A Clinical Study of the Role of Enterococci as Sole Agents of Wound and Tissue Infection

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Patients who had enterococci isolated from wounds or tissues were identified from laboratory records. The charts of patients with pure cultures of enterococci were reviewed to determine the degree of clinically significant infection. We found that the frequency of infections in patients with pure cultures of enterococci was not significantly different from the frequency of infections in a control series of patients with negative cultures, but that it was significantly different from the frequency of infections in a series of patients with pure cultures of Staphylococcus aureus. Our conclusion that enterococci are not by themselves significant pathogens in wound or tissue infections is supported by a few experimental studies of other authors.

INTRODUCTION

Enterococci have long been known as significant pathogens in endocarditis, urinary tract infections, and biliary infections [1,2]. There are rare reports of meningitis [3] and earlier reports of middle ear infections, infections of the female genital tract, and peritonitis from which enterococci were isolated in pure culture [1,2]. Recent reports of similar cases are lacking, however; and there must be some doubt as to whether earlier culture techniques were adequate to recover anaerobic organisms which may also have been present.

Enterococci have been cultured with considerable frequency from surgical and traumatic wounds. In studies where all types of wounds were considered and media selective for streptococci were not employed, they have generally ranked third or fourth in frequency, behind coliforms and staphylococci [4,5,6]. In one series where a selective medium for Gram-positive bacteria was used, they ranked first [7]. The overwhelming majority is found in mixed culture and/or recovered from wounds that do not show clinical signs of infection [4,5]. One study has reported pure isolates from postoperative wound infections: there were only seven in a series of 4,057 wounds, as against 145 pure isolates of Staphylococcus aureus [5]. Furthermore, four of the entero-coccal isolates were from urological patients whose wound sites may have been contaminated with infected urine. Another study has reported a high frequency of entero-coccal colonization (mixed and pure) of burn wounds, but this was related to contamination of porcine xenografts and apparently did not result in wound infection [8].

The question of the pathogenicity of enterococci in wound and tissue infections, and of the need for antimicrobial therapy of these infections, is of obvious importance to the physician. We therefore decided to review records of cultures of wound and tissue sites for the presence of pure isolates of enterococci and to correlate them with the clinical findings in a retrospective study. Since signs of infection may be present in wounds from which no viable organisms can be cultured and since, on the other hand, organisms may be present in wounds without signs of infection, approp-
riate control series were included. Antimicrobial treatment initiated prior to the collection of the specimen was taken into account since it appeared possible that such therapy could suppress other organisms responsible for the infection and leave enterococci as the sole surviving species.

MATERIALS AND METHODS

Cultures of wounds were mostly collected on swabs and submitted to the laboratory in Transport Medium Amies (Difco Laboratories, Detroit, Michigan). In rare instances, i.e., when a large volume of exudate was present, aspirate was sent to the laboratory in a syringe. Tissues were submitted in a Petri dish and ground up with a TenBroeck grinder prior to inoculation.

Specimens were routinely plated onto blood agar (Tryptic Soy Agar [Difco Laboratories] with 5% sheep blood), Deoxycholate Agar (Difco Laboratories), Columbia CNA (colistin-nalidixic acid) Agar (Difco Laboratories) with 5% sheep blood, and Mannitol Salt Agar (Difco Laboratories), and were inoculated also into Fluid Thioglycollate Medium (Difco Laboratories). Deep wound specimens (identified as such on the requisition) were, in addition, cultured on Brain Heart Infusion Agar (Difco Laboratories) with 5% sheep blood and 30 μg neomycin per ml. Cultures were incubated at 37°C for a maximum of 72 hours. Blood and CNA agar plates were incubated in a 5–10% CO₂ atmosphere; Brain Heart Infusion plates, anaerobically in the GasPak 100 Anaerobic System (BioQuest, Cockeysville, Maryland). The other media were incubated aerobically. Enterococci were tentatively identified from growth on blood agar or CNA Agar; the diagnosis was confirmed by growth in Tryptic Soy Broth (Difco Laboratories) containing 6.5% NaCl and by growth plus hydrolysis of esculin on Bile Esculin Agar (Difco Laboratories). Speciation was not undertaken. “Pure cultures” were defined as those growing only one and the same organism on several media.

