

Improved aero-anaerobe recovery from infected prosthetic joint samples taken from 72 patients and collected intraoperatively in Rosenow's broth

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Introduction Recovery of the bacteria responsible for prosthetic joint infections is a major problem, which is due in part to the alteration of their ability to grow by storage during transportation to the laboratory.

Methods In this prospective study, we assessed the benefit of inoculating an enriched liquid medium (Rosenow's broth) with intraoperative samples from 72 patients with prosthetic joint revision due to infection. We compared the results of culture of specimens collected in a standard receptacle with the results for specimens collected in Rosenow's broth.

Results and interpretation 144 samples were taken by each of the 2 collection methods for subsequent culture. Concordance between standard and Rosenow samples was observed for 52 of the 58 strains cultured on agar and for 42 of the 97 strains ($p < 0.001$) which grew only in liquid medium. Infection would not have been diagnosed in 26 patients (almost one-third of all patients) without combining sample collection in Rosenow's broth with standard collection. The bacteria that were not recovered from standard samples but which were recovered from those collected in Rosenow's broth included not only strict anaerobes, in particular *Propionibacterium acnes*, but also coagulase-negative staphylococci and streptococci.

that the antibiotic regimen chosen is the one best adapted to the offending pathogens (Tigges 1993, Tsukayama et al. 1996). Reliable documentation is based on the recovery of bacteria from intraoperative specimens or joint aspirates (Padgett et al. 1995, Duff et al. 1996). In recent series, *Staphylococcus epidermidis* was the pathogen most commonly isolated (40% of cases), followed by *S. aureus*, *Streptococcus* spp., Gram-negative rods, and anaerobes—especially *Propionibacterium acnes* (Hope et al. 1989, Witsö et al. 1999). The bacteria involved in such infections have been shown to exhibit changes in their metabolism, resulting in stationary-phase growth (Norden 1988). The recovery of bacteria whose growth potential has been altered by storage is therefore a major problem. There are currently many transport systems for the maintenance of anaerobic and fastidious aerobic organisms, including Starplex StarSwab II, New Copan Vi-Pack Amies Agar Gel Swabs, Culturette EZ and BBL Port-A-Cul, but although these systems have been evaluated in previous studies, their usefulness has not been established in clinical studies of patients with prosthetic joint infection (Perry 1997, Roelofsen et al. 1997, Hindiyeh et al. 2001). Rosenow's broth is an enriched liquid medium (Rosenow 1914) that has long been used in clinical microbiology laboratories for the culture of anaerobes, but has never been evaluated as a transport system for bacterial specimens from

Microbiological documentation is essential for the management of prosthetic joint infections, to ensure

infected patients. Thus, we assessed whether direct transfer of specimens in the operating room into Rosenow's broth—in addition to collection in a standard receptacle—might improve the recovery of certain pathogens in samples from patients undergoing infected prosthetic joint revision.

Materials and methods

Population

The patients studied underwent revision of infected prostheses in our orthopedic department between 2000 and 2002. The manifestations of total hip or knee prosthesis infection were defined as local signs of inflammation, pain and radiological abnormalities consistent with infection (e.g. intracortical abscess and prosthetic loosening), or drainage from the joint. The specimens examined were all taken during surgical operative procedures, as superficial swabbing of wounds or fistula does not correlate with tissue biopsy culture (Mackowiak et al. 1978). 80 adult patients were considered for this study, but 8 of them (whose 11 intraoperative samples yielded only contaminant bacteria) were excluded. The study population therefore comprised 72 patients who had undergone prosthetic joint revision (mean age 68 years, 37 men). 50 patients had total hip prosthesis and 22 had total knee prosthesis.

