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Adherence of Bacteria to Urinary Catheters

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Summary. The adherence of ^{3}H-labelled gram-negative bacilli to different urinary catheter materials was studied using an in vitro method. Adherence was found to be significantly less to siliconised rubber than to pure latex or teflon coated rubber. Adherence was altered by variations in incubation pH, time, and bacterial concentration; however, incubation temperature did not affect results. Adherence of bacteria to urinary tract catheters may be part of the pathogenesis of certain catheter-related infections. However, in the absence of controlled clinical studies the significance of these findings remains to be determined.

Key words: Enterobacteriaceae, Adherence, Urinary catheters, Adherence of bacteria.

Introduction

Adherence of bacteria to mammalian tissues is thought to play an early role in colonisation and pathogenesis of many infections [2, 5, 11, 13]. Similarly, attachment of microorganisms to acellular surfaces such as external layers of teeth has been implicated in the process by which caries and periodontitis develop [1, 12, 15].

Colonisation and infection of the urinary tract mostly by gram-negative bacilli in catheterised individuals are problems causing considerable morbidity and mortality [4, 8]. An investigation was undertaken to determine the extent of and differences between urinary catheter materials and in vitro bacterial adherence.

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Materials and Methods

Bacteria

Freshly isolated strains of *Escherichia coli* and *Klebsiella pneumoniae* representing the only growth from urine ($\geqslant 10^5$/ml) or blood, or dominant growth in sputum cultures were identified by the API system (Analytab Products, Plainview, NY, USA), subcultured once on trypticase soy agar (BBL, Becton Dickinson, Detroit, MI, USA) and stored at room temperature without further passage.

Bacteria were radiolabelled by incubation at 37 °C for 18 h in Eagle's minimal essential medium (Gibco, Grand Island, NY, USA); methyl-^{3}H-thymidine (New England Nuclear, Boston, MA, USA) with specific activity of 80.1 Ci/mmol was added to yield 5 μCi/ml. Organisms were collected by centrifugation and washed thrice in 0.05 M phosphate buffered saline pH 7.4 without calcium or magnesium (PBS). Bacteria were resuspended in PBS and their concentration was adjusted to 1×10^8/ml (unless otherwise noted) spectrophotometrically; bacterial concentrations were verified by quantitative cultures. This method is similar to one described in greater detail elsewhere (10).

Catheter Material

Commercially obtained urinary catheters of 2 mm wall thickness from several suppliers were cut into 15 x 5 mm strips. Catheter types studied included latex rubber, teflon coated latex and siliconised rubber.

Adherence Assay

On multiple occasions, duplicate strips of catheter material were incubated in 10 ml of bacterial suspensions for up to 90 min on a shaking apparatus at room temperature unless otherwise noted. After incubation, catheters were washed five times in PBS and then placed in 0.1 N NaOH (and vigorously shaken) for 1 h to remove adherent bacteria after which they were discarded. The final PBS wash and discarded catheters were shown not to contain counts per min (cpm) significantly above background. The NaOH solution was adjusted to pH 7 and xylene-surfactant based scintillation fluid was added for determination of cpm in a liquid scintillation counter; calculated counting efficiency was 38%. CPM of known quantities

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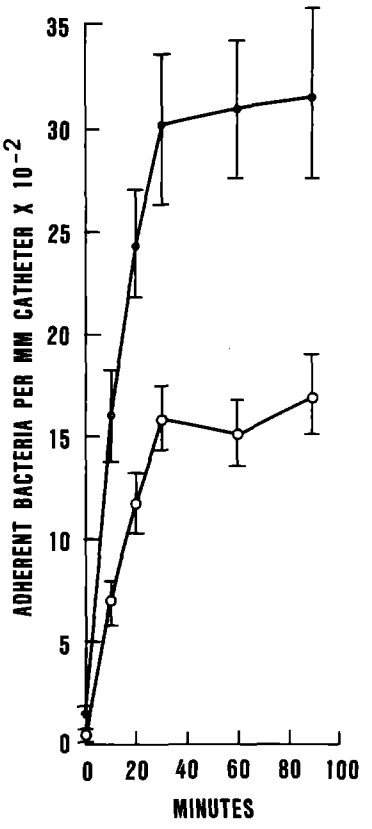

