rate lies below a boundary the bacterial population will not increase its numbers and will eventually be washed out of the bladder. To infer the growth rate required for establishment of an *E. coli* population in the human bladder requires a knowledge of typical micturition dynamics in humans.

In healthy adults, normal urine production ranges from 1–2 litres per day, resulting in flow rates (λ) that span 40–80 ml h^{-1} (Mundy *et al.*, 1984). Micturition results in the release of 200–400 ml of urine (V_x) (Mundy *et al.*, 1984). The volume (V_n) of urine remaining in the bladder following micturition is about 1 ml (Shand *et al.* 1968).

Figure 1a presents the growth-rate boundary for a range of flow rates while assuming that 300 ml of urine is released at micturition (V_x) and 1 ml remains (V_n). This figure illustrates that the growth rate required for establishment is a linear function of the rate of urine production (λ). Figure 1b demonstrates the effect of residual volume (expressed as the fraction V_n/V_x) on the growth-rate boundary. The flow rate was 60 ml h^{-1} and V_x was 300 ml. This figure shows that, except for very small values of V_n, a change in residual volume has less effect on the growth rate required for establishment than an equivalent per cent change in the urine flow rate (λ).

Rates of urine production (λ) and the amount of urine produced at micturition (V_x) normally vary for a number of reasons. To investigate the effect of this variation on the establishment condition, modifications were made to the basic model. The modifications consisted of allowing variation in either the amount of urine voided at micturition (V_x), or the rate of urine production (λ) during the intervals between micturitions. Values of V_x or λ were selected randomly from a normal distribution with a given mean and standard deviation. The simulations were initiated with 100 cells present in the bladder. Establishment occurred if the number of bacteria grew to exceed 10^7 cells, and extinction occurred if the population declined to less than 10 cells. The parameter values used were: $V_n = 1$ ml, $V_x = 300$ ml and $\lambda = 60$ ml h^{-1}. In simulations examining the effect of variation in λ, 60 ml h^{-1} represented the mean flow rate, while V_x was held constant at 300 ml. The

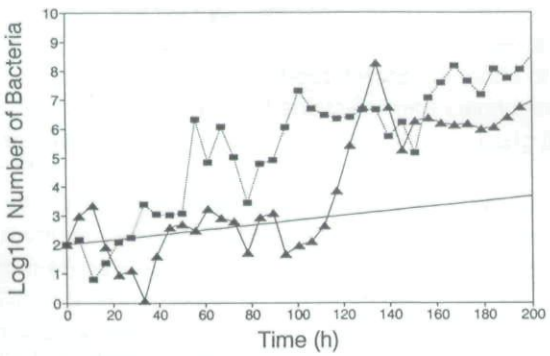

Fig. 2. Simulations of the model to illustrate the effect of variation in the volume of urine voided at micturition on the change, through time, in the number of bacteria (N_k) in the bladder. In these simulations the amount of urine produced at micturition (V_x) was different for every micturition event. The volumes were randomly selected from a normal distribution having a mean of 300 ml and a S.D. of 60 ml. Other parameter values were: $V_n = 1$ ml, $\lambda = 60$ ml h^{-1}, $\psi = 1.0$ h^{-1}. Symbols represent the results of the simulations and the solid straight line the solution of the deterministic model.

opposite was true in simulations investigating the effect of variation in V_x. The effects of three levels of variation (S.D.) in λ or V_x on establishment success were examined. For each level of variation, simulations were carried out for different values of a constant bacterial growth rate. For every growth rate/standard deviation combination, 1000 simulations were run, and the number of times the bacterial population established or went extinct was recorded.

Figure 2 presents the results of two simulations in which V_x was allowed to vary, and illustrates the change in the number of bacteria (N_k) through time relative to the predictions of the deterministic model. In these simulations, bacterial numbers fluctuated considerably but in both cases the populations met the establishment criterion. The results of the complete simulation analysis are presented in Table 1. Given the parameter values used in these simulations, the model predicts that the bacteria should establish and maintain a population in the bladder provided that the growth rate exceeds 1.145 h^{-1}.

Variation in the rate of urine production (λ) had no systematic effect on the growth rate required for establishment as defined by the deterministic model. Populations were more likely to establish when the growth rate exceeded the rate specified by the model (1.145 h^{-1}), and were more likely to become extinct when the growth rate was less than that required. For example, when the growth rate was 1.15 h^{-1}, the majority of simulations resulted in the population becoming established, regardless of the degree of variation in the rate of urine production.

