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Stenotrophomonas maltophilia: an Emerging Global Opportunistic Pathogen

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INTRODUCTION

Clinical microbiologists have long recognized the importance of identifying infectious microbial pathogens as the cause of disease in humans. The emergence of new multiple-drug-resistant (MDR) organisms (MDROs) found in nonclinical environments, the increasing reports of community-acquired infections, and the spread of these pathogens in the clinical setting have all underscored the need to monitor these organisms. The increase in reported cases of MDRO-associated infections has resulted in efforts to examine possible sources of these pathogens, assess the current antimicrobial strategies used for the treatment of infections, and elucidate the molecular mechanisms used by these pathogens during infection and disease.

Gram-negative bacterial pathogens have received much attention, as they are often MDROs due to multidrug resistance pumps, plasmids harboring antibiotic resistance genes, and various gene transfer mechanisms involved in the acquisition of antimicrobial resistance. *Pseudomonas aeruginosa* is an example of such an MDRO that causes respiratory infections in patients, particularly those with cystic fibrosis (CF) or those with chronic lung diseases. *P. aeruginosa* has been reported to survive for months on dry surfaces (180), and it is able to persist and grow in contaminated antimicrobial hand soap containing triclosan, making it a significant issue of concern for hospital staff (192).

Stenotrophomonas maltophilia is an environmental global emerging Gram-negative MDRO that is most commonly associated with respiratory infections in humans. It can cause various serious infections in humans. This current review focuses on the strategies used or being developed to treat infections associated with *S. maltophilia*; the cellular and molecular mechanisms important for its survival, persistence, and pathogenesis; and its mul-

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TABLE 1 *S. maltophilia*-associated infections

Infection	Reference(s)
Pneumonia	115, 310
Acute exacerbations of chronic obstructive pulmonary disease	101, 247
Bloodstream, bacteremia	14, 162, 167, 182, 187, 231, 236, 323, 357, 372
Soft tissue and skin	33, 297, 343, 372
Cellulitis/myositis	94
Osteomyelitis	191
Catheter-related bacteremia/septicemia	97, 188, 310, 372, 376
Meningitis	243, 284, 375
Endophthalmitis/keratitis/scleritis of the eye; dacryocystitis	4, 59, 161, 202, 224, 262, 370
Endocarditis	19, 135, 171, 237, 326
Urinary tract infection	342
Biliary sepsis	261

tiantibiotic resistance and provides a comparison of clinical and environmental *S. maltophilia* isolates.

HISTORICAL AND CLINICAL SIGNIFICANCE OF *S. MALTOPHILIA*

S. maltophilia was first isolated in 1943 as *Bacterium bookeri* and then named *Pseudomonas maltophilia* (154); later, rRNA cistron analysis determined that it was more appropriately named *Xanthomonas maltophilia* (259, 260, 325). In a large study of *Xanthomonas* strains, an analysis of 295 phenotypic characteristics resulted in 7 strains being identified as *X. maltophilia*, with 2 of these 7 being type strains of *Pseudomonas betle* and *Pseudomonas hibiscicola* (341). There is ongoing debate about nomenclature. DNA-rRNA hybridization studies and sequencing and mapping of PCR-amplified 16S rRNA genes have resulted in the classification and naming of *X. maltophilia* as *S. maltophilia* (79, 210, 242, 259).

S. maltophilia is not a highly virulent pathogen, but it has emerged as an important nosocomial pathogen associated with crude mortality rates ranging from 14 to 69% in patients with bacteremia (162, 346). For information about the attributable mortality of *S. maltophilia* infections, the reader is referred to a recent review of the literature (106). The variety of infections associated with *S. maltophilia* is shown in Table 1. Infections associated with *S. maltophilia* include (most commonly) respiratory tract infections (pneumonia [115, 310] and acute exacerbations of chronic obstructive pulmonary disease [COPD] [101, 247]); bacteremia (182, 187, 236); biliary sepsis (261); infections of the bones and joints, urinary tract, and soft tissues (33, 191, 297, 343); endophthalmitis (4); eye infections (keratitis, scleritis, and dacryocystitis [202, 224, 370]); endocarditis (19, 135, 171, 237, 326); and meningitis (243, 284). *S. maltophilia* is a significant pathogen in cancer patients, particularly those with obstructive lung cancer. This review will not address in detail infections of *S. maltophilia* in cancer patients, and the reader is directed to three recent articles that address the implications of infection by *S. maltophilia* in cancer patients (285, 293, 344).

S. maltophilia is an environmental MDRO. It has been isolated from aqueous-associated sources both inside and outside the hospital/clinical setting (Table 2). *S. maltophilia* has been recovered from soils and plant roots, animals (29, 30, 31, 117, 138, 140, 141, 160, 163, 233, 265), invertebrates (286), water treatment and distribution systems (142), wastewater plants (158), sinkholes (75), lakes (279), rivers (239), biofilms on fracture surfaces in aquifers (159), washed salads (273), hemodialysis water and dialysate samples (15), faucets, tap water, bottled water (81, 316, 345, 363, 367), contaminated chlorhexidine-cetrimide topical antiseptic (369), hand-washing soap (176), contact lens solutions (116), ice machines (272), and sink drains (39). A significant feature of *S. maltophilia* is its ability to adhere to plastics and form bacterial films (biofilms). *S. maltophilia* has been identified on the surfaces of materials used in intravenous (i.v.) cannulae, prosthetic devices, dental unit waterlines, and nebulizers (80, 157, 188, 200, 228, 250).

TABLE 2 Sources of *S. maltophilia*

Setting	Reference(s)
Clinical/medical	
Hospital suction tubing	377
Electronic ventilator temp sensors, ventilator inspiratory/expiratory circuits	283
Central venous catheter	188, 228
Nebulizers	80
Endoscopes	179
Dental suction system hoses	250
Dental solid waste	347
Hemodialysis water and dialysate of renal units	15
Contaminated chlorhexidine-cetrimide disinfectant	369
Hand-washing soap	176
Irrigating solutions	4
Sink drains	39, 80, 81, 173
Faucets/faucet aerators, showerheads	80, 81, 173, 355, 363
Water fountain drains	40
Patients' medical charts	327
Cystic fibrosis patient cough-generated aerosols	351
Ice machine	85, 272
Tap water	15, 52, 80, 297, 316, 345
Water treated by filtration, reverse osmosis, UV exposure, or deionization	15
Microfiltered water dispensers	292
Nonclinical	
Plant rhizosphere	29, 30, 160, 233
Washed salads	273
Soda fountain machines	366
Yellowtail fish, snakes, goats, buffalo, West African dwarf crocodile	117, 138, 140, 141, 163, 265
Deep-sea invertebrates	286
Water treatment process and distribution system	142
Returned liquor from wastewater plant	158
Biofilms on fracture surfaces in aquifers	159
Sinkholes of the Yucatan Peninsula	75
Saline subterranean Lake Martel (Spain)	279
River water	239
Water fountain drains and sink drains	40, 81
Showerheads	109
Tap water and bottled water	81, 316, 318, 367
Microfiltered water dispensers	292
Home-use nebulizers of CF patients	157
Contact lens stock solutions	116

The incidence of *S. maltophilia* hospital-acquired infections is increasing, particularly in the immunocompromised patient pop-

ulation, and cases of community-acquired *S. maltophilia* have also been reported. *S. maltophilia* infections can occur in both children and adults. The transmission of *S. maltophilia* to susceptible individuals may occur through direct contact with the source. The hands of health care personnel have been reported to transmit nosocomial *S. maltophilia* infection in an intensive care unit (ICU) (307). *S. maltophilia* has been cocultured with *P. aeruginosa* in respiratory samples obtained from CF patients. Cough-generated aerosols from CF patients have the potential to provide airborne transmission of *S. maltophilia* (351).

Molecular analyses, including ribotyping, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), and enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR), have revealed considerable heterogeneity among *S. maltophilia* isolates (50, 57, 123, 337, 338). *S. maltophilia* clinical isolates have a higher rate of mutation than environmental isolates, suggesting that clinical isolates adapt to their local environment, e.g., within different areas of the lungs of CF patients (31). It was proposed that antibiotic resistance gene acquisition by *S. maltophilia* strains occurs in the environment, and upon gaining access to the clinical setting, the strains retain the gene(s) (30). These observations emphasize the need to continue the current monitoring of reported cases of *S. maltophilia*, the emergence and spread of antibiotic resistance, and the identification of *S. maltophilia* isolates from sources within and outside the hospital setting.

Hospitals in several different countries perform surveillance on infections due to *S. maltophilia* (44, 98, 110, 165, 187, 207, 230, 232, 278, 357, 372). A U.S. multiple-hospital study of patient infections in the ICU during 1993 to 2004 reported *S. maltophilia* as being among the 11 most frequently recovered organisms (4.3% of a total of 74,394 Gram-negative bacillus isolates) (207). A study of bacteremia in adult patients in a medical center in northern Taiwan during 1993 to 2003 reported that risk factors associated with mortality for patients with *S. maltophilia* bacteremia included ICU stay ($P = 0.042$), central venous catheter (CVC) use ($P = 0.003$), and mechanical ventilation ($P = 0.008$) (357). During 1993 to 2003, a study of bacteremic pediatric patients in a university hospital in Taiwan indicated that risk factors associated with mortality in patients with *S. maltophilia* bacteremia included malignancy ($P = 0.049$), failure to remove the central venous catheter ($P = 0.021$), and a lack of effective antibiotic treatment ($P = 0.05$) (372). A study during 1993 to 2003 of adult patients with *S. maltophilia* bacteremia in two hospitals and a medical center in Taiwan identified thrombocytopenia ($P = 0.001$) and *S. maltophilia* shock ($P = 0.013$) as independent risk factors for mortality (187). In a U.S. study of CF sputum microbiology from 1995 to 2008, the prevalence of *S. maltophilia* increased from 6.7% to 12.0% ($P = 0.01$), and *S. maltophilia* was recovered more often from patients with $<$40% than from those with $\geq$40% predicted forced expiratory volume in 1 s (FEV_1) ($P = 0.07$) (98). The data from the CF Foundation Patient Registry from 1995 to 2005 revealed a significant increase ($P \leq 0.001$) in the incidence (range = 3.0 to 13.8%) and prevalence (range = 7.0 to 16.4% increase) of *S. maltophilia* across all age groups of patients studied (age, 0 to $>$25 years) (278). Data from the SENTRY Antimicrobial Surveillance Program during 1997 to 2008 revealed a 3.1% rate of recovery of *S. maltophilia* from hospitalized patients with pneumonia from 2004 to 2008, with regional recovery rates of 3.3% for the United States, 3.2% for Europe, and 2.3% for Latin America (165). In a British study of adult CF patients during 1985 to 2005, the proportion of patients harboring *S. maltophilia* in their respiratory tract increased ($P = 0.02$) over the study period from 1 to 4% and was higher ($P = 0.029$) in patients aged 16 to 25 years (7%) than in patients $>$25 years old (4%) (232). In the 2004 SENTRY Antimicrobial Surveillance Program, among pediatric patient isolates, *S. maltophilia* was among the top 15 pathogens isolated from North America and Latin America but not from Europe (110). Surveillance of Antimicrobial Use and Antimicrobial Resistance in German Intensive Care Units (SARI) monitored *S. maltophilia* as one of the 13 most important organisms associated with nosocomial infections; multivariate analyses of the data from 2003 to 2004 indicated that the use of carbapenems ($P = 0.01$) and being in an ICU with $>$12 beds ($P = 0.037$) were risk factors for *S. maltophilia* infection (230).

MICROBIOLOGY

Characteristics of *S. maltophilia*

S. maltophilia is a Gram-negative obligate aerobe that is rod shaped and motile with a few polar flagella. It is able to persist in nutrient-poor aqueous environments (Table 2). The growth characteristics of *S. maltophilia* are shown in Table 3. Standard microbiology reference data currently indicate that *S. maltophilia* is an oxidase-negative bacterium. Recent data, however, suggest that some *S. maltophilia* isolates are oxidase positive (48).

Burdge et al. reported the misidentification of *S. maltophilia* as *Pseudomonas cepacia* (42). In that study, 3 (9%) of 32 clinical isolates were incorrectly identified as being *P. cepacia* isolates as a result of a delayed reading (3 min instead of within 1 min) of the oxidase test and not holding the tests for DNase production 72 h prior to observation of the results. The misinterpretation of these tests has clinical importance, as *P. cepacia* is a significant pathogen in CF patients.

S. maltophilia has been coisolated with other microorganisms (e.g., *Pseudomonas aeruginosa*, *Burkholderia* species, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Enterococcus* species, *Bacteroides* species, *Corynebacterium* species, and *Candida albicans*) in samples recovered from patients (14, 134, 187, 333, 357). The nonfermenting Gram-negative bacteria *P. aeruginosa*, *A. baumannii*, and *S. maltophilia* are all pathogens of the human respiratory tract. The reader is directed to recent publications for further information about the relationship of *S. maltophilia* to *P. aeruginosa* and *A. baumannii* (35, 205, 309). Selective agar media have been designed to improve the isolation of *S. maltophilia* from polymicrobial cultures (77, 235).