Sampling and culture

Specimens were collected simultaneously into Rosenow's broth (Rosenow samples) and in a standard sterile receptacle (standard samples). The sterile receptacle did not contain any solid or liquid preservative and was hermetically sealed before its transport to the clinical microbiology laboratory within 4 hours of collection. The Rosenow broth (bioMérieux S.A., Marcy l'Etoile, France) was used, which is an enriched medium containing reducing substances that allow both the preservation and growth of strict and facultative anaerobes. The tubes containing Rosenow's broth had a capacity of 12 mL each. Before use, the Rosenow's broth was regenerated by placing the tubes in boiling water for 10 min in order to avoid the presence of any trace of oxygen. On arrival at the laboratory, they were covered with molten paraffin and incubated at 37°C for a week. Standard samples

were used to inoculate agar medium and liquid medium (brain-heart broth). Any solid fragments in the samples were crushed under sterile conditions (Ultra-Turrax, Ika-labortechnik, Germany) and used to inoculate brain-heart broth. Samples on agar were incubated at 37°C for 4 days and the liquid media were incubated for a week. Standard and Rosenow samples from the same intraoperative specimen were called concomitant samples. Specimens were included in the current study if at least one of the two concomitant samples had resulted in a positive culture.

A bacterial strain was considered pathogenic if it was cultured—either on agar or in liquid media—from at least 2 different specimens. In these cases, cultures from other simultaneously collected intraoperative specimens, or from specimens taken during previous surgery by joint aspiration, or from blood cultures, were used to confirm the pathogenic role of the strains concerned. For statistical analysis, the cultures obtained on agar were distinguished from those obtained in liquid media. Assessment of the pathogens isolated from different specimens was based on both species identification using API (Biomérieux S.A., Marcy l'Etoile, France) and on their antibiotic susceptibility. Bacteria isolated from only one specimen cultured in liquid media, but whose pathogenicity was not confirmed by any other bacteriological results in the patient's medical chart, were considered to be contaminants.

We used Fisher's exact test to compare the microbiological results; the significance level was set at $p < 0.05$.

Results

Sampling and culture

Among the 144 samples transported to the laboratory, 52 (36%) resulted in the culture of 58 strains on agar, and 92 (64%) in the culture of 97 strains in liquid media only. The mean number of bacterial isolates per sample was 1.07 (155/144). *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) were the most prevalent bacterial strains isolated from the specimens, and accounted for 66% (102/155) of the total (Table 1). More *S. aureus* than CNS strains were isolated on

Table 1. Bacteria recovered from intraoperative specimens from 72 patients with prosthetic joint revision. Values are number of pathogenic strains and percentage of total

Bacteria	n (%)
Aerobes	141 (91)
<i>Staphylococcus aureus</i>	50 (32)
Coagulase-negative staphylococci	52 (34)
<i>Streptococcus</i> sp.	22 (14)
<i>Enterococcus</i> sp.	6 (4)
<i>Corynebacterium</i> sp.	3 (2)
<i>Enterobacteriaceae</i>	7 (5)
<i>Pseudomonas</i> sp.	1 (0.6)
Anaerobes	14 (9)
<i>Peptostreptococcus</i> sp.	3 (2)
<i>Propionibacterium acnes</i>	10 (7)
<i>Bacteroides fragilis</i>	1 (0.7)
Total	155

Table 2. Proportions of bacterial strains cultured on agar which were isolated in the corresponding Rosenow sample

Bacterial species	Total number of strains	Rosenow samples
<i>S. aureus</i>	35	32
Coagulase-negative staphylococci	14	11
Other Gram-positive cocci ^a	4	4
Gram-negative bacteria	1	1
Anaerobes	4	3
Total	58	51

^a *Streptococcus* sp., *Enterococcus* sp., *Corynebacterium* sp.

agar (35/50 versus 14/52, $p < 0.001$; Table 2). CNS were the most prevalent strains isolated in liquid media, and were found in 38/97 samples, followed by *Streptococcus* spp. (26/97; Table 3).