Variation in the amount of urine voided (V_x) had the effect of slightly increasing the growth rate required for establishment. When the standard deviation in V_x was 60 or 120 ml, a growth rate of 1.2 h^{-1} was required for more than 50% of the simulations to result in establishment.

The results show that random variation in λ or V_x can result in establishment even when the growth rate is less than required and that this occurs more often as the variation in λ or V_x increases. However, in all cases the probability of establishment increases as the growth rate increases. Variation in the amount of urine voided has a greater effect on the frequency of establishment than does variation in the rate of urine production. In these simulations the rate of urine production affects the time available for bacterial growth (T), while variation in the amount voided affects both the time available for growth, and the proportion of the bacterial population lost at micturition (V_n/V_x).

In addition to random variation, the rate of urine production or the frequency of micturition vary in a periodic manner. A series of modifications were made to the basic model that mimicked these periodic effects. For example, micturition often does not occur during the night. Simulations were done that assumed a constant rate of urine production ($\lambda = 60$ ml h^{-1}) and mimimum bladder volume ($V_n = 1$ ml). The intervals between micturitions were varied such that there was one long interval (T) between voidings, followed by several shorter intervals — for example, one 8 h interval followed by four 4 h intervals. By holding the flow rate constant and varying the frequency of micturition we are varying the volume of urine voided at micturition (V_x). All of these simulations had

Table 1. The results of simulations exploring the effect of variation in the rate of urine production (λ) or the amount of urine voided (V_x) on the establishment success of bacteria in the bladder.

	Establishment Success (%)		
Rate of urine production			
	λ, Mean ±S.D.		
Growth rate	60±6	60±12	60±24
1.00	0.0	0.0	28.9
1.10	0.2	23.7	50.3
1.15	75.9	67.0	59.6
1.20	99.6	88.3	68.6
1.25	100.0	97.0	77.6
1.30	100.0	99.3	83.8
Volume of urine voided			
	V_x, Mean ±S.D.		
Growth rate	300±30	300±60	300±120
1.00	0.0	0.1	0.1
1.10	0.0	0.6	10.4
1.15	62.1	35.0	28.9
1.20	99.6	84.3	51.8
1.25	100.0	95.5	63.3
1.30	100.0	98.7	71.1

The frequency of establishment was based on the results of 1000 simulations. Parameter values used in the simulations were: $V_n = 1$ ml, $V_x = 300$ ml, and $\lambda = 60$ ml h^{-1}.

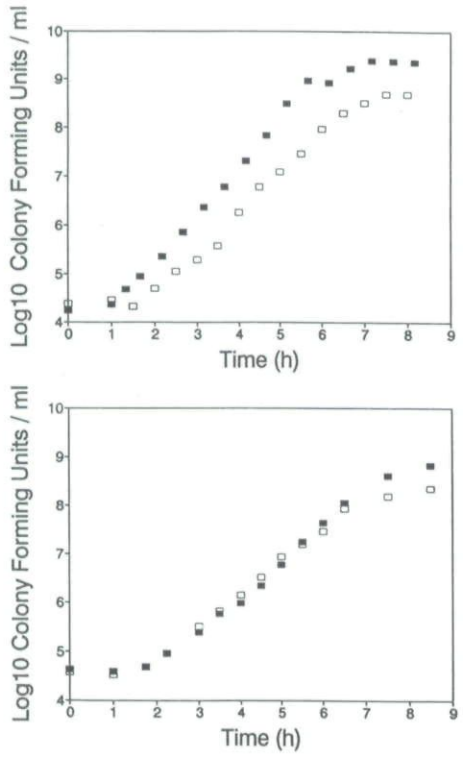

Fig. 3. The growth of urinary tract isolates under different culture conditions.
A. Growth of UTI strain 18 in urine (□) and in broth (■). The growth rate in urine was 1.93 h^{-1}, and in broth, 2.27 h^{-1}.
B. Growth of UTI strain 14 under conditions of good agitation and aeration (■) (growth rate = 1.64 h^{-1}), and no agitation and poor aeration (□) (growth rate = 1.57 h^{-1}). Each point represents the average of three replicates.

similar outcomes (results not presented). There was either no systematic effect on the growth rate (ψ) required for establishment, or establishment occurred when the growth rate was smaller than the rate predicted by the basic model.