To improve the isolation of *S. maltophilia* from CF patient sputum samples, VIA medium, containing vancomycin, imipenem, and amphotericin B, was developed (77). VIA medium consists of a mannitol agar base with a bromothymol blue (BTB) indicator, 5 mg/liter vancomycin, 32 mg/liter imipenem, and 4 mg/liter amphotericin B. A comparison of *S. maltophilia* colony counts recovered from sputum samples on VIA medium with counts on bacitracin (10,000 U/liter) chocolate (BC) medium revealed that VIA medium detected a higher ($P < 0.0001$) number of *S. maltophilia*-positive samples than BC medium with an imipenem disk on its surface. VIA medium was particularly useful for the detection of low colony counts (10^2 to 10^6 CFU/ml) (77).

Gram-negative selective agar (GNSA) medium was later developed by Moore et al. (235) to detect Gram-negative microflora in

TABLE 3 Growth characteristics of *S. maltophilia*[a]

Growth characteristic	Reaction
Straight or curved rods, 0.5 by 1.5 μm	
Oxidase	+/−
Catalase	+
Methionine is required for growth	+
Optimum growth temp of 35°C	
No growth at 4°C or 41°C	
Survival at refrigeration temp	+
Motility	+
Nitrate reduction, but nitrate is not used as nitrogen source	+
Indole	−
Lysine decarboxylase	+
Ornithine decarboxylase	−
Methyl red	−
Voges-Proskauer reaction	−
Hydrogen sulfide	−
Citrate	v
Phenylamine deaminase	−
β-Galactosidase (ONPG)	v
Carbohydrate utilization	
Acid production from maltose	+
Acid production from glucose	−
Carbon source for growth	
Adonitol	−
Arabinose	−
β-Hydroxybutyrate	−
Cellobiose	v
Dulcitol	−
Glucose	+
Fructose	v
Galactose	v
Lactose	+
Maltose	+
Mannitol	−
Mannose	v
Rhamnose	−
Salicin	−
Sorbitol	−
Trehalose	+/−
Esculin hydrolysis	+
Gelatin liquefaction	+
Tween 80 hydrolysis	+
DNase production	+
Starch hydrolysis	−
Urea hydrolysis	−

[a] +, >85% of strains positive; v, 16 to 84% of strains positive; −, ≤15% of strains positive; ONPG, *o*-nitrophenyl-β-D-galactopyranoside. Data are from references 48, 79, 169, 258, and 367.

CF patient sputa. GNSA medium contains novobiocin (5 mg/liter), cycloheximide (100 mg/liter), amphotericin (2 mg/liter), nisin (48 mg/liter), and crystal violet (2 mg/liter) and detects 6.70×10^3 CFU of *S. maltophilia*/ml sputum. Other Gram-negative organisms recovered from adult CF patients and able to grow on this selective medium include *P. aeruginosa*, *Burkholderia cepacia*, *E. coli*, and *Alcaligenes xylosoxidans* (235). This medium is useful for high-throughput specimen screening, as it is compatible with semiautonumeration using digital image capture and processing with transilluminal white light.

Culture media have been developed to differentiate between the bacterial species present in mixed culture samples (e.g., colony color differences between *S. maltophilia* and *P. aeruginosa* reflect their different metabolic abilities). The production of acid from maltose but not from glucose by *S. maltophilia* has been used to distinguish it from *P. aeruginosa*, as *P. aeruginosa* produces acid from glucose and does not use maltose or lactose to a great extent. Colonies of *S. maltophilia* appear yellow and blue on BTB-containing medium containing maltose and glucose, respectively, in contrast to *P. aeruginosa* colonies, which appear blue on BTB medium containing maltose and yellowish green on medium containing glucose (169). A selective and differential agar medium, SM2i, contains Mueller-Hinton agar supplemented with maltose, *dl*-methionine, vancomycin, imipenem, amphotericin B, and bromothymol blue (3). *S. maltophilia* colonies are smooth, round, and green, with an olive green center with a peripheral lighter green area or a dark green center with an olive green peripheral area surrounded by a blue-green halo. The colony appearance of *S. maltophilia* is easily distinguished from those of other Gram-negative bacteria, such as *P. aeruginosa*, which appears white or colored but very often silver, or *E. faecium*, which appears minute and colorless (3). In one study, this medium was successfully used to recover *S. maltophilia* from water samples and cotton swab samples of cold water taps (3). Another study using this medium resulted in an increased awareness by health care workers of the importance of strict adherence to hand hygiene measures, the use of point-of-use (POU) water filtration, and regular maintenance of swan-necked faucets with a regimen of descaling, disinfection, and drying (2).

S. maltophilia may be associated with polymicrobial infections or grow slowly in the host, resulting in some difficulty in isolating this bacterium. Various molecular biology techniques have been used to identify different strains of *S. maltophilia*. PCR amplification of the 16S rRNA gene has been used to detect *S. maltophilia* in blood samples of patients undergoing chemotherapy for acute leukemia or myelodysplastic syndrome (238). That study suggested that PCR analysis of blood would be useful for cases where the bacterial species grows poorly in blood culture medium.

HOST INFECTIONS

Nosocomial and Community-Acquired Infections

S. maltophilia is a waterborne organism, and exposure to this bacterium can occur both in and outside the clinical setting. In the health care environment, *S. maltophilia* has been isolated from several sources, including suction system tubing of dental chair units (DCUs) (250), contaminated endoscopes (179), and tap water (297), all of which present possible patient exposure sources. *S. maltophilia*-contaminated central venous catheters and tap water faucets have been implicated in cutaneous and soft tissue infections in patients with neutropenia (297).

Patients undergoing hemodialysis can be infected by bacterial pathogens or endotoxins through contaminated dialysis machine units (356, 362). MDR *S. maltophilia* isolates have been recovered from hemodialysate, tap water, and treated water samples (15). Treatments of the water included softeners and sand filters, reverse osmosis, bacterial filtration, UV exposure, deionization, and double reverse osmosis. The membrane filter technique was used to detect bacteria present in the samples. Several *S. maltophilia* isolates demonstrated resistance to newer-generation cephalosporins (37% and 58% of the isolates were resistant to ceftazidime and cefepime, respectively) (15). These observations indicate the need

for quality control and assurance measures to screen untreated water, treated water, and dialysate for the presence of *S. maltophilia*. Monitoring of these samples should lead to the more effective disinfection of dialysis machine units.

Central venous catheter (CVC)-related *X. maltophilia* infections have been reported (97). The organism can contaminate the infusate and adhere to the catheter, forming biofilms on the catheter surface. In a study of 149 episodes of septicemia in 131 patients from 1972 to 1986, *X. maltophilia* was the bacterium most commonly isolated in monomicrobial (46%) and polymicrobial (75%) septicemias. That study and several others suggested that the removal of the CVC is essential for the successful treatment of *S. maltophilia* catheter-associated bacteremia, along with antibiotic therapy (14, 97, 187, 188, 372).

CVC-related *S. maltophilia* bacteremia and associated relapsing bacteremia were reported in a study of hematology and oncology patients (188). Nosocomial bacteremia, prior antibiotic therapy, immunosuppressive therapy, and neutropenia were clinical characteristics associated with CVC-related *S. maltophilia* bacteremia. By univariate analysis, two risk factors were revealed, long-lasting neutropenia and failure to remove the CVC upon the initial diagnosis of bacteremia. RAPD analysis of five patients revealed that relapses resulted in recurrent bacteremia.

S. maltophilia CVC-associated infections have been reported for hematopoietic stem cell transplantation (HSCT) recipients (376). These recipients are at a high risk for infection as a result of prolonged neutropenia and breach of the mucocutaneous barrier. Results of a 4-year study of 570 adult patients at the Chaim Sheba Medical Center in Israel indicated that 3.3% of all HSCT patients had *S. maltophilia* isolated from culture samples. Seventeen patients had a CVC during the infection; 15 had bacteremia, and the 2 other patients had different invasive infections. Polymicrobial blood and soft tissue infections were demonstrated for 58% of the patients. *S. maltophilia* was found to be present in addition to coagulase-negative staphylococci, *Corynebacterium* species, *Pseudomonas* species, *Acinetobacter* species, and *Candida* species. After the identification of the organism as *S. maltophilia*, antibiotic therapy was determined by isolate susceptibility; treatment included high-dose ($>$15 mg kg^{-1} of body weight day^{-1}) trimethoprim-sulfamethoxazole (TMP-SMX), ofloxacin, or both. Six patients (31.5%) with *S. maltophilia* bacteremia died. The authors of that study emphasized the importance of proper hand hygiene and handling of the CVCs and encouraged the immediate removal of the CVC upon the initial diagnosis of infection (376).

Bloodstream infections by *S. maltophilia* have been reported to occur during extracorporeal membrane oxygenation used for $>$48 h in adult patients (323). A university hospital study from 1996 to 2007 reported nosocomial infections in respiratory and cardiac support for patients. Of 334 patients, 16.7% had *S. maltophilia* isolated from blood cultures. That study provided evidence that the use of extracorporeal membrane oxygenation can pose an *S. maltophilia* infection risk.

Point-of-use (POU) water filtration has significantly reduced ($P = 0.0431$) health care-associated Gram-negative bacterial infections in bone marrow transplant (BMT) recipients (52). Microbiological screening of 4 unfiltered hospital tap water outlets in the bone marrow transplant unit of a major U.S. teaching hospital identified *P. aeruginosa* in 2 of 4 outlets and *S. maltophilia* in 1 of 4 outlets. Clinical infection rates decreased ($P = 0.0068$) from 1.4 to 0.18 per 100 patient-days in the 9-month period during which the filters were in place. Patient infections during the use of filters were due to coagulase-negative *Staphylococcus* species and *E. coli*. That study provided evidence that POU water filtration may effectively reduce the infection risk for BMT recipients (52). Ultramicrocells (UMC) of *S. maltophilia* are able to pass through a 0.2-μm filter (316). More research is needed to determine the clinical significance of this finding and establish if UMC of *S. maltophilia* are present in hospital tap water. The use of a 0.1-μm filter was suggested to provide better performance for the retention of waterborne bacteria (324).

S. maltophilia is not solely a nosocomial pathogen. There have been reports of *S. maltophilia* associated with community-acquired infections. Studies have identified sink drains, faucets, water, and sponges, etc., as environmental sources of *S. maltophilia* in the homes of colonized and noncolonized CF patients (81); these observations of this opportunistic pathogen are particularly significant for CF or immunocompromised patients.

Community-acquired *S. maltophilia* (defined as infections that occurred 48 or 72 h prior to hospitalization) have been reported for child and adult patients and include bacteremia, ocular infections, respiratory tract infections, wound/soft tissue infections, urinary tract infections, conjunctivitis, otitis, and cellulitis (105). It is common to find that most patients with these *S. maltophilia* infections have some form of comorbidity (e.g., COPD, trauma, central venous catheter, prior antibiotic use, malignancy, prior hospitalization, HIV infection, or other immune suppression).

S. maltophilia can grow and form biofilms in potable water distribution systems, presenting a possible risk of infection for immunocompromised individuals. A recent study compared the abilities of two disinfectants to prevent the contamination of microfiltered water dispensers with *S. maltophilia*. Following the inoculation of water lines with *S. maltophilia*, the lines were disinfected with 10% peracetic acid (PAA) or with 3% hydrogen peroxide. Each line received 3 cycles of disinfection contact times of 10, 30, and 40 min. Disinfection with 10% peracetic acid temporarily reduced the number of bacterial cells up to 2 days posttreatment. After a 40-min contact time, disinfection with 3% hydrogen peroxide was more effective than disinfection with 10% peracetic acid and reduced the number of *S. maltophilia* cells to $<$1 log CFU/100 ml. In that study, *S. maltophilia* was more tolerant to disinfection than *P. aeruginosa*; this is thought to be due in part to a higher level of catalase activity demonstrated by *S. maltophilia* (292). It has been reported that hydrogen peroxide is effective against biofilm growth in dental chair unit waterlines (249).

It is important to identify these environmental sources, as these observations have suggested preventative measures to control the contamination of water supplies with *S. maltophilia* (e.g., the use of filter units and the treatment of water dispensers with peracetic acid and hydrogen peroxide) and increased awareness of the limitations of some of these measures.

Polymicrobial infections with *S. maltophilia* and other organisms such as *P. aeruginosa* in the CF lung environment have been reported (203, 204). Recent studies investigating these organisms for their adherence to and invasion of human bronchial epithelial cells will be described below (see "*S. maltophilia* and the Cystic Fibrosis Lung Environment"). Future research is needed to determine the specific interactions of *S. maltophilia* with other microorganisms during infection and disease.

Risk Factors and Determining Risk of Infection

To prevent the transmission of *S. maltophilia* to susceptible individuals, it is important to identify risk factors for infection by this bacterium. Infection risk assessment must consider criteria that include infectious dose, host immune status, pathogen status, and the ability of the pathogen to cause infection.

Risk factors for *S. maltophilia* infection include underlying malignancy (44), the presence of indwelling devices (e.g., catheters [44, 228]), chronic respiratory disease, immunocompromised host (44), prior use of antibiotics (13, 228), and long-term hospitalization or ICU stay (187). Risk factors for *S. maltophilia* infection-associated mortality include malignancy, severe septic shock, and organ failure (120).

Immunocompromised individuals are at a significant risk for infection by *S. maltophilia*. Orointestinal mucosal damage resulting from anticancer therapies (185), graft-versus-host disease (185), and diarrhea (13) have been reported to be risk factors for infection with *S. maltophilia*. In a Japanese study (14), prognostic factors associated with mortality were neutropenia ($P = 0.008$) and polymicrobial bacteremia with enterococci ($P = 0.022$) (14). Risk factors for mortality of patients with nosocomial *S. maltophilia* pneumonia included stay in an ICU ($P = 0.018$), malignancy ($P < 0.001$), renal disease ($P = 0.001$), and inadequate initial empirical antibiotic therapy ($P = 0.001$) (333).

