

FIG. 1a. *Staphylococcus aureus* grown on membrane placed on agar containing cloxacillin at a concentration equal to 1/3 MIC of this staphylococcus.

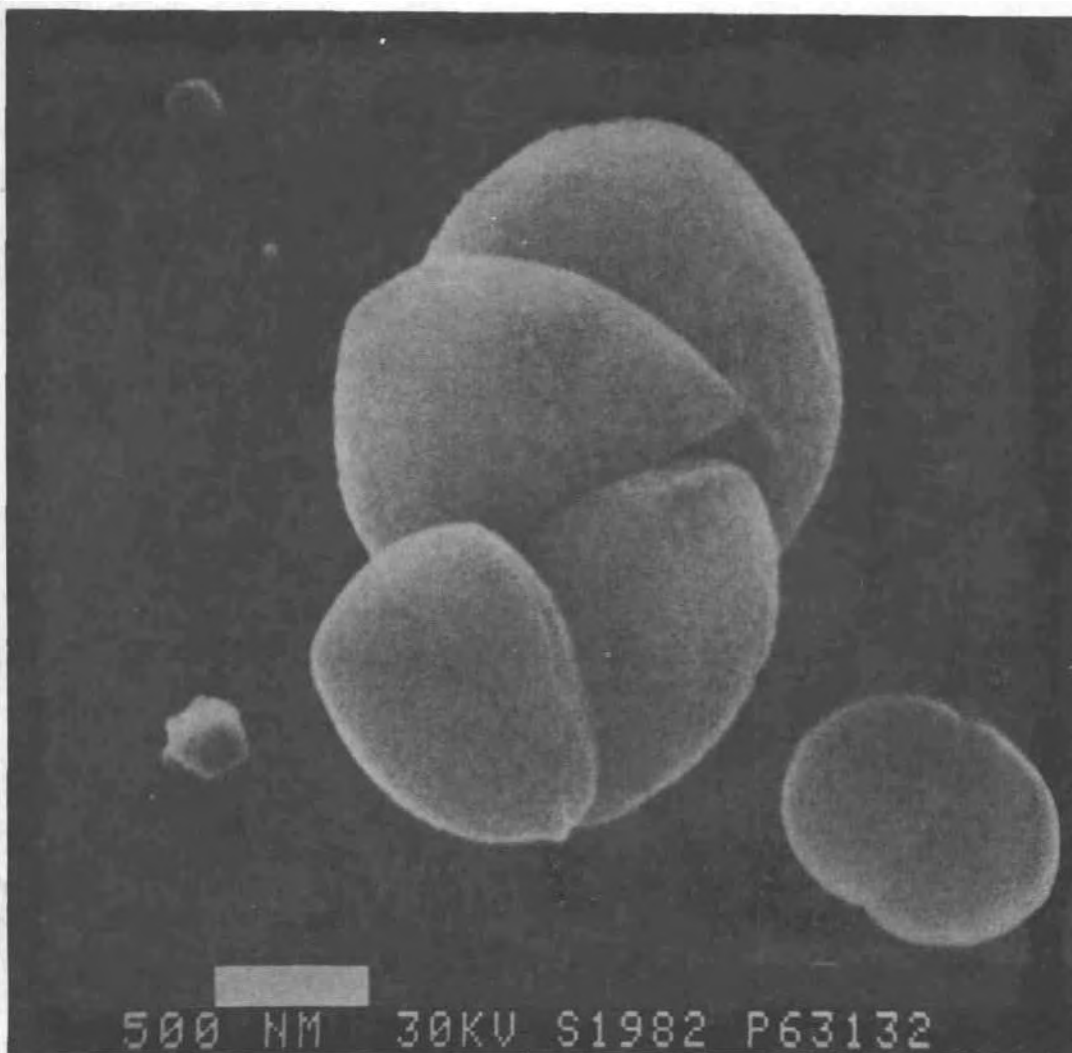


FIG. 1b. (Same as 1a). This staphylococcus has a mass of approximately 50 picograms.

forms of bacteria produced under the effects of low concentrations of antibiotics represent divisions without separation; the antibiotic agent did not inhibit genetic replication but inhibited cross wall formation and its lysis. These giant organisms planted on agar will produce each, one colony forming unit, when in reality each unit contains multiple genomes. While one staphylococcus has a mass of 1.5 picogram (pg) and an *Escherichia coli* has a mass of 3–4 pg, the large staphylococci produced with sub-MICs of antibiotics weigh 18–20 pg while filaments of *E. coli*, up to 100 pg. Since these large forms of organisms have been shown to exist *in vivo* when the host

was treated with a low dose of antibiotics, it must be recognized that bacterial mass could be more relevant than the bacterial number during the investigation of pathogenic effects to a host. Antibiotics at concentrations equal to the MBC eliminate the pathogenicity of any given bacteria inasmuch as these concentrations kill the offending organism. At concentrations equal to the MIC, the antibiotic completely inhibits the growth of the bacteria and, therefore, eliminates their pathogenicity. If antibiotics are successfully to influence certain factors, such as bacterial morphology and/or the synthesis and release of substances which contribute to bacterial virulence/pathogenicity