E. coli *growth rates*

Strains isolated from urinary tract infections grow well in human urine. Figure 3a shows the change in cell density of strain 18 in urine and Luria broth. The exponential-phase growth rate in urine was 1.93 h^{-1} and in broth was 2.27 h^{-1}. In urine, the stationary-phase density averaged 9.9×10^8 cells ml^{-1}, and 1.2×10^9 cells ml^{-1} in broth.

No difference in the exponential phase growth rate of UTI strain 14 could be detected when the cells were grown in well-aerated urine (1.69 h^{-1}), relative to cultures that were poorly aerated (1.57 h^{-1}) (Fig. 3B). The cell-density curves diverged as stationary phase was approached, suggesting that the growth rate declines in the poorly aerated cultures once high cell densities are achieved. This experiment was repeated, using an initial density of about 10^6 cells ml^{-1}, and similar results were obtained.

In urine, the growth rates of 12 *E. coli* clones isolated from urinary tract infections ranged from 1.4–2.2 h^{-1} and lag-phase durations spanned 1–3 h (Table 2). The growth rates of 10 intestinal clones ranged from 0.9–1.6 h^{-1} and their lag-phase durations spanned 2.0–4.5 h (Table 2). The average growth rate (1.91 h^{-1}) of the urinary tract clones was significantly higher than the average growth rate (1.2 h^{-1}) of the intestinal isolates ($F_{(1,64)} = 143.2$, $p<0.001$). This difference in growth rate was not obtained when the growth medium was Luria broth (Table 2); the average growth rate of both the urinary tract and intestinal clones was 1.9 h^{-1}.

Discussion

We have presented a mathematical model of micturition dynamics and bacterial growth. For bacteria to successfully establish a population, without surface growth, they must grow at a rate determined by the rate of urine production (λ), the amount of urine released at micturition (V_x), and the volume of urine remaining in the bladder following micturition (V_n).

The model is a deterministic one; in reality, rates of

Table 2. Growth parameters of *E. coli* in human urine and Luria broth.

		Urine		Luria Broth	
Strain		Growth rate (h^{-1})	Lag phase (h)	Growth rate (h^{-1})	Source
UTI	Type[a]				
14	C	1.99	1.0	1.78	Arthur *et al.* (1989)
17	C	2.16	2.0	2.11	Arthur *et al.* (1989)
18	P	1.93	1.5	2.02	Arthur *et al.* (1989)
23	P	1.93	1.5	2.07	Arthur *et al.* (1989)
36	C	2.08	1.0	1.99	Arthur *et al.* (1989)
45	C	1.91	1.5	1.77	Arthur *et al.* (1989)
175	S	2.04	1.5	1.86	Arthur *et al.* (1989)
475	S	2.23	3.0	1.93	Arthur *et al.* (1989)
70	A	1.92	1.5	1.81	Caugant (1983)
72	A	1.91	1.5	1.93	Caugant (1983)
52	C	1.53	1.5	1.81	Caugant (1983)
67	P	1.36	1.5	1.80	Caugant (1983)
Intestinal					
4		1.23	2.0	1.82	Caugant (1983)
10		1.04	2.5	1.89	Caugant (1983)
14		1.55	3.5	2.07	Caugant (1983)
20		1.41	2.0	2.07	Caugant (1983)
29		1.16	4.5	1.84	Caugant (1983)
42		1.16	2.0	1.67	Caugant (1983)
51		1.45	3.0	1.85	Caugant (1983)
55		0.98	3.0	1.64	Caugant (1983)
140		0.92	4.5	1.87	Arthur *et al.* (1989)
141		1.23	3.0	1.95	Arthur *et al.* (1989)

a. Patient diagnosis: C, cystitis; P, pyelonephritis; S, urosepsis; A, asypmtomatic.