A study of patients and environmental surfaces in two U.S. pediatric chronic-care facilities identified risk factors for colonization by *S. maltophilia* and other antibiotic-resistant Gram-negative bacteria. A strong association ($P \leq 0.01$) with colonization by these bacteria was observed for patients with prosthetic devices (200). That study also revealed that pediatric patients living in chronic-care facilities can serve as sources of antibiotic-resistant Gram-negative bacteria.

S. maltophilia is emerging as a significant pathogen worldwide, and there is a need to continue to monitor its antibiotic resistance, persistence, and spread within the community and health care settings.

TREATMENT OF INFECTIONS

Emergence of Antibiotic Resistance

S. maltophilia exhibits resistance to a broad array of antibiotics, including TMP-SMX, β-lactam antibiotics, macrolides, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, chloramphenicol, tetracyclines, and polymyxins. The low membrane permeability that contributes to resistance to β-lactams including cefepime, ticarcillin-clavulanate, ceftazidime, and piperacillin-tazobactam (5, 68, 229) and the presence of chromosomally encoded multidrug resistance efflux pumps (6, 11, 54, 129, 196, 198, 269, 383), β-lactamases (9, 17, 18, 227, 295, 296, 352, 353), and antibiotic-modifying enzymes (174, 190, 195) all contribute to the intrinsic antibiotic resistance of *S. maltophilia* (298). The intrinsic resistance of *S. maltophilia* was suggested to have been acquired in natural nonhuman environments and is not due solely to the use of antibiotics in medical/clinical settings (218, 298). Environmental intrinsically resistant bacteria such as *S. maltophilia* were suggested to use their metabolic machinery to detoxify and break down harmful compounds (including antibiotics) (218). The biochemical pathways used by these bacteria may enable the use of antibiotics as food sources (218). The contamination of the environment with antibiotics can enrich for antibiotic-resistant bacteria and provide an opportunity for the acquisition of drug resistance by other bacterial pathogens (49).

The drug resistance mechanisms are acquired by the horizontal transfer of antibiotic resistance through plasmids, transposons, integrons, integron-like elements, insertion element common region (IS*CR*) elements, and biofilms (17, 18, 27, 146, 198, 328). The molecular mechanisms of antibiotic resistance in *S. maltophilia* are described in more detail below (see "Antibiotic Resistance").

The Clinical and Laboratory Standards Institute provides guidelines for the testing of antimicrobial agents against *S. maltophilia* using dilution and disc diffusion methods (61, 62). *S. maltophilia* isolates exhibit differences in antimicrobial susceptibility to aminoglycosides when tested at different temperatures (e.g., 30°C and 37°C, with resistance typically observed at 37°C) (275). Differences in resistance rates for *S. maltophilia* have also been reported for observations after 24 h and 48 h of incubation (TMP-SMX, ciprofloxacin, ceftazidime, cefepime, piperacillin, and piperacillin-tazobactam demonstrated significant differences [$P < 0.05$]) (134). It is important to recognize that currently, there is no worldwide standardized guideline for antimicrobial agent testing. As a consequence, the MIC values assigned to antimicrobial agent resistance alter according to the standards used by different countries (e.g., those approved by the European Committee on Antimicrobial Susceptibility Testing and the European Medicines Evaluation Agency for European Chemotherapy) (165). This observation coupled with reports of differences in results obtained after using different methods to assess the susceptibility testing of *S. maltophilia* isolates (133, 199, 223, 244, 337, 357, 372) highlight the need to establish global standard guidelines that will facilitate the monitoring of antimicrobial-resistant organisms.

A comparison of the antimicrobial resistances of *S. maltophilia* isolates recovered from CF and non-CF (NCF) patients revealed that CF strains tended to be more resistant than non-CF strains, with significantly higher ($P < 0.05$) levels of resistance to piperacillin, cefotaxime, cefepime, moxalactam, ciprofloxacin, ofloxacin, sparfloxacin (4-μg/ml resistance breakpoint), gatifloxacin (4-μg/ml breakpoint), and doxycycline (8-μg/ml resistance breakpoint) (45). That study also revealed that isogenic and closely related CF strains displayed differences in susceptibilities to ticarcillin-clavulanate, moxalactam, ciprofloxacin, newer fluoroquinolones, doxycycline, and TMP-SMX.

In 2006, a study of *S. maltophilia* recovered from sputum samples of CF patients in a large German hospital demonstrated that only 34.4% of the isolates were susceptible to TMP-SMX, 25% were susceptible to ciprofloxacin, and all of them were resistant to imipenem (340). Superinfections caused by *S. maltophilia*, *P. aeruginosa*, and *K. pneumoniae* following the use of imipenem to treat patients with severe pneumonia have been reported (112). The treatment of patients with acute exacerbations of severe COPD with imipenem has been associated with a high (30.8%) risk of superinfection, including those caused by *S. maltophilia* and *P. aeruginosa* (91). The identification of imipenem as a risk factor for *S. maltophilia* infection has not been reported for all studies of *S. maltophilia* infections. In a large study of 759 patients, Carmeli and Samore (47) found no significant difference in the rates of acquisition of *S. maltophilia* infection for treatment with imipenem compared to treatment with ceftazidime.

The preferred treatment of *S. maltophilia* infections has been the use of the bacteriostatic compound TMP-SMX (119, 146). During 2004 to 2009, *S. maltophilia* clinical isolates recovered

TABLE 4 New treatment strategies for *S. maltophilia* infections

Antimicrobial approach	Mechanism(s)	Reference(s)
Antimicrobial peptides	Membrane disruption and cell lysis	211, 214, 215
Trimethoprim-sulfamethoxazole and tigecycline	Synergy of antimicrobials	100
Tigecycline and amikacin	Synergy of antimicrobials	100
Aerosolized colistin and doxycycline	Bactericidal combination therapy	371
Aerosolized levofloxacin	Bactericidal	121, 175
Tigecycline	Inhibition of protein synthesis	108
Moxifloxacin	Bactericidal	20, 89, 178, 267
Cationic compounds	Interaction with negative charges on cell membrane and cell wall resulting in disruption of binding sites	132
Nanoemulsions	Membrane fusion and cell lysis	206
Phage therapy	Cell lysis	53, 93
Plant oils	Unknown	103
EGCG from green tea	Membrane damage, inhibition of DNA gyrase	125, 130
Peptide inhibitor of β-lactamase	Inhibitor of β-lactamase L1	305
Triple-β-lactamase inhibitor combination		255

from the adult ICU of a tertiary care center in Saudi Arabia demonstrated major increases in resistance to gentamicin, while >90% of the isolates were susceptible to TMP-SMX (8). Recently, TMP-SMX was used in combination with ciprofloxacin to treat *S. maltophilia* meningitis in a preterm baby (284), but the emergence of resistance to TMP-SMX is forcing physicians to consider alternatives (7, 245, 328, 333, 357). Results from the SENTRY Antimicrobial Surveillance Program in 2004 showed a level of resistance to TMP-SMX of 3.8% for *S. maltophilia* (110), and results from the SENTRY Antimicrobial Surveillance Program in 1997 to 1999 showed a level of resistance of up to 10% across Europe (119).

Ticarcillin-clavulanate has been proposed as an alternate therapy to TMP-SMX, but resistance to ticarcillin-clavulanate has been reported. Antibiotic susceptibility testing of 66 *S. maltophilia* clinical isolates (from clinical specimens [respiratory tract, blood, urogenital tract, cutaneous-mucus specimens, and intravascular devices] in two university hospitals in Rouen, France, and Tunis, Tunisia) collected between 1994 and 1997 revealed that the percentage of isolates resistant to ticarcillin-clavulanate steadily increased from 19% (1995) to 32% (1996) to 42% (1997) (26). The increase in antibiotic resistance did not appear to correspond to antimicrobial use, as the amount of ticarcillin-clavulanate used decreased from 21.7 kg (1995) to 17.1 kg (1996) to 11.5 kg (1997). ERIC-PCR demonstrated a high level of heterogeneity among the *S. maltophilia* isolates, suggesting that the emergence of resistance to ticarcillin-clavulanate was not due to the spread of an epidemic strain but may have been a result of the increased usage of parenteral amoxicillin, amoxicillin-clavulanate, ticarcillin, and piperacillin-tazobactam, at rates of 20%, 58%, 116%, and 48%, respectively, across 1995 to 1997 (26). The levels of resistance to ticarcillin-clavulanate for *S. maltophilia* isolates have been reported to be 17.0% by the SENTRY Antimicrobial Surveillance Program in 2004 (110) and 40.9% and 60.9% in Brazil (244) and Latin American countries (108), respectively.

Antimicrobial cycling using broad-spectrum agents, including carbapenems, has been studied to detect a possible link to increased colonization or infection by *S. maltophilia*. A retrospective study (1992 to 2002) by a U.S. university hospital demonstrated no significant differences between cycling and noncycling periods with broad-spectrum agents including piperacillin-tazobactam, cefepime, and ciprofloxacin (257). No significant differences were noted for antibiotic cycling and noncycling periods in the surgical intensive care unit, medical intensive care unit, and other surgical/medical wards and incidence rates of *S. maltophilia* isolates, but the study did reveal a significant increase ($P = 0.01728$) in the rates of *S. maltophilia* infections in hospitals between 1993 (rate of 0.45 infections/1,000 patient days) and 2002 (rate of 0.57 infections/1,000 patient days) (257). As one would expect, antibiotic cycling was not linked to increased colonization rates of *S. maltophilia*.

New Treatment Strategies

An overview of new treatment strategies for *S. maltophilia* infections is presented in Table 4. A recent review addressed the use of new antimicrobial agents in cancer patients to treat infections of MDR bacteria, including *S. maltophilia* (285). There is ongoing debate about the use of monotherapy versus combination therapy to treat infections of *S. maltophilia*. New treatment strategies have included the use of select antibiotics in synergy. Using the checkerboard method, some synergism has been observed between tigecycline and TMP-SMX, and between tigecycline and amikacin, against *S. maltophilia* (100, 349). *In vitro* pharmacodynamic model results revealed that TMP-SMX in combination with either ciprofloxacin, ceftazidime, or tobramycin demonstrated higher bactericidal efficacy ($P < 0.0001$) against *S. maltophilia* clinical isolates than TMP-SMX alone (379). Synergy testing by Etest revealed that TMP-SMX plus ceftazidime and TMP-SMX plus ticarcillin-clavulanate demonstrated the highest level of synergistic activity against *S. maltophilia* isolates (133). The checkerboard method detected synergy for TMP-SMX plus ceftazidime in only 56% of these isolates and did not detect synergy for TMP-SMX plus ticarcillin-clavulanate in the isolates (133). Synergy or partial synergy was detected by the checkerboard method for combinations of ceftazidime plus ciprofloxacin and for TMP-SMX plus ticarcillin-clavulanate against *S. maltophilia* isolates (199). The effective treatment of those patients with *S. maltophilia* bacteremia and allergy or intolerance to TMP-SMX may be achieved by use of ciprofloxacin in combination with ticarcillin-clavulanate or ceftazidime (107). A combination of doxycycline and aerosolized colistin was successfully used to treat persistent *S. maltophilia* ventilator-associated pneumonia (*S. maltophilia* counts of 500,000

CFU/ml recovered from a bronchoalveolar lavage culture) when high-dose TMP-SMX therapy was ineffective (371).

A recent case report described an *S. maltophilia* isolate with extensive drug resistance (according to the terminology proposed by Falagas and Karageorgopoulos [104]) (306). The isolate colonized and formed biofilms on a bladder device in a patient with myelofibrosis (306). The isolate demonstrated resistance to TMP-SMX, tetracycline, tigecycline, β-lactams, fluoroquinolones, aminoglycosides, colistin, and erythromycin but was unusual in its susceptibility only to chloramphenicol and rifampin. These observations indicate that older antibiotics should still be considered for the treatment of *S. maltophilia* infections (306). Rifampin has shown synergy with gentamicin and carbenicillin in a triple-combination therapy against *S. maltophilia* (378). TMP-SMX and carbenicillin and rifampin together have also shown synergy against *S. maltophilia* (378). Bactericidal activity can be observed when rifampin is used in combination with ofloxacin and ceftazidime (331).

Moxifloxacin shows some promise for the treatment of MDR *S. maltophilia* infections (20, 89, 178, 267). An *in vitro* pharmacokinetic-pharmacodynamic model used to assess the activity of ciprofloxacin and moxifloxacin against *S. maltophilia* suggested that maximum tolerable doses are needed to overcome resistant bacterial populations (20). The use of these antibiotics at concentrations greater than the MIC is needed for the treatment of systemic *S. maltophilia* infections. An *in vitro* assessment of the postantibiotic effect (PAE) of moxifloxacin revealed that the exposure of 20 *S. maltophilia* clinical isolates to high (8× and 10× MIC) concentrations of moxifloxacin resulted in an increased delay of cell growth posttreatment. A PAE of ca. 4 h was achieved, and for some isolates of *S. maltophilia*, the PAE exceeded 24 h (178). The activity of subinhibitory concentrations of moxifloxacin against cell viability in biofilms and against preformed biofilms is addressed in more detail below (see "Antibiotic Resistance").

