Nationwide Epidemic of Septicemia Caused by Contaminated Infusion Products. IV. Growth of Microbial Pathogens in Fluids for Intravenous Infusion

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Septicemia caused by contaminated infusion fluid is a newly appreciated hazard of intravenous infusion therapy. Microorganisms of the tribe Klebsiellae (Klebsiella, Enterobacter, and Serratia) have predominated in these infections. Members of this tribe were found to possess a selective ability over common non-Klebsiellae microbial pathogens to proliferate rapidly in commercial parenteral fluids containing glucose at room temperature. Fifty-one Klebsiellae strains, washed twice before inoculation of approximately 1 organism/ml, attained a mean normalized 24-hr concentration of $1.1 \times 10^6$ organisms/ml in 5% dextrose in water at 25 C. In contrast, 48 of 49 non-Klebsiellae bacterial strains (clinical isolates of Staphylococcus, Proteus, Escherichia coli, Herellea, and Pseudomonas aeruginosa) slowly died (mean 24-hr concentration, 0.2 organism/ml). Five Candida albicans strains grew only very slowly (3.13 organisms/ml). Even with concentrations exceeding $10^6$ organisms/ml, microbial presence was never visibly detectable. The significant increase in cases of nosocomial septicemia caused by Klebsiella, Enterobacter, and Serratia in recent years might be attributable in part to fluid-related sepsis accompanying the expanding use of parenteral therapy.

Septicemia is a recognized and feared complication of iv infusion therapy. Past studies of iv-related infection have focused almost exclusively in the iv cannula [1], despite a report in 1953 implicating contaminated infusion fluid as a cause of septicemia [2]. The hazard of infection from every part of the infusion apparatus became widely recognized abruptly in 1970–1971, when many hospitals in the United States experienced outbreaks of nosocomial septicemia with Enterobacter cloacae and Erwinia (herbicola-lathyri group, proposed designation Enterobacter agglomerans [3]) traced to contamination of the iv fluid of one manufacturer [1, 4–7].

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During the Center for Disease Control's investigations of the epidemic, before identification of the source of contamination as intrinsic (during manufacture), surveys of infusion systems manufactured by Abbott, Baxter, and Cutter laboratories 1 sampled during clinical use had shown 6%–35% rates of fluid contamination with multiple organisms [7]. However, with rare exception, only members of the tribe Klebsiellae (TK) (Klebsiella, Enterobacter, and Serratia) were found in concentrations of >10 organisms/ml. The few published reports of septicemia linked to extrinsic contamination of iv fluid had implicated primarily members of TK [2, 8, 9].

Dextrose in water (5%) (D5%/W) is virtually synonymous with iv therapy and along with other glucose-containing solutions is by far the most frequently used infusion product in United States hospitals [1]; during the nationwide

1 Names of manufacturers and trade names are provided in this paper for identification only, and inclusion does not imply endorsement by the Public Health Service or the U.S. Department of Health, Education and Welfare.
epidemic glucose-containing fluids were utilized in over 90% of iv infusions.

Enterobacter aerogenes is capable of nitrogen fixation [10]. In addition, many members of TK are known to be facultative psychrophiles [11]. Thus it was postulated that members of TK possess a selective ability to proliferate in glucose-containing iv fluids at room temperature. This study was designed to test this hypothesis.

Materials and Methods

Microbial strains. A total of 106 strains, identified at the Center for Disease Control, were selected for study (table 1). Except for six strains of Erwinia and five of E. cloacae that had been recovered from epidemic patients or iv fluid, all strains were isolates from human infections in hospitals uninvolved in the national epidemic.

Fluids. Growth of all 106 strains at 25 C was studied in Abbott D5%/W. Growth in 0.9% NaCl at 25 C was evaluated for 18 strains: eight from three genera of TK, two of Pseudomonas aeruginosa, two of Escherichia coli, two of Herellea, three of Proteus, and one of Staphylococcus epidermidis. One strain each of E. cloacae and E. coli were tested concurrently in D5%/W manufactured by Abbott, Baxter, Cutter, and McGaw.