Fig. 1. Attachment of bacteria to urinary catheters as a function of time. 10^9 *Escherichia coli* (○—○) or *Klebsiella pneumoniae* (●—●) were incubated for various periods of time with latex rubber. I = ± 1 standard deviation

of each bacterial isolate were used for conversion of data to number of bacteria.

Statistical Methods

Means of duplicate determinations were treated as single datum points. Data are presented as the mean ± standard deviation of the mean. Standard t-test analyses and variance ratios of Fisher were used to determine statistical significance.

Results

Effect of Incubation Time, Temperature, and pH

Minimal attachment was observed when the catheter material was added to a bacterial suspension and then removed and washed immediately (time 0). Attachment increased in a non-linear manner with time; over 80% of the 90-min total was noted after 30 min incubation with most bacteria and catheter types tested. Representative data are depicted in Fig. 1. No significant difference ($0.3 < p < 0.5$) was noted after 30 min incubations at 4 °C or 39 °C compared with room temperature (about 24 °C). Attachment of 5 of 6 bacterial strains tested was significantly greater ($p < 0.05$) to latex and siliconised rubber materials at pH 5 compared with pH 7.4 and pH 9. Table 1 depicts the data obtained with latex rubber catheters.

Effect of Bacterial Concentration

Adherence of bacteria increased proportionally with the number of bacteria in the incubating suspension at concentrations between 1×10^7 and 5×10^9 bacteria/ml. Studies to determine the maximum number of bacteria that could adhere were difficult to interpret due to bacterial clumping at high concentrations (data not shown).

Effect of Catheter Material

As shown in Table 2, siliconised rubber was nearly always associated with significantly fewer ($p < 0.05$) bacteria after incubation than was latex rubber with or without teflon. Generally, teflon coated rubber values exhibited intermediate and latex rubber the highest adherence; however, a number of exceptions were noted. Although there were few isolates from each source, no difference in adherence was noted in relation to site of bacterial isolation in this study (Table 2). Barium and non-barium impregnated catheters of the same type from the same supplier were also studied and found to yield results that were nearly identical ($p > 0.3$).

Table 1. Relation between incubation pH and bacterial adherence to catheters[a]

Bacteria and isolate number	Source	pH 5	pH 7.4	pH 9
Escherichia coli 1	urine	3,350 ± 371[b]	2,832 ± 318	2,715 ± 304
Escherichia coli-2	urine	2,794 ± 302[b]	2,017 ± 241	2,103 ± 236
Escherichia coli-5	sputum	1,316 ± 139[b]	986 ± 115	931 ± 104
Klebsiella pneumoniae-1	urine	4,098 ± 517[b]	3,018 ± 425	3,215 ± 491
Klebsiella pneumoniae-2	urine	1,968 ± 240	1,896 ± 212	1,798 ± 237
Klebsiella pneumoniae-5	blood	1,629 ± 163[b]	1,107 ± 138	1,155 ± 161

[a] Results of 30 min incubation of 10 ml of 1×10^8/ml bacteria and latex rubber catheter material expressed as mean number bacteria adherent/mm^2 surface area ± standard deviation of the mean

[b] $p < 0.05$ compared with pH 7.4 values

Table 2. Attachment of gram-negative bacilli to different urinary catheter material[a]