In vitro studies of 1,586 isolates of *S. maltophilia* recovered from global medical centers revealed that isolates were susceptible to tigecycline (95.5% at ≤2 μg/ml) and to TMP-SMX (96.0% at ≤2 μg/ml trimethoprim and 38 μg/ml sulfamethoxazole) (108). Tigecycline demonstrated activity against 938 *S. maltophilia* isolates from North America and Europe (MIC_{50} = 1 μg/ml and MIC_{90} = 2 μg/ml; 94.5 to 95.3% susceptible) and against 648 isolates from the Asia-Pacific region and Latin America (MIC_{50} = 0.5 μg/ml and MIC_{90} = 2 μg/ml; 96.1 to 96.5% susceptible) (108).

Cationic peptides (e.g., esculin-1b) from amphibians can increase the outer membrane permeability of *S. maltophilia* (211, 214). The reported rate of resistance to these antimicrobial peptides (AMPs) is lower than the rate of resistance demonstrated for conventional antibiotics (181). These observations suggest that there is the potential for future treatments to take advantage of the combination of cationic peptides with conventional antibiotics.

The N-terminal region [Esc(1-8)] of the esculentin-1b peptide isolated from *Rana esculenta* skin secretions contains the antimicrobial properties of the peptide (214). Esc(1-18) was effective against MDR *S. maltophilia* clinical isolates at concentrations of 0.5 μM, 8 μM, and 16 μM needed for a bactericidal effect when tested in sodium phosphate buffer (SPB), in 20% heat-inactivated human serum, and in 40% heat-inactivated human serum, respectively (215). Temporins and bombinin peptides showed variability in their bactericidal activities against all three clinical *S. maltophilia* isolates. All of these tested peptides at 2-fold the bactericidal concentration demonstrated a rapid killing of one clinical *S. maltophilia* isolate. The practical value of the Esc(1-18) peptide for use in humans has yet to be determined, as although it has demonstrated reduced cytolytic activity against human red blood cells and retains its bactericidal effects in the presence of human serum, it needs to be tested further (e.g., in animal models of sepsis) to provide further data to support its use in the treatment of human infections (215).

The activity of Esc(1-8) in combination with conventional antibiotics used to treat *S. maltophilia* infections (amikacin, ceftazidime, colistin, and levofloxacin) has been assessed in synergy studies (211). Synergy studies using five clinical *S. maltophilia* isolates and *S. maltophilia* ATCC 13637 were performed in the presence of sodium phosphate buffer and human serum. In sodium phosphate buffer, Esc(1-8) in combination with amikacin or colistin resulted in synergistic activity against five of six *S. maltophilia* isolates. Enhanced killing at subbactericidal concentrations [0.5-μg/ml concentration of Esc(1-8) and colistin] was observed for the combination of Esc(1-8) and colistin against two representative *S. maltophilia* isolates. However, no synergistic effect against these two isolates was observed for the combination of Esc(1-8) and amikacin. No synergy was observed for Esc(1-8) used in combination with ceftazidime or levofloxacin. In the presence of 20% heat-inactivated human serum, against one representative *S. maltophilia* isolate, Esc(1-8) demonstrated enhanced bactericidal activity (4 μg/ml; 1/8 minimal bactericidal concentration [MBC]) when used in combination with colistin (0.125 μg/ml; 1/2 MBC) or with amikacin (1 μg/ml; 1/16 MBC). The observations of an enhanced killing by subbactericidal concentrations of amikacin following the preincubation of *S. maltophilia* with Esc(1-8) suggest that the peptide may reduce the outer membrane permeability barrier of *S. maltophilia*, resulting in an increase in the uptake of amikacin. That study suggested that the use of Esc(1-8) may be helpful for facilitating the antimicrobial activity of drugs (e.g., aminoglycosides) that have difficulty crossing the cell membranes of *S. maltophilia* (211).

The peptide Cys-Val-His-Ser-Pro-Asn-Arg-Glu-Cys has been identified as a specific inhibitor of β-lactamase L1 of *S. maltophilia* through the screening of a phage display library (305). The peptide demonstrated a mixed inhibition of L1 (dissociation constant of complex enzyme inhibitor [$K_{i\ \mathrm{competitive}}$] of 16 ± 4 μM and dissociation constant of complex enzyme-substrate inhibitor [$K_{i\ \mathrm{uncompetitive}}'$] of 9 ± 1 μM) and prevented zinc atoms from an optimal association with L1, altering the functional activity of L1. The peptide is a compound used for the screening and development of small molecules that can inhibit β-lactamases such as L1 (305).

BAL30376 is a triple-β-lactamase inhibitor combination composed of a siderophore monobactam, a specific inhibitor of class C β-lactamases, and clavulanic acid, an inhibitor of most class A and some class D β-lactamases (255). BAL30376 demonstrated an MIC_{90} of 2 μg/ml against *S. maltophilia* (255).

The antibacterial activities of new cationic compounds (e.g., hexamidine diisethionate [HX], chlorhexidine digluconate [CHX], and *para*-guanidinoethylcalix[4]arene [CxI]) have been assessed (132). Cationic compounds act by binding to the negatively charged surfaces of the bacterial cell wall and membranes. The results of that study showed that two strains of MDR *S. maltophilia* demonstrated susceptibility to CHX (MIC < 1 to 32 mg/

liter), some susceptibility to HX (MIC = 32 to 256 mg/liter), and resistance to CxI (MIC = 256 mg/liter) (132).

A surfactant-stabilized oil-in-water nanoemulsion (NB-401) has shown antimicrobial activity against planktonic and biofilm-associated cells of *S. maltophilia* (206). This nanoemulsion consists of emulsified cetylpyridinium chloride, poloxamer 407, and ethanol in water with superrefined soybean oil. The interaction of the nanoemulsion with the cell was suggested to result in the fusion of the outer membrane with the nanoemulsion, leading to cell lysis. The testing of NB-401 against planktonic cells of 15 *S. maltophilia* isolates resulted in MIC values of ≤15.6, 31.2, and ≤15.6 to 62.5 μg/ml cetylpyridinium chloride at 50% MIC, 90% MIC, and the range of MIC values for NB-401, respectively. The *S. maltophilia* isolates demonstrated the greatest susceptibility to NB-401 compared with 135 other bacterial isolates found in cystic fibrosis sputum. Against biofilm cells of *S. maltophilia* and in the presence of 43% CF sputum, NB-401 activity resulted in an *in vitro* minimum bactericidal activity (SMBC) value of 31.2 μg/ml. These observations revealed that the nanoemulsion partially preserved its antimicrobial activity in CF sputum. The antimicrobial action of the nanoemulsion appears to involve the outer membrane lipopolysaccharide (LPS), as the addition of EDTA, a divalent cation chelator that disrupts the stability of lipopolysaccharide on the bacterial cell surface, increases the bactericidal activity of NB-401 against Gram-negative bacteria. NB-401 is suggested to be of use as an inhaled antimicrobial therapy, as indicated by preliminary data from studies in which multiple daily exposures of NB-401 in mice were well tolerated (2,000 $\mu g\ ml^{-1}\ dose^{-1}$). That study suggested that a combination inhalational therapy of NB-401 and hypertonic saline may be of benefit to CF patients and avoids the risk of antibiotic resistance (206). More studies are needed to determine the pharmacokinetics and efficacy of this nanoemulsion treatment in animal models and in clinical trials with CF patients.

Aerosolized antibiotics are of particular significance for use in CF patients' lung infections. Tobramycin was the first antibiotic used for inhalational therapy for CF patients (175). The delivery of antipseudomonal aminoglycoside therapy by nebulizer has been associated with an increased risk for colonization by *S. maltophilia* (82). The intermittent delivery of aerosolized tobramycin by a nebulizer (cycles of 300 mg tobramycin or taste-masked placebo twice daily for 28 days followed by 28 days without treatment) did not increase the selection of tobramycin-resistant *S. maltophilia* and resulted in persistent treatment-emergent *S. maltophilia* in a very low number of patients (43, 276). A retrospective study of tobramycin in two placebo-controlled trials revealed that most *S. maltophilia* isolates occurred intermittently and were rarely persistent isolates (131); this occasional appearance of *S. maltophilia* in CF patients has been reported by several studies (76, 168, 338). As *S. maltophilia* has been recovered from nebulizers of CF patients (80, 157), a small study tested the biofilm-forming abilities of environmental and clinical *S. maltophilia* isolates after exposure to tobramycin at a concentration (16,000 μg/ml) found inside the nebulizers (234). All five biofilm-associated *S. maltophilia* isolates remained viable after exposure to tobramycin (234).

Levofloxacin, a broad-spectrum fluoroquinolone, has been reported to demonstrate an MIC range of 0.25 to 8 μg/ml against 51 *S. maltophilia* clinical isolates from CF patients (175). That study suggested the potential for the use of levofloxacin as an aerosolized antibiotic in CF patient infections. An additional attractive feature of levofloxacin is the higher maximum concentration of the drug (C_{max})/MIC and area under the curve (AUC)/MIC values obtained through aerosolized delivery, in contrast to those values obtained for intravenous or oral delivery (121). Observations of CF subjects receiving nebulized formulations of MP-376 (levofloxacin inhalation solution; Aeroquin) at a dose of 180 mg followed by 7 days of daily treatment doses of 240 mg demonstrated high sputum and low serum levofloxacin concentrations. Patients tolerated the MP-376 formulations well, with no serious adverse events reported and no patients excluded during the study due to an adverse event (121). Clinical trials using MP-376 are needed to assess the efficacy and tolerance of this antibiotic in CF subjects with *S. maltophilia* infections.

As an alternative to the use of antibiotics, essential oils from plants (e.g., orange, bergamot, cinnamon, clove, cypress, eucalyptus, fennel, lavender, lemon, mint, rosemary, sage, and thyme) were investigated and found to demonstrate antibacterial activity against *S. maltophilia* (103). Vero cell assays were performed to determine the cytotoxicity of the oils. Clinical isolates of *S. maltophilia* that were resistant to phosphomycin, imipenem, piperacillin, and aztreonam demonstrated susceptibility to the oils at nontoxic concentrations ranging from 0.0005 ml/ml to 0.00005 ml/ml. Cinnamon, thyme, and clove demonstrated the highest level of antimicrobial activity and inhibited all tested strains of *S. maltophilia*. It has been suggested that despite the observation of the MIC of thyme (0.003125 ml/ml) above the noncytotoxic concentration, thyme has potential use for the treatment of respiratory tract infections in humans (103). The toxicity of these oils against respiratory epithelial cells needs to be assessed. At nontoxic concentrations, these oils may show potential application for inhalation therapy to treat respiratory tract infections. Future research is needed to elucidate the precise chemical composition of the oil that determines the mechanism of action (bactericidal/bacteriostatic activity) of these oils.

The use of phage therapy may be an alternative to the use of antibiotics to treat *S. maltophilia* infections. To the best of my knowledge, phage therapy is not used in ordinary clinical practice for the treatment of *S. maltophilia* infections. *S. maltophilia* phages have been isolated from sputum samples, pleural effusions, and catheter tips (53). One of these phages, phage ϕSMA5, has been further characterized and exhibits ultrastructural features similar to those of phages of the family *Myoviridae* or Bradley's group A1. This phage was tested against 87 *S. maltophilia* strains isolated from hospitals and was found to have a narrow host range. These observations suggest that further research is needed to isolate and identify multiple *S. maltophilia* phages that can be used as a cocktail against heterogenous strains of *S. maltophilia*. A recent review suggested that the use of phages to treat biofilms has potential (93). Research is needed to determine if phage-coated catheters demonstrate significantly reduced numbers of viable cells when the catheters are exposed to *S. maltophilia*, if the *S. maltophilia* biofilms can be reduced or removed, and if *S. maltophilia* develops resistance to the phage.

Together, the observations from the studies described above suggest that it is possible that a cocktail of surfactant, antimicrobial peptides, and phage may provide a suitable alternative to the administration of antibiotics.

The green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) has demonstrated antimicrobial activity against clinical isolates of *S. maltophilia* (125). EGCG is the major polyphenol component of green tea (*Camellia sinensis*). The testing of 40 *S.*

maltophilia clinical isolates with EGCG resulted in a range of MICs (128 mg/liter to >512 mg/liter) (125). Future work is needed to determine whether EGCG inhibits the adherence of *S. maltophilia* to epithelial cells and if exposure to EGCG reduces or alters the biofilms of *S. maltophilia*.

