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Adherence of Bacteria to Suture Materials[1] (41141)

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Abstract. Sutures were incubated in suspensions of radiolabeled Enterobacteriaceae or *Staphylococcus aureus* and nonadherent bacteria were removed by washing. Adherence of bacteria to gut was up to 100 times greater than to nylon; adherence to polyglycolic acid or silk was intermediate. These results correlate with laboratory and clinical investigations which have suggested that gut sutures have the highest frequency of association with surgical wound infection, followed by silk and nylon in descending order of frequency. Braided materials had increased adherence compared to nonbraided materials, probably due to increased surface area. Adherence of Enterobacteriaceae to suture material was saturable and time dependent and was blocked by addition of unlabeled bacteria. Adherence of bacteria to sutures may be an integral part of the pathogenesis of certain surgical infections.

Certain suture materials have been said to be more often associated with wound infection than others. Although some controversy persists since many of the studies have not been well controlled, both clinical and laboratory observations suggest that gut sutures are more often associated with infection than silk, and both of these more often than nylon (1–5). Braided sutures have also been implicated as more likely causes of infection than monofilamentous suture of the same material and diameter (1).

In recent years adherence of bacteria to mammalian surfaces has been found to play a role in the pathogenesis of bacterial colonization and infection (6–8). Specific ligands mediating adherence include teichoic acids for certain streptococci and staphylococci (9, 10), mannose-sensitive pilus sites on many strains of Enterobactericeae family members (6–8), and mannose-resistant (perhaps not always pilus related) ligands of other strains (11–14).

We thought that it would be useful to examine adherence of bacteria to various types of suture, especially since several commonly used suture materials are composed of glycoproteins, substances related to those present on cell surfaces. The purpose of these studies was to see whether differential adherence of bacteria might perhaps relate to the reported frequency with which various suture materials have been implicated in surgical infections.

Methods. *Bacteria.* Freshly isolated Enterobacteriaceae representing the dominant growth in wound or sputum cultures, or the only growth from blood or urine ($\geq 10^5$/ml in urine) were identified by the API system (Analytab Products, Plainsville, N.J.), subcultured once on trypticase soy agar (BBL, Becton Dickinson, Detroit, Mich.), and stored at 23° without further passage. *Staphylococcus aureus* isolated from blood cultures or as the dominant organism from wounds, were identified by colonial morphology, hemolytic growth, and coagulase production. Organisms were subcultured onto trypticase soy agar slants and stored at 4° without further passage.

Bacteria were radiolabeled by incubation at 37° for 18 hr in Eagle's minimal essential medium (Gibco, Grand Island, N.Y.) for the Enterobacteriaceae or Mueller–Hinton broth (Difco, Detroit, Mich.) for the *S. aureus;* [*methyl*-^{3}H]thymidine (New England Nuclear, Boston, Mass.) specific activity of 50.5 Ci/mmole was added to yield 5 μCi/ml. Organisms were collected by centrifugation

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and washed three times in 0.05 *M* phosphate-buffered saline, pH 7.4, without calcium or magnesium (PBS). *S. aureus* were sonicated gently for 90 sec to break up clusters. Bacteria were resuspended in PBS and their concentration was adjusted spectrophotometrically; bacterial concentrations were verified by quantitative cultures.

Suture material. Plain gut, chromic gut, monofilament silk, braided silk (siliconized and unsiliconized), braided polyglycolic acid, and monofilament nylon, all of 0.2-mm diameter were studied. In some cases, suture material from several different suppliers was compared without significant differences being noted.

Adherence assay. On at least two separate occasions, 50- to 100-mm strips of suture materials were incubated in 5 to 10 ml of bacterial suspensions for up to 2 hr on a shaking apparatus at various temperatures. Duplicate sutures were usually processed separately in each experiment. After incubation, sutures were washed five times in PBS, fluid from the final wash was shown not to contain counts per minute (cpm) significantly above background. Sutures were placed in 0.1 *N* NaOH to remove adherent bacteria and shaken for 1 hr, after which the sutures were discarded. The NaOH solution was adjusted to pH 7 and xylene-

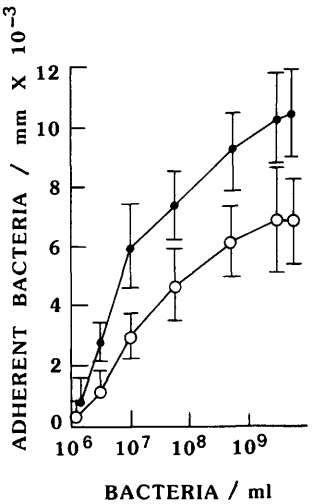

FIG. 2. The relation between bacterial adherence to gut suture and the concentration of bacteria in the incubating suspension. 50 mm of plain gut sutures was incubated for 60 min at 23° in the presence of 10 ml of increasing concentration of *E. coli* (○——○) or *K. pneumoniae* (●——●).