Microbiologic methods. After two washings in sterile 0.9% NaCl, sufficient organisms were introduced into a freshly opened bottle to give an initial (zero time) concentration of between 1 and 10 organisms/ml. Bottles were incubated without agitation at 25 C and sampled at 3, 6, 12, 24, and 48 hr. Quantitative cultures were performed by serial 10-fold dilutions, in triplicate, with use of trypticase soy agar pour-plates that were incubated at 37 C and counted on an illuminated colony counter. After the sampling at 48 hr, all remaining viable organisms were identified to confirm growth and to exclude experimental contamination. At 24 and 48 hr, each bottle was inspected for turbidity, schlieren, or other visible evidence of microbial growth. Fluid pH was determined in each bottle with an electronic pH meter before inoculation and after incubation for 48 hr.

Analysis of growth data. The mean concentration at each designated time for each strain was normalized to an initial concentration of 1 organism/ml by dividing the observed concentration by the concentration at zero time.

Results

Growth in D5%/W. Desired zero-time concentrations in fluid were attained (mean, 1.8; range, 0.1–9.8 organisms/ml).

As seen in table 1 and figure 1, TK strains proliferated rapidly at 25 C, reaching logarithmic growth by 12 hr and attaining mean concentrations (normalized for the group) of $1.11 \times 10^5$/ml at 24 hr and $1.70 \times 10^5$/ml at 48 hr. E. cloacae and Erwinia, the major pathogens in the nationwide epidemic, were among those that grew most rapidly (table 1). Only one of 51 TK strains tested, an E. cloacae strain, failed to grow; this was confirmed by repeated testing.

In contrast, only one of 49 non-TK bacterial strains initiated substantial growth; a herellea

Table 1. Growth of 105 strains in 5% dextrose in water at 25 C.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mean concentration at 24 hr, organisms/ml*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. strains tested</td>
</tr>
<tr>
<td>Tribe Klebsiellae</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>11</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>10</td>
</tr>
<tr>
<td>Erwinia (Enterobacter agglomerans)</td>
<td>6</td>
</tr>
<tr>
<td>Enterobacter liquifaciens</td>
<td>3</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10</td>
</tr>
<tr>
<td>Enterobacter hafnia</td>
<td>5</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
<tr>
<td>Non-tribe Klebsiellae</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11</td>
</tr>
<tr>
<td>Herellea species</td>
<td>3†</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10</td>
</tr>
<tr>
<td>Proteus species</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus species</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>5</td>
</tr>
</tbody>
</table>

* Normalized to an initial zero-time concentration of 1.0 organism/ml.
† Excludes one strain of Herellea that grew well (1.3 × 10^9 organisms/ml at 24 hr).
isolate repeatedly attained 24-hr concentrations of \(10^2\)-\(10^4\) organisms/ml. The remaining 48 strains either remained static (seven strains) or lost viability (41 strains) over 48 hr (figure 1). The five strains of *Candida albicans* grew only very slowly (mean normalized 24-hr concentration, 31.3 organisms/ml).

**Visible signs of growth.** Evidence of microbial growth visible to the unaided eye could not be detected at any time, even at 48 hr when many strains of TK had attained concentrations exceeding \(10^6\)/ml.

**Changes in pH during incubation.** Only small decremental changes in pH occurred during incubation (mean, 0.20 units). Freshly opened Abbott DS5%/W was consistently in the pH range 4.80-4.90; Baxter, 4.25-4.70; Cutter, 4.55-4.70; and Mc Graw, 4.50-5.00.

**Growth in DS5%/W from various manufacturers.** The *E. coli* strain failed to grow in any of the four brands tested; however, the *E. cloacae* strain grew well in all four brands (mean normalized 24-hr concentrations: Cutter, \(2.7 \times 10^5\); Abbott, \(6.0 \times 10^5\); Mc Graw, \(5.1 \times 10^5\); and Baxter, \(2.4 \times 10^5\) organisms/ml).