Laboratory records were reviewed to identify hospital inpatients having cultures of wound or tissue sites. The following groups were established:

Enterococcal Incidence. Wound/Tissue cultures yielding enterococci, either pure or mixed, were tabulated over a six-month period (July through December, 1975).

Pure Cultures of Enterococci. Wound/Tissue cultures yielding pure isolates of enterococci in an 18-month period (October 1974 through March 1976) were tabulated.

Negative Controls. Wound/Tissue cultures yielding no growth in a three-month period (July through September, 1975) were tabulated. At the same time, the total number of wound/tissue cultures without enterococci taken during that period was determined.

Positive Controls. Wound/Tissue cultures from 17 random inpatients yielding pure cultures of *S. aureus* were selected. Identification had been performed using microscopic morphology and the coagulase reaction (with Coagulase Plasma, Rabbit [BioQuest]).

The clinical charts of patients with pure cultures of enterococci and of patients in the control groups were obtained for review. Patients were subdivided into three categories: “definite infection,” “possible infection,” and “doubtful infection.” Only one site per patient, and one culture per site, were eventually included.

Criteria of “definite infection” included well-documented evidence of at least three of the following signs: significant erythema, tenderness, purulent drainage, fever and/or leucocytosis (the latter two only if not due to other causes). Patients with only one or two of the local signs of infection were placed in the “possible infection”
category. Patients who had no documented evidence of wound/tissue infection were placed into the “doubtful infection” category. Patients who had one culture yielding pure enterococci and an additional culture from the same site (and within 48 hours) yielding other organisms or a mixed flora with enterococci were also placed into the latter category, since it was uncertain that their infections were due to enterococci alone. The presence or absence of antimicrobial therapy immediately prior to the collection of a specimen yielding pure enterococci was documented from the medication record.

RESULTS

In the six-month period, 343 wound/tissue cultures yielded enterococci, only 31 (9.1%) in pure culture (Table 1). Of all wound/tissue cultures without enterococci, 11.6% showed no growth at all in the three-month study period (Table 2).

Over the 18-month period, there were 66 patients with pure isolates of enterococci from wound/tissue sites. The clinical charts of 58 of these patients could be obtained for review. Seven patients were excluded because their wounds were obviously contaminated with infected material such as sputum or urine from which enterococci had been cultured. Three patients were eliminated due to inadequate records. The remaining 48 patients constitute the study group and were subdivided into the “definite,” “possible,” and “doubtful” categories (Table 3). Of these patients, 39 had cultures obtained from surgical or other wounds, and nine from abscesses, osteomyelitis, or other tissue infections.

Of the charts of 36 patients with negative wound cultures, 30 could be obtained for review.

A large proportion of the patients with pure enterococcal isolates (71%, as against 37% in the negative control series, \( p < 0.01 \) in the \( X^2 \) test) had been on antibiotics at the time their cultures were obtained. The use of cephalosporin antibiotics (mostly cephalothin) had been especially frequent; at least 21 of the 34 patients with pure enterococcal isolates on antibiotics had been receiving drugs of this class.

Comparisons of the “definite infection,” “possible infection,” “definite” and “possible” infection combined, and “doubtful infection” categories, whether or not the patients were on antibiotics, revealed no statistically significant differences (all \( p > 0.05 \)) between the patients with pure enterococcal cultures and the negative control group. Statistically significant differences (\( p < 0.01 \), however, exist in the “definite,” “definite” plus “possible,” and in the “doubtful” infection categories between the patients with pure enterococcal and those with pure staphylococcal cultures; only the numbers in the “possible” categories are not significantly different (\( p > 0.05 \)).