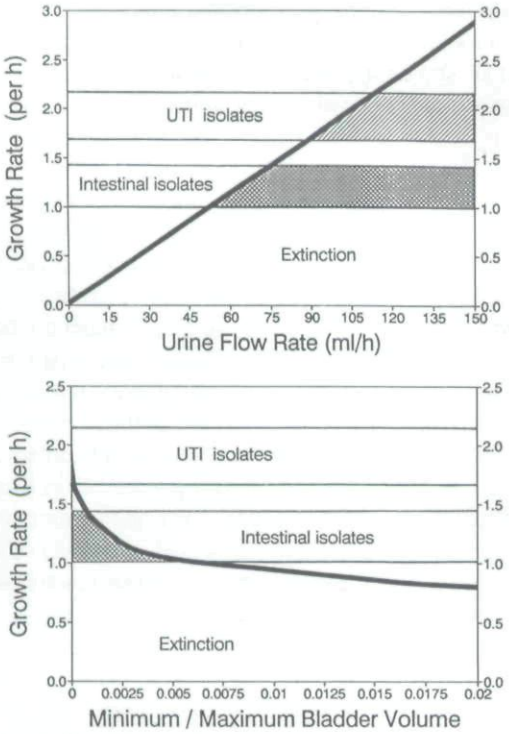

Fig. 4. Growth rates of urinary tract and intestinal strains in relation to the extinction–establishment boundary. Micturition parameter values defining the boundary are as in Fig. 1. The parallel lines bound the mean growth rate ±1.0 S.D. of the urinary tract and intestinal isolates. The shaded region indicates the range of flow rates (A) or minimum/maximum bladder volumes (B) where the model predicts the bacteria would fail to establish a population in the bladder.

urine production and the volume of urine released at micturition vary for many reasons. Simulation results demonstrate that incorporating random or periodic variation in the rate of urine production (λ) or the volume of urine voided at micturition (V_x) has a minor effect on the establishment condition defined by the deterministic model.

Our model does not predict the equilibrium number of bacteria in the bladder, i.e. the value of N_{k+1}, when $N_{k+1} = N_k$. The system described by the model is not self-regulating. No combination of growth rate, flow rate or bladder volumes will result in a stable population. The model predicts that the number of bacteria in the bladder will either increase without limit, or decline until extinct. This result demonstrates that to achieve an equilibrium population requires some form of resource limitation. The equilibrium density would depend on the dynamics of micturition and the functional relationship between the growth rate and resource concentration. Further empirical work is required to describe the relationship between the population growth rate and the concentrations of the resources upon which the bacteria are growing.

Although the equilibrium density will depend on resource concentration, the establishment condition is essentially independent of the relationship between the growth rate and resource concentration. Establishment success is determined by the growth rate during the early phase of colonization. During this stage, bacterial density is low and resources enter the bladder at a rate greater than the rate at which the bacteria can consume them. Once the bacteria are at a density sufficient to cause significant resource depletion, thereby reducing their growth rate, successful establishment has occurred. Our assumption that the bacterial growth rate is constant during the early phase of colonization is supported by the empirical evidence. When urine is inoculated with about 10^4 cells ml^{-1} the growth rate remains constant until cell density has increased by more than four orders of magnitude, an increase that requires about 4 h (Fig. 3B).

Previous studies have demonstrated the importance of bladder hydrokinetics on clearing bacterial infections from the bladder (Boen and Sylwester, 1965; Cox and Hinman, 1961; Dugdale, 1969; Hinman, 1968; Hinman and Cox, 1966; Mackintosh *et al.*, 1975a,b; O'Grady and Cattell, 1966). However, our interest was in determining if the growth rate of *E. coli*, in urine, is sufficient to overcome the mortality associated with micturition. In normal adults, typical values for the parameters of the model ($\lambda = 60$ ml h^{-1}, $V_x = 300$ ml, $V_n = 1$ ml) suggest that *E. coli* attempting to establish a population in the human bladder must grow at a rate that exceeds 1.2 h^{-1}. Is the growth rate of *E. coli* sufficient to allow it to colonize the bladder in the absence of surface growth?

The empirical results suggest that the *E. coli* clones isolated from urinary tract infections would be successful in establishing a population in the bladder. Figure 4 presents the extinction–establishment boundaries assuming typical micturition parameter values. The parallel lines encompass the range defined by the average growth rate ±1.0 S.D. Given the observed growth rates, these strains would fail to establish only if normal daily urine production exceeded 2 l d^{-1} (Fig. 4a), or if the residual volume of urine was unrealistically low (Fig. 4b).