Bacteria	Source	Treatment of Rubber					
		None (Latex)	None[b]	Teflon	Teflon[b]	Silicone	Silicone[b]
Escherichia coli							
1	urine	2,832 ± 318	2,989 ± 321	2,107 ± 204[c]	2,016 ± 192[c]	1,103 ± 94[c]	1,081 ± 98[c]
2	urine	2,017 ± 193	1,926 ± 253	2,089 ± 225	1,987 ± 214	651 ± 73[c]	594 ± 75[c]
3	urine	1,635 ± 157	1,607 ± 165	917 ± 86[c]	854 ± 90[c]	813 ± 94[c]	901 ± 104[c]
4	urine	703 ± 101	528 ± 97[c]	481 ± 52[c]	509 ± 43[c]	412 ± 36[c]	641 ± 85
5	sputum	986 ± 115	1,053 ± 149	1,105 ± 149	1,029 ± 117	1,027 ± 103	1,048 ± 136
6	sputum	2,513 ± 268	2,591 ± 216	2,287 ± 249	2,401 ± 201	941 ± 108[c]	802 ± 113[c]
7	blood	684 ± 82	617 ± 72	523 ± 64[c]	478 ± 41[c]	312 ± 45[c]	339 ± 41[c]
Klebsiella pneumoniae							
1	urine	3,018 ± 425	3,194 ± 396	1,912 ± 215[c]	942 ± 184[c]	1,156 ± 104[c]	1,107 ± 99[c]
2	urine	1,896 ± 212	1,759 ± 228	1,801 ± 200	1,831 ± 213	623 ± 70[c]	548 ± 65[c]
3	sputum	2,957 ± 314	3,015 ± 308	2,245 ± 281[c]	2,937 ± 310	2,014 ± 257[c]	1,182 ± 203[c]
4	blood	2,245 ± 279	2,298 ± 294	881 ± 102[c]	796 ± 84[c]	2,172 ± 294	1,626 ± 197[c]
5	blood	1,127 ± 138	1,070 ± 115	873 ± 76[c]	992 ± 107	385 ± 26[c]	514 ± 48[c]

[a] Results of 30 min incubation of 10 ml of 1 x 10^8/ml bacteria and catheter material expressed as mean number of bacteria adherent/mm^2 surface area ± standard deviation of the mean

[b] Different manufacturer of same type catheter

[c] $p < 0.05$ compared with first brand of latex

Discussion

Pili (fimbriae) of the *Enterobacteriaceae* (especially pili of type I) are thought to mediate attachment to a variety of mannose-like mammalian surface receptor sites in a passive, non-energy requiring process: however, not all studies are in agreement with this [7, 9]. It has also been reported that other bacteria attach to non-cellular dental surfaces [1, 12, 15]. In these studies it was noted that members of the *Enterobacteriaceae* family readily attach to catheter material in a pH, time, and concentration-dependent fashion. Marked differences were noted between individual strains of bacteria and various catheter materials.

While the sample number was too small to draw firm conclusions, it is of note that different strains of the same bacteria displayed a variety of adherence characteristics, in spite of identical handling of all isolates. Also, the site of isolation did not appear to influence attachment to catheters. These observations suggest that intrinsic bacterial adherence capabilities may be important.

Most urinary catheter-related infections are thought to arise from retrograde spread of bacteria [3]. Multiple pathogenetic mechanisms are involved in the development of bacterial colonisation and infection in such instances [3, 6, 14]. Reviewed elsewhere [6] are results suggesting that siliconised urinary catheter material may be less associated with encrustation and perhaps infection than other rubber materials. However, why this may be so is still speculative. The decreased association of bacteria with siliconised rubber presented here could be related. It is suggested that physical attachment of bacteria to urinary tract catheters can be an early step in the pathogenesis of colonisation and infection in certain instances.

Exact types or amount of rubber treatment were not controlled in this investigation. Instead, commercially obtained urinary catheters were examined; of these, the siliconised rubber catheters displayed decreased bacterial association. The few exceptions noted may reflect variations in processing of the same catheter material by the same manufacturer. Alternatively, undefined intrinsic bacterial properties may vary amongst certain strains sufficiently to account for some difference. Further work to determine whether there is a definite relationship between infection and the in vitro findings reported here appears warranted.

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