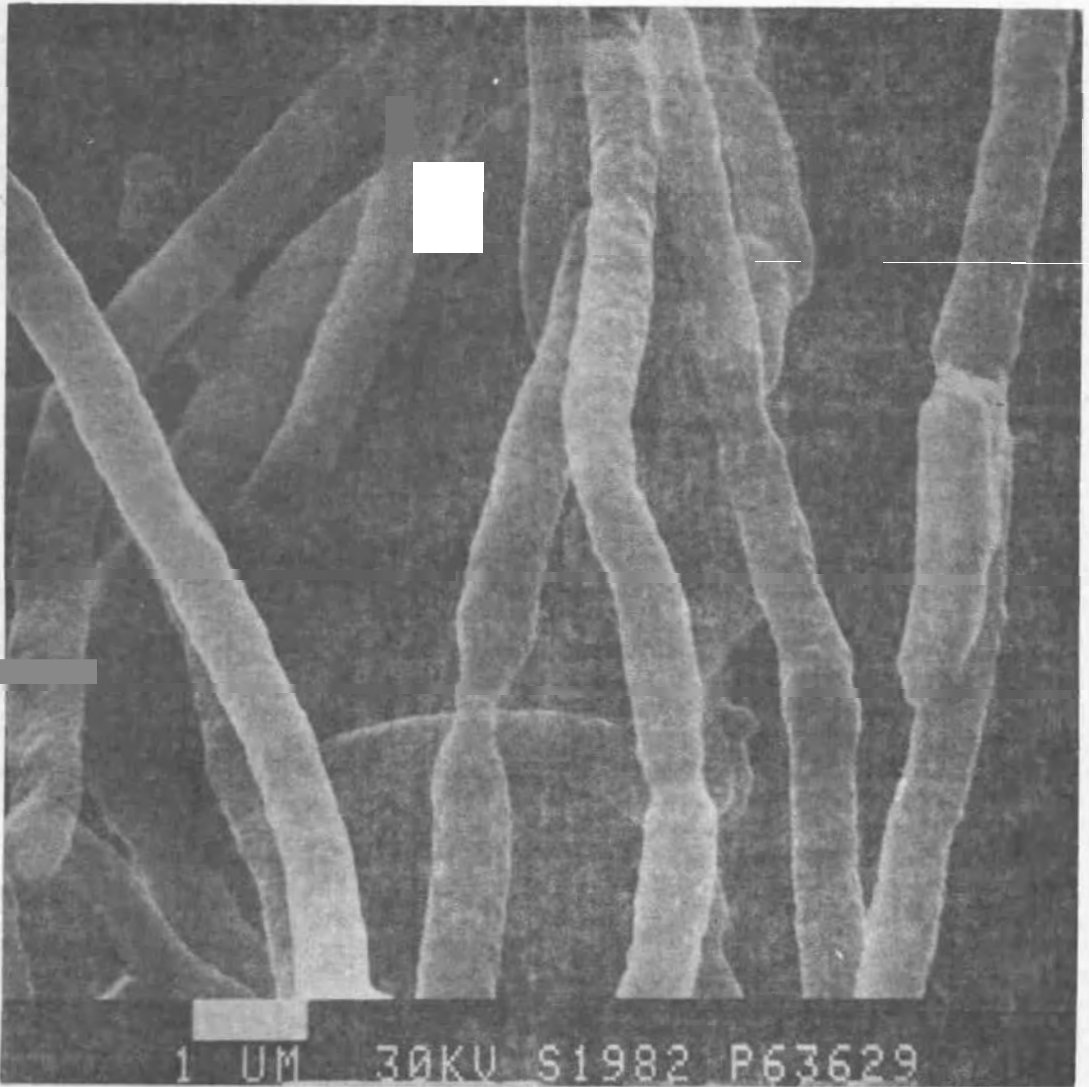


FIG. 2b. *Escherichia coli* grown on a membrane placed on agar containing ampicillin at a concentration equal to $1/3$ MIC of this *E. coli*. Each of these filaments has a mass of 30–100 picograms.

have been shown in experimental animals to have therapeutic efficacy. Zak and Kradolfer produced peritonitis in rabbits with *E. coli*.⁹ They showed that a $1/3$ MIC of gentamicin reduced mortality in a proportion of 80% as compared to a concentration above the MIC. A group of 120 patients with gram negative bacteremia were treated with gentamicin or tobramycin. It was later identified that 22 patients had aminoglycoside concentrations in the serum that were less than the MIC of the organism producing their infection. In fact, half of them had less than $1/4$ of the MIC of aminoglycosides. The overall

mortality for the entire study groups was 37% while the mortality rate among the group of patients with sub-MICs in their serum was 36%.¹⁰ Another example that antibiotics administered at very low dose and producing sub-MICs at the site of infection can result in lasting therapeutic effects is shown in a prospective study with a low dose of ampicillin in urinary infections produced with *E. coli*.¹¹