Concomitant samples yielded the same strains in 51/58 cases when samples were cultured on agar, and in 42/97 cases when they were only cultured in liquid media ($p < 0.001$). *S. aureus* strains cultured on agar were found in the corresponding Rosenow samples in 32/35 of cases, and CNS strains in 11/14 (Table 2). Among the 97 strains which grew in liquid medium only, 56 were isolated from standard samples and 83 were from Rosenow samples. The bacterial strains responsible for these discrep-

Table 3. Number of bacterial strains in samples cultured in liquid media only ^a, according to the method of collection of intraoperative specimens from 72 patients with prosthetic joint revision

Bacterial species (total number of strains)	Standard samples only	Rosenow samples only	Both
<i>S. aureus</i> (15)	12	10	7
Coagulase-negative staphylococci (38)	25	31	18
Other Gram-positive cocci (26) ^b	18	25	17
Gram-negative bacteria (4)	1	3	0
Anaerobes (14)	0	14	0
Total (97)	56	83	42

^a Rosenow's broth and brain-heart broth;

^b *Streptococcus* sp., *Enterococcus* sp., *Corynebacterium* sp.

ancies were CNS, other Gram-positive cocci (i.e. *Streptococcus* spp., *Enterococcus* spp. and *Corynebacterium* spp.) and strict anaerobes, especially *P. acnes* (Table 3). Of the 144 concomitant samples analyzed, 41 (26%) taken from 26 patients (36%) would have been considered sterile if standard specimen collection alone had been used.

Cultures of Rosenow samples were completed on average one day later than cultures of standard samples in 62 cases (43%), 2 days later in 4 (3%), and simultaneously in 78 (54%).

Discussion

We found that in about one-third of the patients studied, infection would not have been diagnosed without the combined use of Rosenow's broth and standard sample collection. Not only strict anaerobes (the detection of which was the initial reason for using Rosenow's broth for sample collection and transportation), but also facultative anaerobes such as *Streptococcus* spp. and CNS, were cultured from more samples collected in Rosenow's broth than from samples collected by the standard method. The enriched culture medium of Rosenow's broth may have helped to maintain bacterial viability in stationary growth phase during sample transport to the microbiology laboratory (Norden 1988). Our aim was to explore the bacteriological

improvement which could be expected from transporting samples in Rosenow's broth, rather than to demonstrate its superiority over standard sample media since the latter does not constitute a transport system. *Candida* species and mycobacteria which can rarely cause prosthetic joint infections were not identified in our patients. However, these bacteria are unlikely to be cultured from Rosenow's broth mainly because they are strict aerobes.

Two-thirds of the strains isolated from the intraoperative specimens only grew on liquid media. As reported elsewhere, most of these strains were facultative anaerobes such as CNS, streptococci and enterococci (Padgett et al. 1995, Duff et al. 1996). Other authors have suggested that only bacteria cultured on solid media should be considered for the diagnosis of infection (Duff et al. 1996). However, liquid media have been shown to be useful for the diagnosis of infections in cases of reduced bacterial inoculum (Padgett et al. 1995). As most of the bacterial strains not recovered from the standard collection of samples but recovered from Rosenow's broth were CNS and *P. acnes*, and were cultured in liquid media only, it could be argued that these specimens were contaminated by skin flora. However, a review of the medical charts of the subgroup of patients concerned revealed the presence of the same bacteria in at least one previous, reliable, sample—as shown by species identification and the antibiotic susceptibility pattern of the bacterial strain. If the specimen is being used to inoculate Rosenow's broth, no further manipulation is required in the laboratory except for overlay of paraffin over the broth. Other transport systems with semisolid media are less prone to the risk of false positive results due to contamination, because interpretation of the cultures is easier when they are plated on agar rather than being grown in liquid media. However, contamination can also occur during the manipulation of solid specimens—as they must be removed from their receptacle. Specimens are probably less contaminated when they are manipulated in the operating room under sterile conditions, than in the clinical microbiology laboratory.

In conclusion, the results of the present study support the notion that inoculation of Rosenow's broth with intraoperative specimens in the operating room facilitates not only the recovery of anaer-

obes but also isolation of CNS and *Streptococcus* spp. from patients with revision of infected total joint arthroplasty. Since Rosenow's broth was not designed for the culture of strict aerobes, sample collection in this broth should be combined with collection into a standard receptacle. However, inoculation of Rosenow's broth should only be done in the operating room to reduce the risk of specimen contamination.

Author contributions

ES has full access to all the data in the study and takes responsibility for the integrity of the data. CS and ES: study concept and design. IS and ES: acquisition of data. CS, ES, YY, HM: analysis and interpretation. YY, LC, FG, RC, YM: critical revision.

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