SURVIVABILITY AND PERSISTENCE

Surfaces and Solutions

S. maltophilia is associated with wet surfaces and aqueous solutions. Cells of *S. maltophilia* have the ability to survive with minimal nutrients, e.g., in drinking water, ultrapure water, treated water (after water treatment of filtration, reverse osmosis, UV exposure, or deionization), and dialysate effluent (15, 184). In response to starvation or stress, *S. maltophilia* in tap water reduces the energy cost of chemotaxis by forming UMC (0.1 to 0.2 μm) that can pass through a 0.2-μm filter (316). UMC are formed in water (potable water, mineral water, and reverse osmosis water) by several genera of bacteria, including *Stenotrophomonas*, *Pandoraea*, *Microbacterium*, *Afipia*, *Pseudomonas*, *Vibrio*, *Sphingomonas*, and *Aeromonas* (95, 145, 164, 209, 221, 241, 316). It has been reported that biofilm and UMC can pass through these filter units, demonstrating that filtration has limited efficacy for the removal of these potential pathogens from water (316). Cultivated UMC capable of forming biofilms on polyvinyl chloride (PVC) pipe walls have been recovered from chlorinated drinking water samples and grown on Alpha agar plates containing 0.005% peptone of soybean meal. These ultramicrobial cells would not be detected by using the standard and total heterotrophic plate counts (HPC). These observations suggest that UMC can act as a potential source of infection if they come into favorable environmental conditions, e.g., through the leaching of nutrients from the PVC pipe (220). Medical devices with PVC are prepared by combining PVC with components such as phthalic esters, organic tin compounds, epoxidized soy bean oil, esters, and organic phosphate compounds. The leaching of these components from PVC may contribute to the adherence of nonmucoid *P. aeruginosa* strains to PVC. The adherence of these strains was reported to be greater ($P < 0.05$ at 5 days and $P < 0.01$ at 7 days) than their adherence to polyurethane and siliconized latex (220).

Water treatment processes are designed to prevent the waterborne distribution of pathogens to humans. The treatment typically consists of coagulation, flocculation, sedimentation, filtration, and chlorination. HPC are often used to assess the bacteriological quality of water but do not support the growth of all bacteria that can inhabit chlorination distribution systems. Viable but nonculturable (VBNC) bacteria are not detectable by HPC. Flow cytometry in combination with dyes to measure numbers of active bacteria together with HPC can be used to determine the presence of active and culturable bacteria. PCR-denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene nested PCR, fluorescence *in situ* hybridization (FISH), and DNA sequencing methods are useful to assess bacterial diversity. *S. maltophilia* has been identified by PCR-DGGE to survive water treatment, avoid detection by HPC, and remain active in distribution system water (142).

The iron-reducing activity of *S. maltophilia* has been applied to phosphate removal from the returned liquor of a municipal wastewater treatment plant (158). The removal of phosphate from the returned liquor of wastewater is important, as it reduces the possibility of eutrophication, dissolved oxygen depletion, and a decreased value of the water supply. *S. maltophilia* BK is able to reduce Fe(III) to Fe(II) using xenobiotics as sole sources of carbon under anaerobic conditions. The production of Fe(II) resulted in the removal of dissolved phosphate and the increased precipitation of phosphate by *S. maltophilia*. *S. maltophilia* BK exhibited a rate of phosphate removal of 33 mg/g volatile suspended solids/day (158).

Tap water can harbor opportunistic pathogens at levels that are significant for immunocompromised individuals. Municipal tap water can contain 10^7 bacteria/liter (109). The numbers of *S. maltophilia* cells in water samples vary with environmental conditions, including how frequently the water source is used and the temperature of the water (81). Hospital water sources can serve as reservoirs of nosocomial pathogens such as *Pseudomonas* spp. and *S. maltophilia*. Showerheads equipped with 0.2-μm filters may select for UMC that pass through the filter and form biofilms on the showerhead filter surface, where they can act as a source of infection. Showerhead biofilms have been reported to enrich opportunistic pathogens such as chlorine-resistant nontuberculous mycobacteria (109). Increased exposure to aerosolized bacteria due to increased shower use was hypothesized to correlate with the rising rates of infection by nontuberculous mycobacteria (248). Several studies have reported that point-of-use filtration reduced exposure to the waterborne pathogens *Legionella* species, *Mycobacterium gordonae*, and *Pseudomonas* species in health care facilities (52, 102, 144, 254, 311, 332). At the time of writing, I found no studies that investigated a possible correlation between showerhead aerosols of *S. maltophilia* and infection rates.

The dispensers of soda fountain machines have been shown to harbor microorganisms that include MDR *S. maltophilia* (366). Of the beverages (sugar sodas, diet sodas, and water) sampled for microorganisms, 48% contained coliform bacteria and >11% contained *E. coli* (366). Since the water supply used for the soda fountain machines was reported to be in compliance with U.S. Environmental Protection Agency (EPA) standards, this implicated the soda fountain machines as the source of the microbial contamination of the beverages. It was suggested that communities of the bacteria form biofilms inside the fountain dispensing machines. The contamination of soda fountain machines with potentially pathogenic microorganisms, including *S. maltophilia*, is of concern for immunocompromised individuals consuming these beverages. It is recommended that the dispensing unit and fittings of soda machines be regularly inspected for physical wear and the presence of microbial biofilms and be disinfected to reduce microbial contamination.

Nebulizers used for the delivery of aerosolized therapy to CF patients in an adult cystic fibrosis unit have been reported to be contaminated with *S. maltophilia* (80). Environmental sampling of sites including taps, tap water, sink drains, and ice-making machines on the unit yielded *S. maltophilia* in sink drains, taps, and water samples; however, none of these isolates shared a genotype with isolates recovered from the nebulizers. It was suggested that the rinsing of reusable nebulizer equipment with tap water may result in the adherence and contamination of *S. maltophilia* on the wet surface of the nebulizer. That study used ERIC-PCR and PFGE profiling to compare the genotypes of environmental and clinical *S. maltophilia* isolates. That study did not rule out the possibility of or provide direct evidence identifying the ward environment as the source of contamination but pointed to the need

for caregivers of CF patients to ensure that nebulizer equipment washed with tap water be thoroughly dried before its next use (80). *S. maltophilia* has also been recovered from the surface of home-use nebulizers of CF patients (157). Patients who regularly dried their nebulizers after use demonstrated no or minimal contamination of their nebulizers (157).

Recent observations in my laboratory indicate that *S. maltophilia* is highly susceptible to drying. Cell suspensions (10 μl) of *S. maltophilia* on stainless steel surfaces following 1 h of air drying showed a >3-log reduction in cell viability (my unpublished observations).

Biocide Tolerance

Hypochlorite cleaners have been recommended to reduce biofilms of heterotrophic plate count bacteria, coliforms, and fecal coliforms in drains (289). Recent experiments in my laboratory have shown that *S. maltophilia* clinical isolate X26332 forms biofilms in PVC microtiter wells containing Luria-Bertani (LB) broth with ≤0.006% bleach after 18 h of incubation at 35°C (22). This study did not take into account the inactivation of the chlorine by the broth medium. These observations underscore the importance of using bleach at a concentration that will eliminate the *S. maltophilia* biofilm, to remove the possibility of a regrowth of the biofilm.

The effect of sodium hypochlorite disinfection was tested on *S. maltophilia* present in suction tubing used for sputum suction (377). Suction tubing samples containing *S. maltophilia* at 5.5×10^6 to 6.5×10^8 CFU/tube were exposed to 0.1% (1,000 ppm) sodium hypochlorite for 2 h. Following this disinfection treatment, counts of 5.1×10^5 to 4.8×10^6 CFU/tube were recovered. Tubing containing counts of 6.4×10^4 to 1.0×10^7 CFU/tube was cleaned with an automatic cleaner, effectively reducing counts to <20 CFU/tube (377). These data suggest that in preference to sodium hypochlorite, automatic cleaners should be recommended for the disinfection of suction tubing. It should be noted that one should always follow established guidelines for the reprocessing of endoscopes, where a precleaning step is performed at the point of use to remove bioburden and visible debris prior to manual or automated high-level disinfection (264).

S. maltophilia has demonstrated tolerance to the biocides triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) (193, 303) and sodium dodecyl sulfate (SDS) (22). Repeated exposure to triclosan, which acts primarily on Gram-positive bacteria, resulted in a slight decrease in the susceptibility of domestic drain biofilm isolate *S. maltophilia* M9.13 (193). SDS has been assessed for its ability to reduce bacterial biofilms (197). Data from my laboratory indicate that *S. maltophilia* clinical isolate X26332 survives and persists in a 0.02% solution of SDS for 14 days at 30°C; biofilms of this isolate have been observed to form in Luria-Bertani broth containing 0.02% SDS (22; my unpublished observations).

S. maltophilia has been recovered from a contaminated deionized-water-diluted hospital antiseptic solution (Savlon concentrate; 1.5% chlorhexidine and 15% cetrimide) (369) and from contact lens preservative solutions (116). The preservative solutions ReNu (BLJ Co., Ltd., Japan), Complete (Abbott Medical Optics Japan, Inc., Japan), and Opti-Free (Alcon Japan, Ltd., Japan), used for contact lens storage, varied in their bactericidal activities against suspensions of *S. maltophilia*, and no bactericidal activity against *S. maltophilia* cells adhered to polystyrene was observed for these preservative solutions (116). These results emphasize the importance of maintaining good hygiene practices when handling antiseptics and preservative solutions.

The *qacE*Δ*1* gene, encoding tolerance to antiseptics containing quaternary ammonium compounds, has been detected in association with IS*CR1* elements as a part of complex class 1 integrons. The *qacE*Δ*1*gene has been detected in *S. maltophilia* clinical isolates from China by PCR amplification (354).

Resistance to Metals

Silver acts as an antimicrobial agent by binding sulfur groups of proteins in bacterial cell walls, ultimately resulting in cell death. Silver has been used in catheters in attempts to prevent biofilm formation (60, 118, 122). The use of an Environmental Protection Agency (EPA) (http://www.epa.gov/safewater/mcl.html)-approved level (100 μg/liter) of silver nitrate as a disinfectant in drinking water did not significantly prevent ($P \leq 0.05$) bacterial biofilm formation in modified Robbins devices with polyvinyl chloride and stainless steel surfaces in comparison to the control treatment (317). The recorded silver concentrations (90 to 122 μg/liter) in samples entering the devices were reduced to 14 to 20 μg/liter after exit from the devices, suggesting that the biofilms absorbed silver. Data from my laboratory are in agreement with those reported by Silvestry-Rodriguez et al. (317), revealing no dramatic inhibition of biofilm formation by an *S. maltophilia* clinical isolate cultured overnight at 37°C in polyvinyl chloride microtiter plate wells in LB broth containing 100 μg/liter of silver nitrate (156). Under the conditions of that study, the inhibition of biofilm formation was achieved at a silver nitrate concentration of ≥10,000 μg/liter (156).

In a study of central venous catheters impregnated with minocycline and rifampin (M-R), with silver platinum and carbon (SPC), or with chlorhexidine and silver sulfadiazine (CHX-SS), compared with noncoated catheters, only the M-R catheters inhibited ($P < 0.005$) the adherence and biofilm formation of MDR *S. maltophilia* clinical isolates recovered from catheter-related bloodstream infections in cancer patients (274). The M-R catheters also showed more prolonged antimicrobial durability against the *S. maltophilia* isolates in comparison with the SPC and CHX-SS catheters.

The biocidal efficacies of three silver-impregnated contact lens storage cases (Microblock, i-clean, and Nano-case) against *S. maltophilia* were tested (70). Case wells contained cell counts (10^3 to 10^6 CFU/ml) that were incubated for 6 to 24 h at 25°C. Antimicrobial activity was noticeable only after 24 h of incubation. For the three cases examined, Microblock, i-clean, and Nano-case, the antimicrobial activities were different ($P \leq 0.001$), with the Nano-case demonstrating the greatest activity, decreasing cell counts by 0.2 ± 0.3 logs. Only the Microblock case showed silver release over 28 days (70).

All of these observations of the persistence of *S. maltophilia* following exposure to silver are significant, as this organism demonstrates resistance to metals in clinical and environmental settings. The genome of clinical isolate *S. maltophilia* K279a contains gene clusters used for the import, storage, and efflux of metals (67). A comparison of clinical isolate K279a and environmental isolate R551-3 of *S. maltophilia* revealed that some metal resistance is common to both isolates and that some operons are found only in K279a (281).

Both environmental and clinical strains of *S. maltophilia* have been found to contain genes encoding resistance to metals. Environmental strain O2, isolated from East Fork Poplar Creek in

TABLE 5 Comparison of metal resistances demonstrated by *S. maltophilia* O2 and Sm777

Metal	Metal resistance tolerated by *S. maltophilia* (mM)[a]	
	O2	Sm777
Hg(II)	0.25	0.05
Cd(II)	0.33	0.50
Cu(II)	5.00	5.00
Au(III)	0.25	Not available
Ag(I)	0.03	0.02
Cr(VI)	8.00	Not available
Se(IV)	40.0	50.0
Pb(II)	Not available	5.00

[a] Data from references 143 and 256.

Tennessee, grows in toxic acidic wastes containing gold, mercury, platinum, lead, cadmium, chromium, silver, copper, and selenium salts (143). *S. maltophilia* strain Sm777, isolated as a contaminant of a *Pseudomonas* culture, tolerates the presence of silver, mercury, copper, lead, cadmium, and selenium salts (256). The metal resistances demonstrated by both *S. maltophilia* isolates are shown in Table 5. *S. maltophilia* Sm777 was proposed to use different mechanisms to protect itself against metal toxicity. In the presence of selenite and tellurite, *S. maltophilia* Sm777 accumulated cytoplasmic electron-dense Se^0 granules and Te^0 granules, indicating that active efflux pumps probably are not the sole mechanisms used to control heavy metal tolerance in this strain. *S. maltophilia* Sm777 demonstrated tolerance to cadmium through the use of cysteine and the production of CdS particles from Cd(II) when grown aerobically on solid agar containing 500 μM $CdCl_2$. These observations of metal resistance in environmental isolates suggest that similar to the acquisition of antimicrobial drug resistance, the acquisition of metal resistance occurs in the natural environment. Environmental isolates of *S. maltophilia* found in the clinical/medical setting may simply be maintaining metal resistance genes when challenged with antimicrobials containing metals.