A total of 20 patients with symptomatic urinary tract infections produced by *E. coli* (10^5 cfu/ml of urine) and pyuria received 10 mg of ampicillin and 2 liters of fluid daily for three days. The ampicillin concentration in

Clostridium difficile has been implicated as the etiological agent in pseudomembranous colitis in humans. The organism produces an exotoxin within the gastrointestinal tract which may be detectable *in vitro* by cytopathic activity. Six clinical isolates of *C. difficile* from patients with proven pseudomembranous colitis were grown in trypticase nitrate broth (TNB) for 4 days in the presence of $\frac{1}{2}$ - $\frac{1}{4}$ the MIC of clindamycin; whereas the number of CFU remained unaffected, the *C. difficile* strains, actively produced high titers of toxin.^{21,22} A strain which could produce toxin *in vivo* but not *in vitro* could be induced to elaborate cytotoxin in culture when clindamycin was added to the medium.

The Growth Rate as a Pathogenicity Factor. Sub-MICs of various antibiotics have been shown to inhibit the rate of growth *in vitro*.²³ The number of infecting organisms is extremely important to the clinical outcome of urinary, lung and wound infections; therefore, a 90% reduction in bacterial populations by sub-MICs of antibiotics could have clinical consequences. Since the minimum antibiotic concentration (MAC) reflects the lowest-acting concentration of an antibiotic, the ratio MIC/MAC can be used to indicate the magnitude of the concentration range for effective activity.

INDIRECT EFFECTS OF ANTIBIOTICS ON PATHOGENICITY

Antibiotic resistant bacteria are equal or less pathogenic than the sensitive organism, never more virulent. A study of 188 clinical isolates of *S. aureus* showed that the strains resistant to five to seven antibiotics differed from those resistant to a smaller number of drugs. They were shown to produce less lecithinase, hyaluronidase and hemolytic activity.²⁴ Exposure of a large inoculum of *S. aureus in vitro* to progressive concentrations of gentamicin which exceeded the MIC resulted in aminoglycoside-resistant bacteria which grew as very small nonhemolytic colonies. Most were coagulase, deoxyribonuclease and mannitol negative. When injected into rats, they produced less extensive disease than the parent strain. Gentamicin-resistant staphylococci were also shown to be less virulent to mice since they produced a milder degree of pyelonephritis than the gentamicin-sensitive parental strains.²⁵ Gentamicin-resistant colonies of *S. aureus* were selected from clinical isolates and their infectivity and pathogenicity were compared to those of the sensitive variant of the same strain. In a rabbit model of

endocarditis it was shown that the mortality rate was 10% for the rabbits injected with gentamicin-resistant staphylococci but 75% for the animals injected with the sensitive parent strain.²⁶ A study of 43 newborn infants infected with *S. aureus* showed no difference in pathogenicity between gentamicin-sensitive and gentamicin-resistant strains. A 6-year study at the Mayo Clinic showed that, of 144 infections with *P. aeruginosa*, 21% of strains were resistant to gentamicin. The pathogenicity of these gentamicin-resistant isolates appeared to be less than that of susceptible organisms. The number of bacteremias produced by resistant organisms was significantly smaller than that produced by the sensitive strain.²⁷ In a rat model, gentamicin-sensitive *P. aeruginosa* (MIC less than 10 ug/ml) were shown to produce a significantly higher mortality than medium-sensitive and highly resistant *P. aeruginosa* strains (MIC 12.5-312 ug/ml). A slower rate of growth in gentamicin-resistant strains of *P. aeruginosa* was observed in both the lag and log phase than in sensitive strains. The decreased pathogenicity of gentamicin-resistant strains was attributed also to the decreased ability of the organism to produce heat-labile toxic components. Streptomycin-resistant mutants of *Pseudomonas phaseolytica* were also less virulent. In a mouse model, the pathogenicity of fosfomicin-resistant *Salmonella enteritidis* was found to be 1000 times less than that of sensitive strains. Resistance to erythromycin also reduced pathogenicity.

Clostridium perfringens resistant to clindamycin showed changes in morphology and biochemical activity, a decrease in the lecithinase and a decrease in pathogenicity for animals. INH-resistant *Mycobacterium tuberculosis* is usually catalase-negative. It is also less virulent or completely avirulent for guinea pigs. The loss of pathogenicity is related to the loss of catalase which would otherwise inactivate the bactericidal effects of peroxides in the guinea pig tissues.

Gonococci isolated from urogenital lesions have developed some drug resistance, but gonococci causing disseminated infection have retained exquisite sensitivity to penicillin. The most virulent strains have been found to be the most sensitive.²⁸ One of the mechanisms of resistance is an alteration of bacterial permeability. In some cases antibacterial agents are prevented from gaining access to their sites of action or are excreted from the bacteria at a significantly higher rate. Such alterations in the permeability of resistant bacteria also affect the penetration