DISCUSSION

Enterococci belong to the normal flora of the human intestine [1,2] and would be expected to contaminate wounds fairly frequently. If one extrapolates the total number of wound/tissue cultures without enterococci from Table 2 to a six-month period and combines this figure with the total number of cultures with enterococci from Table 1, the percentage of wound/tissue cultures yielding enterococci is 36% (343 of 963), not significantly (\( p = 0.05 \)) different from earlier percentages (42 of 100) recorded in this laboratory [7]. We assume that the use of the selective CNA-blood agar (which inhibits Gram-negative organisms and grows Gram-positive ones only) resulted in the relatively high recovery rate not reported by other authors (1.6% [5], 8.8% [4], 9.8% [6]). An extrapolation similar to the one above yields a percentage of 3.2% (31 of 963) of wound/tissue cultures with pure enterococcal isolates, also higher than that found by other authors (0.2% [4,5]).
TABLE 1
Wound/Tissue Isolates of Enterococci
(6-month period)

|                |        |
|----------------|--------|
| Mixed          | 312    |
| Pure           | 31     |
| Total          | 343    |

TABLE 2
Wound/Tissue Cultures without Enterococci
(3-month period)

|                |        |
|----------------|--------|
| With Growth    | 274    |
| With No Growth | 36     |
| Total          | 310    |

TABLE 3
Wound/Tissue Infections and Antibiotic Therapy in Patients
with Pure Isolates of Enterococci and in Control Patients

|                          | Definite Infection | Possible Infection | Doubtful Infection | Total Patients |
|--------------------------|--------------------|--------------------|--------------------|----------------|
| Patients with pure       |                    |                    |                    |                |
| cultures of enterococci  |                    |                    |                    |                |
| On antibiotics           | 4                  | 10                 | 34                 | 48             |
| Not on antibiotics       | 0                  | 2                  | 12                 | 14             |
| Patients with cultures   |                    |                    |                    |                |
| yielding no growth       |                    |                    |                    |                |
| On antibiotics           | 4                  | 5                  | 21                 | 30             |
| Not on antibiotics       | 2                  | 2                  | 15                 | 19             |
| Patients with pure       |                    |                    |                    |                |
| cultures of S. aureus    | 12                 | 4                  | 1                  | 17             |

*Almost all of them on antibiotics

In this connection, it must be emphasized that our patients with pure enterococcal isolates had received antibiotics more frequently than the patients with negative cultures. Many patients had been on cephalothin, which is largely ineffective against enterococci [8]. This treatment may well have suppressed the more significant pathogens, leaving enterococci as the only microorganisms recovered. Incomplete recovery of other organisms, particularly of anaerobes, due to faulty collection and delay in transportation, is another possibility to explain the relatively high rate of pure cultures (cultures which eventually yielded no growth would have been at least equally influenced by these factors, however).

This frequency of isolation does not imply pathogenicity, for enterococci could be mere colonizers or may only be able to act synergistically with other organisms to cause infection. Table 3 shows that the presence of enterococci alone made no difference in the clinical infection rate compared to the negative controls while S. aureus was recovered with significantly higher frequency from infected than from non-infected wounds. Coliforms and S. aureus were recovered with higher frequency from septic than from non-septic wounds in another study [4] which also showed that S. epidermidis and non-hemolytic streptococci (a group presumably including the enterococci) were cultured with equal frequency from both types of lesions.

Experimental animal studies further support the notion that enterococci alone lack pathogenic significance in wound or tissue "infections." The MLD for enterococci in mice is very high, in the order of $10^9-10^{10}$ [9]. Subcutaneous injection of an entero-
cocal broth culture into guinea pigs did not result in definite infection but only in a small area of induration, and the animals survived [10]. Intraperitoneal injection of 5 x 10⁷ enterococci into rats neither killed them nor led to abscess formation. If enterococci were injected along with *Bacteroides fragilis* or *Fusobacterium varium*, abscesses were produced, although mortality was associated only with injection of *Escherichia coli*, alone or in combination with these bacteria [11]. In experimental abdominal sepsis following intraperitoneal injection of colonic contents in rats, gentamicin reduced the mortality rate from 37% to 4%, while clindamycin had no effect on the mortality rate [12]. Surviving untreated as well as gentamicin-treated rats developed intra-abdominal abscesses, while surviving clindamycin-treated animals did not. Both drugs are ineffective against enterococci, which could be cultured from the abscesses together with *B. fragilis* or *F. varium* [12]. These results suggest that mortality and abscess formation were not associated with enterococci alone, which could at best act as synergistic agents together with other bacteria. Synergism of enterococci and certain anaerobic bacteria in producing skin necrosis in mice has been demonstrated [13].

Although the presence of enterococci may call for antimicrobial therapy if the danger of systemic spread looms, our findings do not support any significant pathogenic role of these bacteria alone in wound or tissues.

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