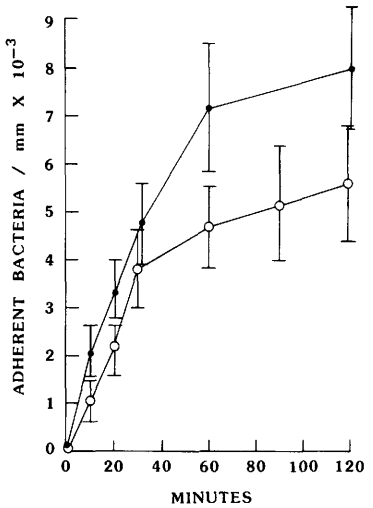

FIG. 1. The relation between bacterial adherence to gut suture and length of incubation. Strips of plain gut suture, 100 mm length, were incubated in 5 ml PBS containing 5×10^8 *K. pneumoniae* (●——●) or *E. coli* (○——○) per milliliter for varying time intervals at 23°. Brackets indicate the standard deviation of the mean. The vertical axis represents the number of bacteria adherent per millimeter suture.

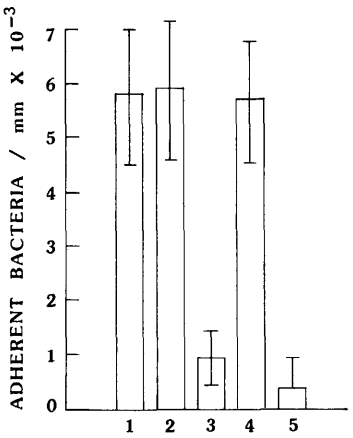

FIG. 3. Blocking of bacterial adherence to gut suture. 50 mm of plain gut sutures was incubated in a 5-ml suspension of 1×10^9 radiolabeled *E. coli*/ml for 60 min at 23° after the addition of 5 ml of (1) PBS (control); (2) unlabeled *E. coli*, 1×10^9/ml; (3) unlabeled *E. coli* 2×10^{10}/ml; (4) unlabeled *K. pneumoniae*, 1×10^9/ml; (5) unlabeled *K. pneumoniae*, 2×10^{10}/ml. $P < 0.01$ comparing (3) or (5) with (1) using *t* test analyses.

TABLE I. ADHERENCE OF ENTEROBACTERIACEAE TO VARIOUS SUTURE MATERIALS[a]

	Nylon Monofilament	Silk			Polyglycolic acid Braided	Gut	
		Monofilament	Braided	Braided siliconized		Chromic	Plain
Source							
E. coli							
Blood	43 ± 23	517 ± 81	940 ± 132	736 ± 97	3787 ± 610	2952 ± 317	3951 ± 604
Urine	106 ± 24	397 ± 52	806 ± 102	704 ± 98	935 ± 182	825 ± 153	1038 ± 217
Wound-1[b]	32 ± 19	835 ± 68	1124 ± 187	1056 ± 143	2865 ± 402	3130 ± 495	4156 ± 813
Wound-2	13 ±11	196 ± 28	402 ±73	311 ± 50	1236 ± 195	687 ± 99	981 ± 182
K. pneumoniae							
Blood	18 ± 11	1021 ± 185	1624 ± 215	1397 ± 196	1618 ± 401	1879 ± 354	2941 ± 513
Sputum[b]	87 ± 41	3966 ± 821	5248 ± 730	5149 ± 788	4825 ± 601	4579 ± 584	7318 ± 1085
Wound-1	49 ± 17	917 ± 102	1217 ± 185	1104 ± 187	1399 ± 218	1186 ± 179	1324 ± 194
Wound-2	28 ± 10	1194 ± 213	1308 ± 214	1098 ± 139	1670 ± 243	1218 ± 188	2012 ± 391
Proteus mirabilis							
Wound	9 ± 5	184 ± 26	316 ± 50	238 ± 49	689 ± 117	602 ± 98	1371 ± 212

[a] Strips of sutures 50-mm long were incubated in 5 ml of PBS containing 5×10^8 bacteria/ml for 60 min at 23°. Results are presented as the mean number of bacteria adherent/mm suture length ± standard deviation of the mean. $n = 3$.