The treatment of *S. maltophilia* planktonic cells and biofilms with copper-silver ionization has demonstrated efficacy (312). These studies are addressed in more detail below (see "Biofilms").

MOLECULAR MECHANISMS INVOLVED IN PATHOGENESIS

Biofilms

A significant feature of *S. maltophilia* is its ability to form biofilms on surfaces including Teflon, glass, and plastics and on host tissues (83, 84, 166, 268). Biofilms have been estimated to be associated with 65% of hospital-acquired infections (270). Scanning electron microscopy (SEM) images in a study by Di Bonaventura et al. (89) showed that *S. maltophilia* SM33 cells can adhere to polystyrene surfaces within 2 h of inoculation and can form biofilms by 24 h (Fig. 1).

One of the early steps of biofilm formation is the adherence of bacterial cells to a surface. Transmission electron microscopy (TEM) and SEM identified the presence of flagella on 46 clinical isolates of *S. maltophilia* (84). Studies of the kinetics of adherence of *S. maltophilia* SMDP92 to polystyrene in minimal medium indicated that from 30 min to 18 h postinoculation, the bacterial cells attached and formed small clumps, with three-dimensional clumps being formed at 6 h, and bacterial adherence reached a maximum level at 18 h (84). High-resolution SEM of bacterial cell monolayers adhered to plastic suggest that flagella and other thin fibrillar structures are involved in bacterial cell adherence to plastic (Fig. 2) (84).

S. maltophilia biofilms have been studied using *in vitro* tissue culture assays. TEM, high-resolution SEM, and immunogold labeling have identified the *S. maltophilia* fimbriae 1 (SMF-1) protein as being important for adherence to cultured HEp-2 monolayers (83). Adherence to eukaryotic cells was inhibited in the presence of anti-SMF-1 antibodies. The antibodies were most effective at blocking adherence during the early stages (the first half hour) of infection. The adherence and biofilm formation of *S. maltophilia* SMDP92 on glass were inhibited by anti-SMF-1 antibodies in a dose-dependent manner, implying that the fimbriae facilitate interactions between the *S. maltophilia* cell surface and the host cell/abiotic surface (Fig. 3) (83).

S. maltophilia can form biofilms on lung cells (73, 83, 268). Confocal microscopy of biofilms formed by CF isolate *S. maltophilia* OBGTC9 on CF sputum-derived bronchial epithelial IB3-1 cell monolayers revealed that *S. maltophilia* formed microcolonies embedded in a matrix (268). SEM of a 24-h-old biofilm formed by *S. maltophilia* OBGTC9 on an IB3-1 cell monolayer revealed microcolonies of *S. maltophilia* in the presence of an extracellular matrix (268). It is of interest that the degrees of adherence of *S. maltophilia* clinical isolates to the bronchial epithelial cell monolayer varied and did not correspond with the degrees of biofilm formed on the cell monolayer. Even more interesting is the observation that the biofilm formed by *S. maltophilia* CF isolates on polystyrene did not correspond with the biofilm formation of the isolates on the cell monolayer. The latter evidence supports the view that biofilm formation on abiotic surfaces may not reflect the biofilm formation observed on biotic surfaces in animal models or human patients.

Environmental factors that can influence the biofilms of *S. maltophilia* include phosphate (38), chloride concentrations (66), pH, temperature, aerobic or anaerobic conditions (90), and the presence of copper and silver ions (312). *S. maltophilia* can form films on moist surfaces that make direct or indirect contact with patients, including hospital water plumbing systems, respiratory tubing, dental suction tubing and unit waterlines, catheters, i.v. lines, dialysis equipment, clinical sink drains, domestic sink drains (40), and faucets (52).

The presence of sodium phosphate was reported to alter the biofilms of clinical *S. maltophilia* isolates (38). In a study of 11 clinical isolates, 9 demonstrated altered biofilm formation when cultured in Luria-Bertani (LB) medium supplemented with 0.1 M sodium phosphate buffer (SPB) (pH 7.0). Five isolates showed increased biofilm formation ($P < 0.008$) in the presence of sodium phosphate, in comparison to their biofilm formation in LB medium without SPB supplementation. This increased biofilm formation occurred with no increase in culture growth. Four isolates showed decreased biofilm formation ($P < 0.03$), probably as a result of a decrease in culture growth (8.5% less than that of the same isolates cultured in LB medium without SPB supplementation) (38). These findings have relevance for applied situations where *S. maltophilia* may be present and form biofilms. Biofilms and the selection of *S. maltophilia* isolates will likely lead to an increased resistance of the bacterium to water-diluted disinfectants. This bacterial pathogen has been isolated from water-diluted antiseptic solutions (369). Levels of sodium and phos-

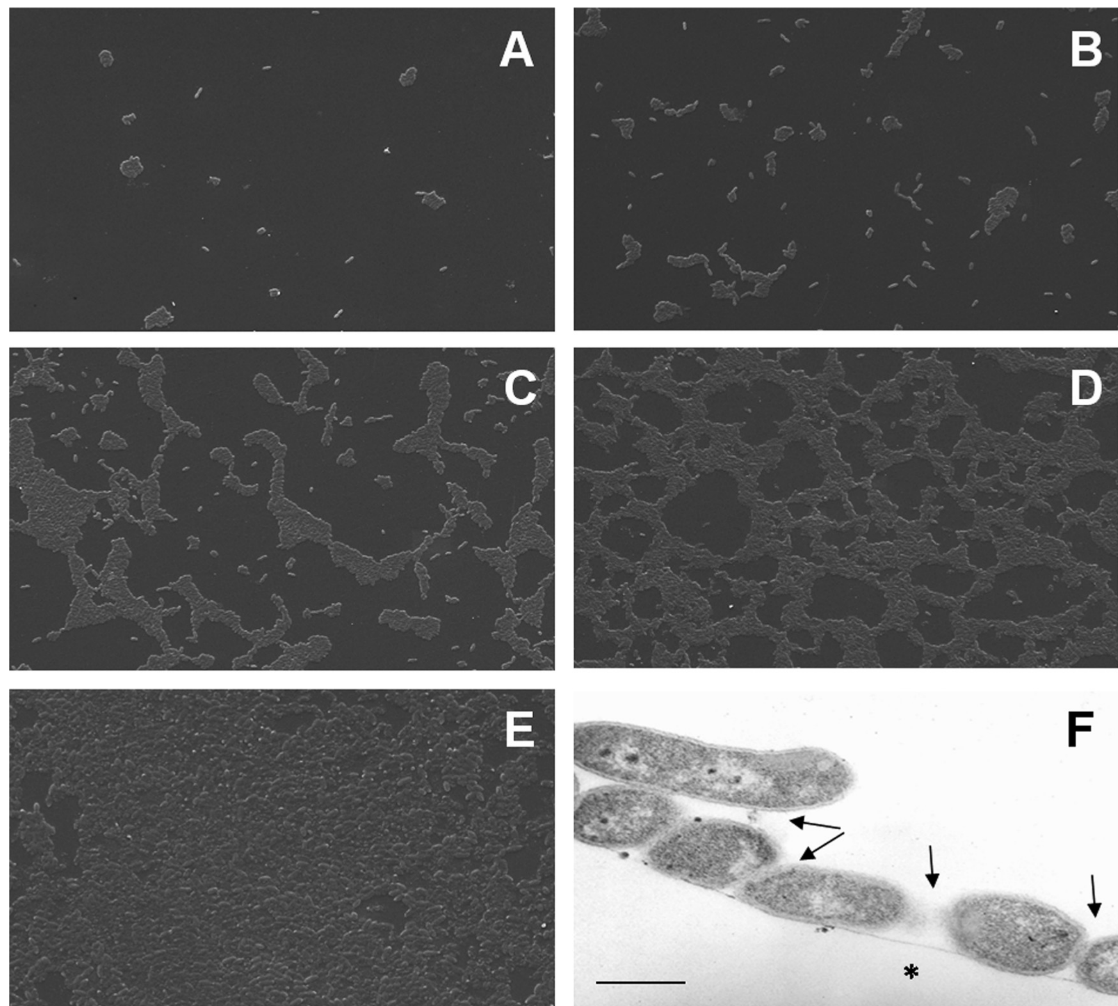

FIG 1 (A to E) Scanning electron micrographs of *S. maltophilia* SM33 biofilms formed on polystyrene surfaces at 2, 4, 8, 16, and 24 h, respectively. Magnifications, ×1,000 (A to D) and ×2,000 (E). (F) Transmission electron micrograph of a 24-h biofilm produced by *S. maltophilia* SM33. Arrows indicate glycocalyx surrounding bacteria. The asterisk indicates the biofilm limit line in contact with the polystyrene surface. Bar, 0.5 μm. (Reprinted from reference 89 with permission.)

phate in hospital water plumbing systems should be monitored, as phosphate has been reported to alter the microbial communities in the human water supply (172).

Clinical *S. maltophilia* isolates have been observed to form more biofilms at 32°C than at 37°C and 18°C (90). The level of biofilm production was higher under aerobic conditions and in a 6% CO_2 atmosphere than the level of biofilm production under anaerobic conditions. The *S. maltophilia* isolates produced comparable biofilms at pH 8.5 and 7.5 but larger amounts of biofilm than those produced at pH 5.5.

Biofilms in copper plumbing systems may increase the concentration of soluble copper (cuprosolvency) in drinking water (66). A concentration of 2 mg/liter of copper in water has been associated with gastrointestinal distress (266). The effect of the chemical composition of water (e.g., total organic carbon [TOC] and pH) on cuprosolvency by bacteria commonly isolated from biofilms in copper plumbing, including *S. maltophilia*, revealed an inverse correlation of cuprosolvency with increasing chloride concentrations for pure-culture *S. maltophilia* biofilms formed on copper coupons. That study suggested two reasons for this observation, that the increase in chloride could stress the bacteria and result in altered biofilms and that chloride ions may block exopolysaccharide groups important for cuprosolvency activity. No significant correlations were found between the biofilms of *S. maltophilia* and water pH or TOC (66). The results of that study demonstrated that in comparison to pH and the TOC concentration, the cuprosolvency activity of *S. maltophilia* is more sensitive to the level of chloride present in its aqueous environment. It is of interest that *S. maltophilia* has been reported to survive and persist in chlorinated water distribution systems. Taken together, these observations suggest that *S. maltophilia* should not be considered a major culprit responsible for the corrosion of copper plumbing systems.

A recent study examined the use of copper-silver ionization in a model plumbing system to control biofilms and planktonic cells of the waterborne pathogens *P. aeruginosa*, *A. baumannii*, and *S. maltophilia* (312). A 72-h exposure to copper-silver ion concentrations of 0.2 mg/liter-0.02 mg/liter to 0.8 mg/liter-0.08 mg/liter resulted in the inactivation of biofilm-associated and planktonic *S. maltophilia* cells (3-log reduction and >6-log reduction for

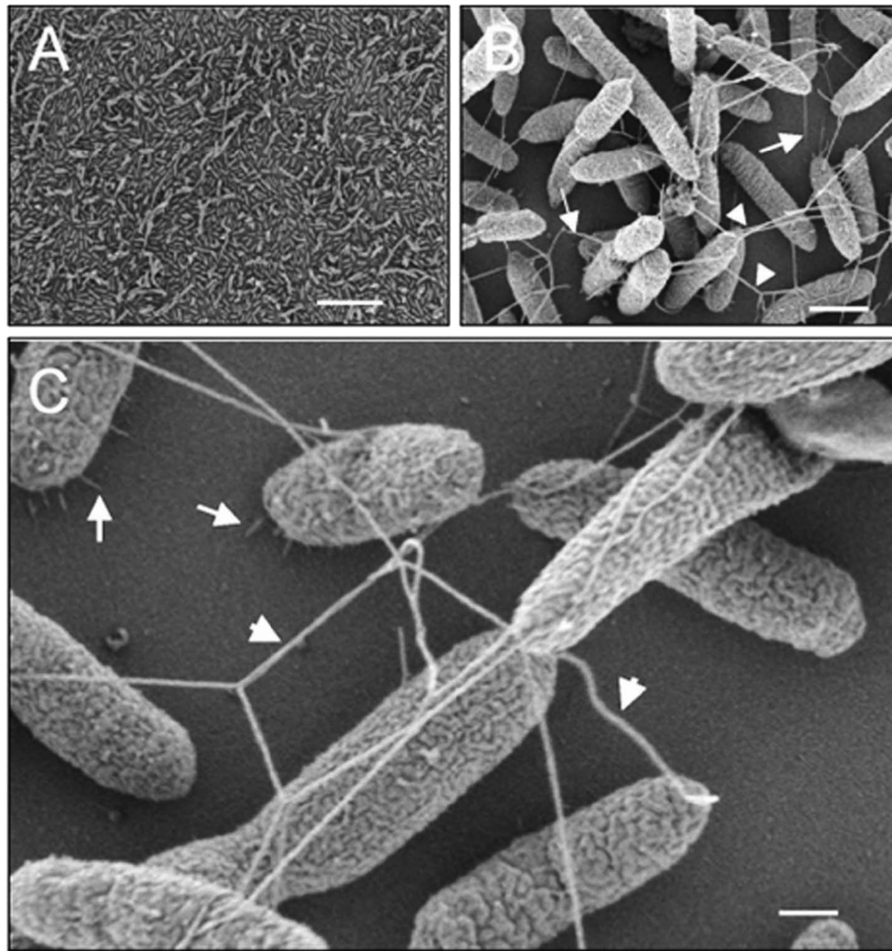

FIG 2 Scanning electron micrographs of *Stenotrophomonas maltophilia* adhering to plastic. (A) SMDP92 cells adhere tightly to the plastic surface. (B) Structures resembling flagella appear to protrude from the cell surface and interconnect bacteria (arrowheads) or connect bacteria to the plastic (arrows). (C) In addition to the flagellum-like filaments (arrowheads), high-power magnification shows the presence of thin fibrillar structures (arrows) connecting the bacterial cells to the abiotic surface. Bars, 10 μm (A), 1 μm (B), and 2 μm (C). (Reprinted from reference 84.)

biofilm-associated and planktonic cells, respectively) in 48 h. Higher concentrations of copper-silver ions (0.4 mg/liter-0.04 mg/liter and 0.8 mg/liter-0.08 mg/liter) caused a reduction beyond the 72-h exposure to the copper-silver ionization system. *S. maltophilia* showed a greater sensitivity to copper-silver ionization than *P. aeruginosa* and *A. baumannii*. These data suggest that the use of copper-silver ionization for the disinfection of water plumbing systems may be effective against *S. maltophilia*. More studies are needed to determine if strain-to-strain differences in susceptibility exist for *S. maltophilia* isolates. Silver ions have been hypothesized to disrupt biofilms by binding to biological molecules and disrupting binding sites that are important for electrostatic and hydrophobic interactions on these molecules (58).