Less clear is the fate of the clones isolated from the intestinal flora attempting to colonize the bladder. Their growth rates result in significant regions of extinction (Fig. 4). Although it cannot be concluded that the *E. coli* clones isolated from intestinal flora would fail to establish stable populations in the bladder, their low growth rates and long lag phases do indicate that the clones from this source would be less likely to become established than strains isolated from the urinary tract.

Our model represents a 'worst-case scenario' in that it assumes no surface growth. Any surface growth, regardless of its nature, would result in a smaller fraction of the population being lost during micturition and, as a result, the growth rate required for establishment would be lower. However, even in the worst-case situation, the empirical results suggest that the growth rates of *E. coli*

clones isolated from urinary tract infections are more than sufficient to overcome losses due to micturition even without adherence to the bladder wall. *E. coli* isolated from faecal flora are significantly less likely to successfully establish infections in the absence of surface growth.

Although it is possible that our *in vitro* growth-rate estimates may not accurately reflect the growth rate of *E. coli* under *in vivo* conditions, such a discrepancy does not seem to result from the physical conditions found in the bladder. The growth-rate estimates were made under culture conditions quite different from those in the bladder, i.e. well agitated and aerated. However, when the cells were cultured under conditions of very poor aeration and no agitation, the estimated exponential-phase growth rate was less than 8% lower than when the cultures were well aerated. Successful establishment depends on the growth rate observed when cell density is low. As a result, the observed divergence in the cell-density curves as the cultures approached stationary phase is not significant (Fig. 3b).

The urine samples used for the growth-rate estimates were collected on different days, at different times, and from both sexes. Differences between urine samples did not appear to have a substantial effect. The growth rate of urinary tract strain 14 was measured on three occasions, namely in urine from a female, and in two samples collected from the same male: the average growth rates were 1.98, 1.69, and 1.78 h^{-1}, respectively. The growth rate of strain 36 was also estimated twice, in urine from a female (1.96 h^{-1}) and a male (2.08 h^{-1}). Urine osmolality and pH can influence *E. coli* growth rates, factors which depend on sex, diet, and the time at which the urine is produced (Asscher *et al.*, 1966; Kaye, 1968). However, the normal ranges of pH and osmolality in healthy adults, particularly women, are such that they do not significantly influence *E. coli* growth rates (Asscher *et al.*, 1966; Kaye, 1968).

There have been several studies investigating *E. coli* growth in urine (Asscher *et al.*, 1966; Kaye, 1968; Stamey and Mihara, 1980). For a number of reasons, meaningful comparisons of the results reported here and those of previous studies are not possible. In some studies the urine was filter-sterilized, centrifuged, or stored prior to use. Often, a single urinary tract or laboratory strain of *E. coli* was used in the study. Another problem with some studies was that the experiments were started at a high cell density (10^7), a density close to the point at which the growth rate starts to decline (Fig. 3a). However, rough qualitative comparisons suggest that the growth rates reported in previous studies fall within the range of growth rates we observed for the urinary tract strains.

The difference observed between the average growth rates, in urine, of *E. coli* isolated from urinary tract and intestinal habitats does not appear to reflect intrinsic growth-rate differences between these two groups of strains. When grown in Luria broth, the average growth rates of strains isolated from the two habitats were the same. Kaye (1968) also observed that faecal isolates of *E. coli* tended to grow more poorly in urine than did urinary tract isolates.

E. coli strains isolated from urinary tract infections normally originate from the intestinal flora (Bollgren and Winberg, 1976; Caugant, 1983; Svanborg-Eden *et al.*, 1989; Turck *et al.*, 1962; Vosti *et al.*, 1964). Therefore, the growth-rate differences do not so much reflect the habitat from which they were isolated, but instead whether they had experienced urine as a growth medium prior to being isolated. The intestinal strains had perhaps never, or at least not recently, been exposed to urine. We do not know the nature of the adaptation that permits *E. coli* strains previously exposed to urine as a growth medium to grow 150% faster than intestinal isolates. However, there are at least two possible explanations for these results — one physiological and one genetic.