[b] Strains depicted in Fig. 1–3.

surfactant-based scintillation fluid was added for determination of cpm in a liquid scintillation counter; calculated counting efficiency was 39%. The cpm of known quantities of each bacterial isolate were used for conversion of data to number of bacteria.

Statistical methods. Means of daily determinations were treated as single datum points. Data are presented as the mean ± SD of the mean.

Results. *Effect of incubation time and temperature.* No bacterial adherence to suture materials was detectable when sutures were placed into bacterial suspensions and then immediately removed (0-min incubation). Adherence of Enterobacteriaceae increased in nonlinear fashion with increasing duration of incubation; data obtained using gut sutures are shown in Fig. 1. With all Enterobacteriaceae studied, over 50% of the total 2-hr adherence was noted within 30 min. No significant differences in adherence were observed when incubations were carried out at 4, 23, or 37° when compared using *t* test analyses.

Effect of bacterial concentration during incubation. Association of Enterobacteriaceae with sutures was greater with increasing concentrations of bacteria in the incubating suspension (Fig. 2). Saturation of plain gut occurred in suspensions containing about 5×10^9 bacteria/ml; under these conditions 10^3 to 10^4 bacteria adhered/mm of suture. Because progressive agglutination of *S. aureus* occurred at concentrations $\geq 10^8$ bacteria/ml and/or with prolonged incubation, these variables were not studied in the case of staphylococci. Agglutination of Enterobacteriaceae used in these studies was not observed during *in vitro* incubations.

Competition for attachment. Experiments were performed in which increasing concentrations of unlabeled *E. coli* or *K. pneumoniae* were incubated with 5×10^8/ml radiolabeled *E. coli* and sutures. With 10^{10}/ml unlabeled bacteria, adherence of labeled *E. coli* was significantly reduced (Fig. 3).

Effect of suture material. Adherence of Enterobacteriaceae was greatest to gut suture, less to silk or polyglycolic acid, and least to nylon. Adherence to braided silk was greater than to unbraided silk of the same diameter. Bacteria adhered more readily to native than to siliconized or chromium-treated materials (Table I). A similar relation between adherence and the type of suture material was observed with *S. aureus* (Table II).

Discussion. These studies show that bacterial adherence to suture material is saturable and time dependent. No adherence was observed after 0-min incubation and over 50% of total Enterobacteriaceae adherence was noted after 30 min. These data, in conjunction with the lack of effect of different incubation temperatures, suggest that the process is passive, nonenzymatic and nonenergy requiring. Sugars and glycoproteins are thought to mediate adherence of certain bacteria to mammalian receptor cells (6–8, 15). In our studies, glycoprotein suture materials had a higher degree of association with bacteria than nylon. Adherence of Enterobacteriaceae or *S. aureus* was greatest to gut, less to silk, and least to nylon. Chromium-tagged gut and siliconized silk were associated with

TABLE II. ADHERENCE OF *Staphylococcus aureus* TO VARIOUS SUTURE MATERIALS[a]

	Nylon (monofilament)	Silk (monofilament)	Gut (plain)
Source			
Wound 1	12 ± 7	2691 ± 526	3025 ± 519
Wound 2	108 ± 38	1754 ± 302	1814 ± 307
Wound 3	143 ± 49	7612 ± 1425	9187 ± 2004
Blood	68 ± 20	1694 ± 287	2135 ± 513

[a] Strips of suture 50 mm long were incubated in 5 ml of PBS containing 5×10^8 bacteria/ml for 60 min at 23°. Results are presented as the mean number bacteria adherent/mm suture length ± the standard deviation of the mean. $n = 3$.

fewer bacteria than native sutures; it is likely that processing of these materials shields or alters potential sites for adherence.

Bacteiria isolated from wounds did not display increased association with sutures as compared with bacteria from other sites of isolation. Although only a small number of isolates was studied, this suggests that attachment to sutures is a nonselective property which is not limited to certain isolates.

The greater degree of bacterial adherence to braided than to nonbraided silk is probably secondary to the increased surface area of the braided material. However, surface area is clearly not the major determinant of attachment since more than 100 times as many bacteria adhered to single-stranded plain gut as to single-stranded nylon of the same diameter.

Many factors determine which surgical wounds become infected. This study investigated interactions between bacteria and sutures in an isolated model. Certain *in vivo* factors such as host inflammatory response and interactions with serum proteins were not examined. However, the correlation between bacterial attachment to different sutures and other data relating the incidence of infection with type of suture suggest that bacterial adherence to sutures may be a pathogenetic mechanism in the etiology of certain surgical wound infections.

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