In a study of 41 dental chair units (DCUs) in a hospital, *S. maltophilia* was isolated from biofilms in 14.6% of the DCU suction host orifice baseplates and was recovered from the internal lumens of the attachment ends of the high-volume suction hose and connectors in 21.6% of 37 DCUs (250). The bacterial contamination and corrosion of the baseplates and suction hoses were discovered within 6 months of the opening of a new hospital. The bacterial contamination appeared to be due to the seepage of liquid from suction hoses that were poorly connected to the DCU baseplates, leading to the corrosion of the baseplates. The liquid seepage problem was solved by replacing the suction hose connectors with interlocking connector collars and bushings that resulted in tightly fitting suction hoses that could not be loosened during use. Additional corrective measures included the replacement of the connectors with new fittings that replaced the steel baseplates with new aluminum baseplates. These new fittings were observed and sampled over the next 36 months, and no bacterial contamination was observed. That study suggested that most DCU suction systems contain bacterial biofilms due to an inadequate disinfectant contact time. The presence of high cell densities of *Pseudomonas* spp. and related bacteria in the suction systems despite regular disinfection is of concern. Some studies have reported the entry of liquid from the low-volume suction line into the patient's mouth during use, leaving open the possibility of the transfer of biofilm-containing microorganisms into the mouth from the suction line (25, 216, 361). The closing of the lips around the saliva ejector tip can cause a backflow of liquids into the mouths of patients. The recovery of viable bacteria from suction lines (361), stresses the importance of the disinfection of the suc-

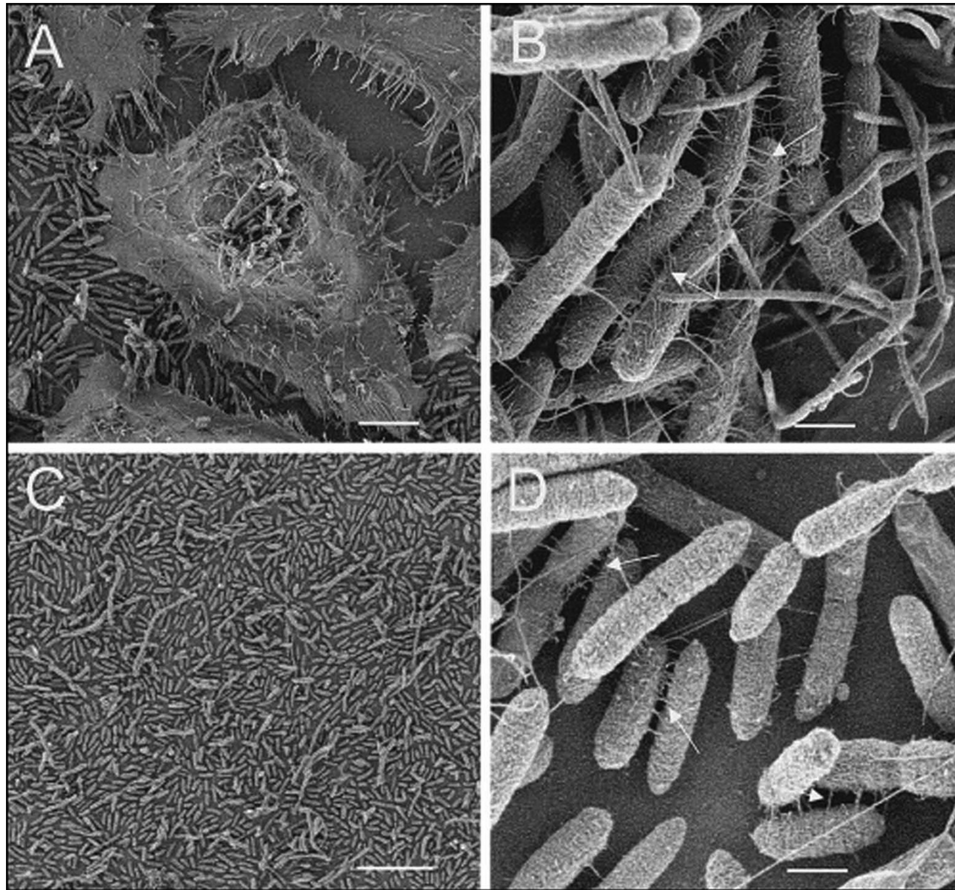

FIG 3 High-resolution scanning electron microscopy. (A) Adherence of SMDP92 cells to HEp-2 cells. In addition to the association of bacteria with eukaryotic cells, many bacteria adhere to the glass substratum. Bar, 10 μm. (B) High-magnification image of adhering SMDP92 cells with lateral fimbriae protruding from the bacteria (arrows). Bar, 1 μm. (C) SMDP92 cells adhering to the glass surface (biofilm formation) without epithelial cells. Bar, 10 μm. (D) High-resolution image of biofilm-forming bacteria showing peritrichous fibers attaching to bacteria. Long and thick filaments, probably flagella, are also shown. Bar, 2 μm. (Reprinted from reference 83 with permission of John Wiley & Sons.)

tion lines between patients to reduce the possibility of the transmission of potentially pathogenic organisms.

The American Dental Association (ADA) has set a water quality standard of ≤200 CFU/ml for dental chair units. Regular weekly disinfection of dental chair units using Planosil and Planosil Forte, two waterline disinfectants (Planosil contains 1.5% hydrogen peroxide, 0.003% silver, and 0.0015% phosphoric acid; Planosil Forte contains 2.5% hydrogen peroxide, 0.012% silver, and 0.0025% phosphoric acid), demonstrated the ability to nearly eliminate water unit biofilms (249). Biofilm regrowth was present at 7 days posttreatment. The regular use of these disinfectants maintained bacterial counts in the water unit lines at levels below the ADA water quality standard (249). These data indicate that the efficient removal and inhibition of bacterial biofilm regrowth can be achieved by using disinfectants containing multiple active antimicrobial agents.

Eight biocides (1% sodium dodecyl sulfate, 35% hydrogen peroxide, 5.25% sodium hypochlorite, 1% phenol, 4% Tween 20, 1% EDTA, 0.2% chlorhexidine gluconate, and 1% povidone-iodine) were studied for their effects on biofilm bacteria present in dental unit water lines (197). The flushing of the tubing for 48 h with a combination of 5.25% sodium hypochlorite and 1% phenol resulted in lower biofilm bacterial counts present in the unit water line tubing than in those with flushing with each biocide separately. The combination of sodium hypochlorite and phenol reduced biofilm bacterial counts on plate count agar, for the air and water tubing (from control counts of 6,384 ± 98 CFU/cm^2 to 156 ± 12 CFU/cm^2), for the main water pipe tubing (from control counts of 7,838 ± 61 CFU/cm^2 to 248 ± 79 CFU/cm^2), and for patient tubing (from control counts of 5,103 ± 78 CFU/cm^2 to 736 ± 44 CFU/cm^2). This combination of biocides effected an almost complete removal of biofilm, as demonstrated by epifluorescence microscopy of the tubing samples.

Peracetic acid (PAA) has a wide range of applications, including disinfection of ultrapure water systems, disinfection of industrial systems, reprocessing of hemodialyzers for reuse, disinfection of dialysis machines, and high-level disinfection of endoscopes (179). In a fetal bovine serum-coated polystyrene microtiter plate test system, a 10-min exposure to a 1% concentration of the PAA-based disinfecting agent Neodisher Septo PAC (W. Weigert, Hamburg, Germany) inhibited the growth of monoculture *S. maltophilia* and dual-culture *S. maltophilia* and *Candida parapsilosis* biofilms. At the minimum bactericidal concentration, Neodisher Septo PAC inhibited the growth of the monoculture and dual-culture biofilms, but in the absence of drying, biofilm regrowth was observed at 48 h postdisinfection. The drying (2 h at

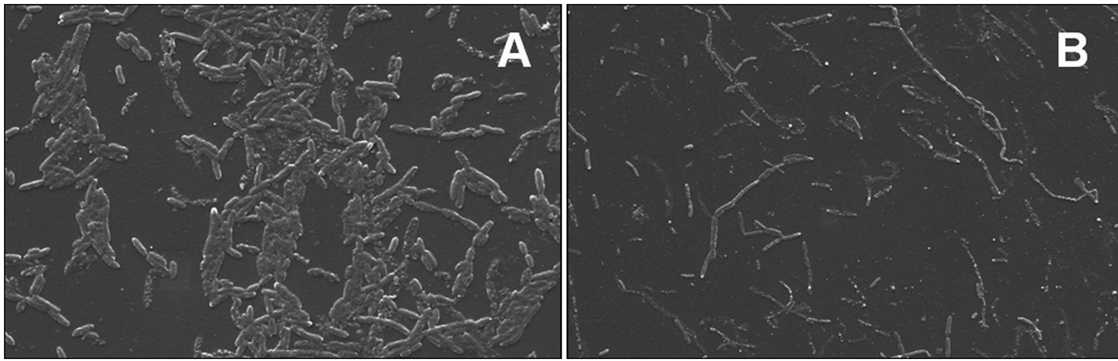

FIG 4 Scanning electron micrographs of antibiotic activity against *S. maltophilia* SM33 biofilm. Shown are the effects of rufloxacin at 100 μg/ml (A) and 500 μg/ml (B) against preformed *S. maltophilia* biofilm. Magnifications, ×2,500 (A) and ×2,000 (B). (Reprinted from reference 89 with permission.)

50°C) of the microtiter plates resulted in no biofilm regrowth (179). These observations emphasize the importance of a thorough drying of endoscopes after disinfection and before their next use.

The adherence and subsequent biofilm development of *S. maltophilia* on surfaces are affected by both the physicochemical properties of the bacterial cell (e.g., the presence of outer membrane proteins and lipopolysaccharide) and the surfaces to which the cell attaches (e.g., prosthetic devices covered with host extracellular matrix polymers or relatively hydrophilic glass and Teflon, in contrast to hydrophobic polyvinyl chloride). A study of the adherence of two relatively hydrophilic *S. maltophilia* isolates obtained from a model laboratory drinking water system revealed that the *S. maltophilia* isolates showed no or weak adherence to polyvinyl chloride (PVC), weak or moderate adherence to polyethylene (PE), and no adherence to ASI 316 stainless steel (318). It is very difficult to construct human implant materials that deter bacterial adhesion, as the implant devices become covered with host substances, including proteins and carbohydrates, that can facilitate the attachment of bacteria.

Recently, the interaction of antimicrobial drugs with biofilms of *S. maltophilia* has been more closely examined. MDR and non-MDR *S. maltophilia* clinical isolates have been compared for their abilities to form biofilms (198), and the effects of antibiotics at MICs and at concentrations below the MICs on *S. maltophilia* cell adherence to surfaces and biofilm formation have been studied (89, 90, 267). In a study of 70 *S. maltophilia* clinical isolates (40 MDR and 30 non-MDR isolates), the MDR isolates demonstrated a higher level of biofilm formation (average optical density at 540 nm [OD_{540}] of 0.52) than the non-MDR isolates (average OD_{540} of 0.15), and biofilm was correlated ($P \leq 0.01$) with resistance to ceftazidime, cefepime, ticarcillin-clavulanic acid, piperacillin-tazobactam, aztreonam, and gentamicin. Biofilm formation did not correlate with resistance to ciprofloxacin, levofloxacin, TMP-SMX, or meropenem (198).