**Growth in normal saline.** None of the 18 strains initiated growth during 24 hr of incubation; however, by 48 hr, three TK strains had multiplied one log (mean normalized concentration, 23.3 organisms/ml).

**Discussion**

This in vitro experiment conclusively demonstrated differential abilities of representative nosocomial pathogens to grow in commercial in-
fusion fluids; this had been predicted on the basis of known biochemical properties and clinical and epidemiologic observations. Study of primarily D5%/W and a broad variety of microbial species (nearly all isolated from clinical infections), minute inoculum size (approximately 1 organism/ml), washing the inoculum twice to exclude an organic source of nutrients, and incubation at room temperature were all designed to simulate, as closely as possible, the microbial ecology attending contamination of infusion fluid. Fifty (98%) of 52 tested strains of TK exhibited an impressive ability to proliferate rapidly in D5%/W, whereas 48 of 49 non-TK strains failed to grow or, more commonly, died ($P < 0.001$). Normal saline was unable to support significant growth, even of members of TK. The four major commercial brands of D5%/W supported growth comparably.

Microbial physiologists have long appreciated the wide range of nutritional requirements and adaptability to environmental extremes among the various microorganisms [12, 13], findings reaffirmed in this study. Commercially produced D5%/W might be characterized as a relatively minimal microbiologic medium. It is acidic with a pH range of 3.5–5.5 [14] and contains only dissolved atmospheric $N_2$ as a nitrogen source; glucose and dissolved $CO_2$ provide the sole sources of carbon. Trace amounts of inorganic elements, such as manganese, zinc, and copper, have been demonstrated in commercial iv fluids [15]. E. aerogenes is able to utilize atmospheric nitrogen [10], as are many other members of the TK [16, 17]. Although many pseudomonads also can fix molecular nitrogen [18], and some strains of P. aeruginosa not only subsist but actually proliferate in tap water and even in distilled water [19, 20], some inhibitory property of D5%/W prevented growth. Total inability of Pseudomonas, Proteus, and three of the four strains of Herellea to grow in D5%/W was unexpected, since these organisms are frequent nosocomial pathogens and thrive in the inanimate hospital environment.

Favero et al. [19] found that “naturally occurring” strains of Pseudomonas, i.e., strains inoculated directly from water sources in the hospital, multiply rapidly in distilled water, whereas strains precultured in broth before inoculation grow poorly. It is unlikely that this phenomenon is applicable in our studies with D5%/W despite an obvious logistic necessity to preculture all tested strains. Although 22 microbial species, mostly non-TK, were ultimately isolated from intrinsically contaminated bottle closures and the epidemic strains E. cloacae and Erwinia were recovered from only 20% of culture-positive closures [5], nearly all clinical infections in the national outbreak were caused by TK organisms [1, 4–7]. In addition, a large variety of “naturally occurring” organisms, again including many non-TK, were isolated from Abbott, Baxter, and Cutter infusions sampled during use, yet with rare exception, only members of the TK were present in concentrations suggesting multiplication (>10 organisms/ml) [1, 7].

Other investigators have recently also studied microbial growth in conventional iv fluid in vitro [6, 21–23]; however, clinical applicability of these studies is compromised in part by limited numbers of species and strains tested [6, 21–23], by failure to wash inocula [22], and especially by the large inoculum size of $>10^4$ organisms/ml [6, 22, 23]. Despite these experimental drawbacks, results have been in general concordance with the findings of the present study, in which a large number of species and strains were employed and stringent attempts to duplicate in vivo conditions were made.

