the genotypically identical cells [2]. Several studies have highlighted extracellular components within *M. tuberculosis* aggregation, including mycolic acids [3], complex sugars [4], cellulose, proteins, lipids and DNA [5,6]. In addition, *M. tuberculosis* residing within organized pellicle-like structures exhibits drug tolerance to antitubercular agents [3]. Thus, criteria of a structure to what is interpreted as biofilms are given.

M. Tuberculosis Biofilms in Humans

The clinical role of *M. tuberculosis* biofilms in humans is not fully understood. Basaraba and Ojha [7] provide convincing arguments that extracellular *M. tuberculosis* in necrotizing lesions likely grows as biofilms. Hence, mycobacterial biofilms may participate in the process of caseous necrosis and cavitation formation in lung tissue [5–7].

M. Tuberculosis Biofilms on Metal Surface

The vast majority of studies investigating *M. tuberculosis* biofilms uses polystyrene plates [8]. Ha et al. [9] compared the adherence and the biofilm formation of *Staphylococcus epidermidis* (*S. epidermidis*) with those of *M. tuberculosis* on four types of metal segments. In contrast to *S. epidermidis*, *M. tuberculosis* rarely adhered to metal surfaces and showed discrete biofilm formation. Similar results were reported by Chen et al. [10] who compared *S. aureus* and *M. tuberculosis* in vitro and in vivo. Adetunji et al. [11] analyzed *M. tuberculosis* biofilm formations on cement, ceramic or stainless steel coupons. The experimental settings in this study are difficult to transfer in an in-vivo implant model (e.g., more biofilms were formed when media containing 5% liver extract was used). However, more biofilms were formed on cement than on ceramic and stainless steel coupons [11]. Taken together, the few available data from in-vitro and in-vivo studies indicate that biofilm formation of *M. tuberculosis* on metal segments is poor in comparison to Staphylococcus spp.

Among the 66 cases reported by Veloci et al. [12], 13 (19.6%) were treated with antitubercular agents only. Hence, in these cases no surgical intervention was performed to reduce the mycobacterial load or to remove mechanically the biofilm adhering to the implant. One patient died because of far-advanced tuberculous meningitis, miliary tuberculosis of the lungs, femoral osteomyelitis and extended cold abscesses along the femoral shaft [13]. In the other cases, no failure was reported. Though only in 6 (50%) of 12 cases, follow-up results of ≥18 months after the end of therapy was available. Treatment duration ranged from 6 to 18 months. These data indicate that tubercular biofilm eradication is possible with chemotherapy only. Whether this is due to poor biofilm formation on metal implants or due to effective anti-biofilm activity of antitubercular agents cannot be assessed.

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QUESTION 7: What is the role of the microbial synergy in polymicrobial infections?

RESPONSE: In polymicrobial infections, a complex environment may be formed in which microbiological interactions exist between microorganisms. Scientific evidence exists to show that combinations of bacterial species may exist whereby these can protect each other from antibiotic action via the exchange of virulence and antibiotic resistance genes, and this may be evident in adverse outcomes for polymicrobial orthopaedic implant-related infections. It is also probable that polymicrobial infections may be more likely in patients with poor immunity and tissue healing.

LEVEL OF EVIDENCE: Strong

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

PRE-MEETING RATIONALE

Varying incidences for polymicrobial infections have been reported with rates ranging from 6% to 37% [1–5]. The literature consistently demonstrates that patients with a polymicrobial infection demonstrate inferior treatment outcomes. Tan et al. reported that patients with polymicrobial periprosthetic joint infection (PJI) had a higher failure rate (50.5%) compared with monomicrobial PJI (31.5%) and a higher rate of amputation (odds ratio [OR] 3.80), arthrodesis (OR 11.06), and mortality (OR 7.88) [2]. Similarly, Wimmer et al. demon-

strated that the infection free rate after two years was 67.6% for polymicrobial infections vs. 87.5% for monomicrobial infections in a series of 77 polymicrobial PJIs [6]. In addition, Marculescu et al. demonstrated that the 2-year cumulative probability of success of polymicrobial PJIs was 63.8% compared to 72.8% for monomicrobial PJIs [7].

There are several explanations for the increased rate of failure in patients with polymicrobial PJI. Some explanations of polymicrobial infection include the following: the association with a sinus tract or a soft tissue defect; the frequent presence with difficult to treat organisms, such as Enterococcus spp and gram negatives [2,7,8]; increased comorbidities [2,7]; and microbial synergy.

Microbial synergy is defined as an interaction of two or more microbes in an infection site that results in enhanced disease by creating a more favorable condition for one another, compared to infections containing a single organism [9,10]. According to this definition, it can be appreciated that polymicrobial infection have less optimal outcome over that of monomicrobial infections because of the enhanced pathogen persistence in the infection site, increased disease severity and antimicrobial resistance [10,11]. While microbial synergy results in an enhancement of the disease, real experimental data supporting this phenomenon is still limited [12–14], which may be attributed to the complex and dynamic web of interactions that occur in natural systems [15].

Identified types of polymicrobial infections are due to: (1) changes in relative composition of individual species of microbiota [16]; (2) colonization of a pathogenic microbe of an infection site that already contains commensal microbes; and (3) colonization of a pathogenic microbe on a body they don't usually habit [17].

Several mechanisms of microbial synergy have been proposed in order to explain microorganisms interactions during polymicrobial infections: (1) metabolite cross-feeding: reported as the consumption of metabolic end-products by one of the microbial communities involved and optimization of local environment with the metabolic end-products [9,18,19]; (2) dedicated signaling systems: capacity of many microorganisms to communicate and coordinate activities as a group through low molecular weight signals, called "quorum sensing" [20]; (3) stimulation of resistance to the immune system: production of chemical substances that induce resistance to immune system like outer membrane proteins that inhibits immune pathways [9,18]; (4) suppression of the immune system by commensal bacteria: promotion of growth environment for commensal pathogens [9,21,22]; (5) direct contact: formation of biofilm by membrane-bound structures (adhesins) between microbes [23,24]; and (6) increased virulence of the organisms: production of substances that enhance the virulence of other bacteria [9].

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