The urinary tract isolates may grow better in urine because, having been previously been exposed to urine, they have been induced in some manner that permits faster growth in urine. However, it is unlikely to be a case of conventional induction. The effect would have had to persist during the procedures involved in isolating and identifying the strains, their long-term storage in stabs or at –70°C, subsequent subculturing, and, finally, several growth cycles on or in Luria broth prior to our growth-rate experiments. Alternatively, the clones that grow well in urine may form a genetic subpopulation of the intestinal *E. coli* flora. The superior growth rate of this subpopulation would result in these strains being selected for in the bladder, and they would then become urinary tract isolates. If these strains represent a subpopulation of the intestinal flora, then in a large sample of intestinal isolates, we would expect to find strains that grow well in urine.

The results of our theoretical and empirical studies suggest that the growth rates of *E. coli* isolated from urinary tract infections are sufficient to overcome the losses due to micturition and that these clones have the ability to establish a population in the bladder without adhesin-mediated surface growth. The growth rates of the clones isolated from faecal flora, strains which may have never experienced urine as a growth medium, are sufficiently low that the ability to adhere to the wall of the bladder would increase their likelihood of establishing populations.

What role do adhesins play in urinary tract infections? Adhesin-mediated surface growth is required for colonization of the upper urinary tract (O'Hanley *et al.*, 1985) and may also play an important role in allowing *E. coli* to ascend the urethra. In addition, adhesion is responsible

for some of the symptoms associated with infection. The results of this study do not allow us to conclude that surface growth is unneccessary for the establishment and persistence of *E. coli* populations in the human bladder. However, the predictions of the model together with the observed growth rates of clones isolated from urinary tract infections do suggest that surface growth is not required to explain the maintenance of *E. coli* populations in the bladder.

There have been many studies describing the nature and frequency of virulence determinants in *E. coli* causing urinary tract infections. Our results suggest that the ability to survive and efficiently utilize the resources available in urine is an important adaptation found in *E. coli* clones inhabiting the urinary tract. Further *in vivo* experimental studies are required in order to fully understand the role of adhesins in the dynamics of urinary tract infections.

Experimental procedures

Strains and media

The *E. coli* strains used in this study were isolated from the urinary and intestinal tracts of humans as part of epidemiological surveys (Arthur *et al.*, 1989; Caugant, 1983). From these two large collections 22 strains were selected. The urinary tract strains chosen represented isolates from patients diagnosed with asymptomatic infections, cystitis, urosepsis, and pyelonephritis.

Cells were cultured in urine or Luria broth. Urine was supplied by the authors and a colleague, and was from the first or second micturition of the day; it was collected under aseptic conditions and used within the hour. The absence of bacteria in the urine samples was confirmed by plating 0.1 ml of the sample on a Luria-broth plate.

Growth-rate determinations

The growth rates, in urine, of the 22 strains were estimated *in vitro* by monitoring the change in the density of colony-forming units over time. Fifty-millilitre Erlenmeyer flasks containing 20 ml of urine were inoculated with cells from overnight Luria-broth cultures at an initial density of about 10^4 cells ml^{-1}. The cultures were incubated at 37°C and shaken at 150 r.p.m. The flasks were sampled after 1 h, and then at half-hourly intervals. Cell densities were estimated by plating on Luria broth agar following serial dilution. For comparative purposes, the growth rate of one of the strains in Luria-broth was estimated in the same manner. The growth rates of all 22 strains were estimated in Luria broth by measuring the change in the optical density of cultures (675 nm). The initial density of these cultures was about 10^6 cells ml^{-1}. The cultures were incubated at 37°C and shaken at 150 r.p.m.

Under the culture conditions used in our growth-rate experiments the cells are thoroughly suspended and the cultures well aerated. Cells growing in the bladder may not be so well mixed and the concentration of dissolved oxygen will be lower. To confirm the validity of our growth-rate estimates, the growth rate of one strain was measured, in urine, under two culture conditions: low and high aeration. To achieve good aeration, the flasks were shaken at 200 r.p.m. and closed with foam stoppers. For the low-aeration treatment, the flasks were closed with rubber stoppers and not shaken. In addition, to minimize the amount of atmospheric oxygen dissolving in the urine, an alcohol flame was introduced into these flasks and allowed to extinguish itself. This step was performed prior to the start of the experiment, and at each sampling time. The flasks were briefly vortexed prior to being opened to remove a sample, as the cells had to be uniformly suspended in order for cell density to be estimated.

The growth rates reported are based on the average of three replicates. Statistical analyses were performed on untransformed data using analysis of variance.

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