Since 1970, five other outbreaks besides the United States epidemic have been traced to contaminated fluid [8, 9, 24–26]. Members of TK were implicated in 16 of the 21 cases in four outbreaks. Citrobacter freundii caused three cases of septicemia in a small outbreak traced to intrinsically contaminated 5% dextrose in lactated Ringer’s solution [26]; as might be expected, the epidemic strains of C. freundii were found to proliferate rapidly in this specific infusion product. In the single epidemic of iv fluid-related sepsis in which organisms of the TK did not predominate [25], infections with Pseudomonas cepacia were linked to contaminated distilled water used to cool infusion bottles after autoclaving; contamination of iv fluid never exceeded 10 organisms/ml. Favero et al. [20] have shown that P. cepacia multiplies rapidly in distilled water; it may also be one of the rare non-TK species capable of rapid growth in D5%/W [23].

Most contamination of infusion fluid is prob-
ably extrinsic, excluding isolated outbreaks caused by intrinsic contamination such as the recent nationwide one. Reported rates of in-use contamination have ranged from 3.0% to 38% [1]. Microorganisms from without probably are introduced most frequently during manipulation of the apparatus by personnel or by the patient [1]. Members of the TK have been demonstrated in high frequency on the hands of both hospital personnel [27] and patients [28]. In studies of in-use iv systems, rates of fluid contamination were directly proportional to the duration of infusion therapy [1, 7, 9]. Once introduced into a functioning infusion system, microorganisms capable of growth in iv fluid can persist for days in the delivery tubing, despite serial replacements of the bottle [1, 2].

All of these data indicate that iv infusions suffer a significant risk of in-use contamination, frequently by TK organisms that by their unique growth properties pose an increased hazard. Thus, in addition to the cannula, infusion fluid must now be recognized as a source of nosocomial sepsis, and TK organisms must be expected to predominate. Realizing the cumulative nature and potential exponential amplification of in-use contamination of fluid, we recommend that all bottles and delivery apparatus be changed at least every 24 hr, and that at each change of cannula all equipment be totally replaced [1, 4].

Routine buffering of intrinsically acidic parenteral solutions by addition of sodium bicarbonate immediately before use has been advocated to diminish the incidence of infusion phlebitis [29]. We found that neutralization of D5%/W to pH 7.35 alone permits rapid growth of Pseudomonas and Herellea in addition to TK. Broadening the spectrum of microorganisms capable of growth in glucose-containing fluids and the added risk of introducing organisms with routine added manipulations argue against pre-use neutralization.

Minute quantities of blood (1:500 by volume) added to glucose-containing solutions also buffer fluid significantly [30] and provide organic nutrients for more fastidious organisms incapable of growth in unaltered fluid. In addition, blood products are a source of introduced organisms, often members of TK [31, 32]. Thus, after administration of blood products the entire delivery apparatus should be replaced.

Inability to detect microbial growth visually in infusion fluid, even with counts of >10⁹/ml, is noteworthy. While inspection of the bottle and delivery apparatus at setup and thereafter is recommended for identification of malfunction or particulate contamination, it cannot be relied upon for detection of bacterial contamination of fluid. Molds, usually introduced through minute cracks in the bottle, may produce faint turbidity or filmy precipitates [33].

In the past 15 years there has been a striking rise in number of nosocomial cases of septicemia caused by members of the TK, especially Klebsiella pneumoniae, E. aerogenes, and Serratia marcescens [34–38]; this trend has been generally attributed to increasingly heavy antimicrobial pressure selecting for multiply resistant pathogens. However, parenteral therapy has also found even greater use and wider applications in recent years [1]. Accordingly, the increasing prominence of TK in infections of the bloodstream might also be related in part to a rising incidence of iv fluid-associated septicemia, with lack of recognition in many cases of the relation to fluid contaminated by rapidly proliferating organisms of the TK. The inordinate preponderance of Candida in infections complicating total parenteral nutrition [39] and the differential ability of Candida to multiply luxuriantly in hypertonic glucose solutions utilized in this new therapeutic modality [40] may well be similarly related.

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