Some antibiotics at suboptimal MICs (e.g., moxifloxacin) have demonstrated efficacy for reducing the adherence and biofilm formation of *S. maltophilia* (89, 267). A study of 20 biofilm-producing *S. maltophilia* clinical isolates revealed that at one-half the MIC, all tested fluoroquinolones (ciprofloxacin, grepafloxacin, levofloxacin, moxifloxacin, norfloxacin, ofloxacin, and rufloxacin) effectively ($P < 0.01$) reduced the biofilm mass of *S. maltophilia*, and at one-quarter the MIC, they reduced the biofilm mass (with the exception of levofloxacin) (89). Moxifloxacin was the most effective fluoroquinolone at preventing the adherence of *S. maltophilia*. All of the tested fluoroquinolones, with the exception of norfloxacin, reduced preformed biofilm biomass. Moxifloxacin was most effective at reducing preformed biofilm biomass. Treatment with moxifloxacin at 500 μg/ml eradicated biofilm biomass in 50% of the *S. maltophilia* isolates and reduced biomass up to 95% for 60% of the isolates. SEM studies have revealed no significant changes in the cellular morphologies of *S. maltophilia* cells after exposure to subinhibitory concentrations (sub-MICs) of moxifloxacin that inhibit biofilm formation on polystyrene (89). An analysis of cell viability in preformed biofilms treated with antibiotics revealed that rufloxacin was the most effective antibiotic, significantly reducing ($P < 0.01$) bacterial cell counts to 0.6%, 5.4%, and 17.1% for concentrations of rufloxacin at 500, 100, and 50 μg/ml, respectively. The treatment of preformed 18-h *S. maltophilia* SM33 biofilms on polystyrene with rufloxacin (500 μg/ml) results in ultrastructural changes in the bacterial cells (Fig. 4) (89). The treatment of preformed biofilms of *S. maltophilia* with ceftazidime was ineffective at removing biofilms. A high concentration (500 μg/ml) of TMP-SMX was needed to significantly reduce ($P < 0.01$) preformed biofilm biomass.

Moxifloxacin at sub-MICs was tested against *S. maltophilia* strains SM132 and Sm144, recovered from CF patients not treated previously with this antibiotic (267). At sub-MICs, moxifloxacin reduced the adherence of the *S. maltophilia* strains to polystyrene and inhibited biofilm formation. At a concentration of 0.06× MIC, the cell surface hydrophobicity of *S. maltophilia* strain SM144 changed from hydrophobic (observed at 0.03× MIC) to hydrophilic, in contrast to strain SM132, which remained hydrophilic at 0.03× MIC and 0.06× MIC of moxifloxacin. The hydrophobicity of the cell surface is likely an important factor to consider in the adherence and biofilm formation of *S. maltophilia*, but as this appears to be a strain-dependent phenomenon, individual strains would need to be evaluated to determine the efficacy of moxifloxacin activity against biofilm formation by this opportunistic pathogen. SEM micrographs did not reveal any ultrastructural changes of *S. maltophilia* SM132 cells treated with these sub-MICs of moxifloxacin. That study suggested that clinically attainable concentrations (e.g., 0.015 μg/ml corresponds to 0.03× MIC for both SM132 and SM144) of moxifloxacin will effectively inhibit *S. maltophilia* adherence and biofilm formation (267).

TABLE 6 Molecular mechanisms of antimicrobial resistance in *S. maltophilia*

Mechanism	References
β-Lactamases chromosomally and plasmid encoded and on mobile elements, e.g., Tn*1*-like transposon	9, 17, 18, 227, 295, 296, 352, 353
Multidrug efflux pumps, e.g., SmeDEF, SmeABC, and SmrA, associated with resistance to quinolones, tetracycline, chloramphenicol, erythromycin, aminoglycosides, and β-lactams	6, 10, 11, 54, 129, 196, 198, 269, 383
Class 1 integrons and IS*CR* elements associated with resistance to trimethoprim-sulfamethoxazole	27, 146, 198, 328
Phosphoglucomutase (SpgM) associated with resistance to polymyxin B, polymyxin E, nalidixic acid, gentamicin, vancomycin, ceftazidime, ticarcillin-clavulanic acid, and piperacillin-tazobactam	198, 225
Reduction in outer membrane permeability	5, 229
SmQnr determinants associated with resistance to quinolones	126, 300, 358
Modification of antibiotics	174, 195
Mutations of bacterial topoisomerase and gyrase genes	126, 339

Further studies are needed to test this hypothesis with an animal model of *S. maltophilia* infection.

The roles of extracellular DNA and D-amino acids in the biofilm matrix surrounding *S. maltophilia* cells need to be studied. If extracellular DNA is a major structural component of the biofilm matrix, as seen for other bacterial biofilms (365), it could serve as a target for treatment with DNase I. DNase I has been reported to disrupt biofilms (65). D-Amino acids have been reported to prevent biofilm formation (177). Further work is needed to establish whether D-amino acids have the same effects on *S. maltophilia* biofilms and if they and DNase I could be considered alternative treatments for patients with *S. maltophilia* biofilm infections.

Antibiotic Resistance

Several molecular mechanisms of *S. maltophilia* contribute to its multiantibiotic resistance, including plasmids, integrons, and transposons (27). A summary of these antibiotic resistance mechanisms is shown in Table 6. *S. maltophilia* has two chromosomally encoded β-lactamases, L1 and L2. The β-lactamase L1 is a metallo-β-lactamase (352), and L2 is a clavulanic acid-sensitive cephalosporinase (353). Several studies have reported differential β-lactamase activities among *S. maltophilia* isolates (16, 17, 56, 78, 147, 148, 153, 170, 227, 251, 374). The L1 β-lactamase uses a Sec export system, while the L2 β-lactamase uses a Tat export system for periplasmic translocation (194, 271).

A study to examine the heterogeneity of β-lactamase production among 17 clinical and 9 environmental *S. maltophilia* isolates using PFGE, the MICs of six β-lactam antibiotics, and isoelectric focusing found no correlation between MIC, isoelectric focusing electrophoresis (IEF), and genotyping data. The results of that study suggested that the mechanism underlying the variation in β-lactamase expression was unclear and needed to be further investigated (78).

Both the L1 and L2 β-lactamase genes have been found on 200-kb plasmids in *S. maltophilia* (17). There is allelic variation among L1 and L2 β-lactamase genes. The L1 and L2 β-lactamase genes showed levels of sequence changes as high as 20% and 25%, respectively, with the corresponding amino acid sequence divergences for L1 and L2 β-lactamases being as high as 21% and 32%, respectively. For these clinical isolates, changes in amino acid residues important for the binding of the L1 β-lactamase to its substrate were reported to alter its activity (17). *S. maltophilia* clinical isolates have demonstrated considerable heterogeneity for β-lactamase induction upon exposure to three antibiotics (imipenem [50 μg/ml], cefoxitin [50 μg/ml], or ampicillin [1 mg/ml]) (227). In that same study, the L1 β-lactamase genes from clinical isolate 39/95 and from reference strain *S. maltophilia* ULA-511 were cloned and sequenced; a comparison of the deduced amino acid sequences revealed a high level of homology (98%) between the enzymes (227). These observations suggest that β-lactamase activity is not just a result of the gene being present in the *S. maltophilia* isolate but point to another mechanism for the control of β-lactamase expression.

The isolation of *S. maltophilia* mutants and the generation of isogenic L1 and L2 gene knockout mutants have shown that β-lactamase L1 and L2 expressions are differentially regulated (16, 148). The expression of the β-lactamases is controlled at the level of transcription by the *ampR* gene, positioned upstream of L2, as part of an *ampR*-L2 module (201, 251). AmpR is needed for the basal-level expression of L1 but not L2 and is needed for the induced expression of L1 and L2 (201). The binding of AmpR to the intergenic sequence positioned between *ampR* and the L2 gene induces the expression of lactamase (56). The genetic diversity of selected L2 proteins and the intergenic sequences are relatively high (up to 32%), in contrast to the highly conserved AmpR proteins, suggesting that the expression of the chromosomal β-lactamase gene is mediated by changes in the sequences of the intergenic region or in the L2 gene (56). AmpR is a transcriptional regulator of *ampC* expression. AmpC is associated with the recycling of bacterial cell wall components. AmpC expression is activated when AmpR is bound with anhydro-*N*-acetylmuramyl-peptide, and the expression of AmpC is repressed when AmpR is bound with UDP-*N*-acetylmuramic acid-pentapeptide (374). Additional proteins used in cell wall recycling include AmpG, which is involved in the transport of degraded cell wall components into the cytoplasm, and AmpD, which is associated with the cleavage of the components into 1,6-anhydromuramic acid and peptide (374). Two *ampD* homologues encoding $AmpD_I$ and $AmpD_{II}$ have been identified in *S. maltophilia* K279a and in R553-1, and $AmpD_I$ is an anhydro-*N*-acetylmuramyl-*l*-alanine amidase and is involved in the regulation of both lactamases L1 and L2, while $AmpD_{II}$ is not associated with the regulation of expression of the β-lactamases (374).

In addition to the *ampR*, *ampC*, *ampN*, and *ampD* genes, an *ampN-ampG* operon is needed for the expression of lactamases L1 and L2 in *S. maltophilia* (153). The disruption of the *ampN* gene exhibits a polar effect on the expression of the downstream *ampG* gene. The partial complementation of *S. maltophilia* strain $KJN2_{xylE\Omega}$ containing an *ampN* polar mutant with *ampG* from *E. coli* indicates that *ampG* is needed for the expression of functional lactamases. It was suggested that AmpN is a cytosolic protein that interacts with AmpG to form a permease and interacts with AmpG-associated ligands to form the inducer for lactamase expression (153).

Charge variants of L2 β-lactamases have been identified in *S.*

maltophilia isolates (147). IEF analysis of the isolates revealed two major patterns, with most isolates exhibiting pattern I (with one band of pI <7.0 [L1] and one band of pI >7.0 [L2]) and some exhibiting pattern II (multiple bands representing two acidic β-lactamases [pI <7.0] and several β-lactamases [pI >7.0]). The different β-lactamase charge variants are products of the same L2 gene, suggesting that *S. maltophilia* has the ability to maintain the production of β-lactamase activity even in environments with a changing pH (147).

The multiantimicrobial resistance of *S. maltophilia* is in part due to the activity of multidrug efflux pumps (6, 10, 11, 54, 129, 196, 198, 269, 383). These multidrug efflux pump systems consist of a membrane fusion protein, an energy-dependent transporter, and an outer membrane protein. The cloning and sequencing of the *Stenotrophomonas* multiple-efflux (*sme*) *smeDEF* operon from *S. maltophilia* and the expression of the operon in *E. coli* indicated that *smeDEF* encodes a multidrug efflux pump (11). The SmeDEF efflux pump contributes to resistance to β-lactams, tetracycline, erythromycin, quinolones, aminoglycosides, and chloramphenicol. Experiments that examined the intracellular accumulation of ethidium bromide and norfloxacin in the absence and in the presence of the proton uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) revealed that the activity of the multidrug efflux pump SmeDEF is linked to the membrane potential. The *smeF* gene encodes an outer membrane protein that displays immunological cross-reactivity with an antibody generated against outer membrane protein 54 (Omp54), a diagnostic protein for multidrug resistance in *S. maltophilia* (11).

A MexAB-OprM-like multidrug efflux system was identified in *S. maltophilia* by Zhang et al. (383). Studies of gene knockout mutants of the β-lactamase L1 and L2 genes in multidrug-resistant strain K1385 of *S. maltophilia* containing MexAB-OprM efflux systems revealed that L1 knockout mutants were unable to hydrolyze imipenem but were able to hydrolyze nitrocefin. The L2 knockout mutants did not show an altered hydrolytic activity against imipenem but did demonstrate a decreased hydrolytic activity against nitrocefin. In the knockout mutants of L1 and L2 and the L1 L2 double mutant, the remaining β-lactam resistance was due to the overexpression of the multidrug efflux system responsible for the resistance of *S. maltophilia* to quinolones, chloramphenicol, and erythromycin (383).

The *smeABC* operon of *S. maltophilia* was identified and cloned by using a PCR-amplified probe for the *mexB* sequence within the *mexAB-oprM* multidrug efflux operon of *P. aeruginosa* (196). The putative proteins encoded by the SmeA, SmeB, and SmeC genes are an inner membrane fusion lipoprotein, a resistance-nodulation-cell division (RND) transporter, and an outer membrane efflux lipoprotein, respectively. The *smeABC* operon is regulated by a two-component regulatory system encoded by the *smeS* (encodes a sensory kinase) and *smeR* (encodes a response regulator) genes. An analysis of *smeABC* in multidrug-resistant mutants of *S. maltophilia* revealed that the expression of *smeC* is important for drug resistance and that SmeABC does not act as a multidrug efflux system in *S. maltophilia* (196).

When introduced into *P. aeruginosa*, SmeC of *S. maltophilia* restored antibiotic resistance, indicating that it was able to function as a component of a MexAB-SmeC multidrug efflux system in *P. aeruginosa* (196). The *smeC* gene has its own weak promoter similar to that of the *oprM* gene of the *mexAB-oprM* multidrug efflux operon of *P. aeruginosa*. SmeR positively regulates the *smeABC* and *smeSR* operons. The β-lactam resistance of an SmeABC-overexpressing strain is due to increased β-lactamase activity and not efflux. A deletion of *smeC* in *S. maltophilia* alters the hydrolysis of nitrocefin. There was a difference in the hydrolysis rates of nitrocefin by parental MDR *S. maltophilia* strain K1668 (ΔL1 ΔL2 MDR) of 14 nmol g^{-1} min^{-1}, compared with a hydrolysis rate of 0.016 nmol g^{-1} min^{-1} by *S. maltophilia* strain K1785 (Δ*smeC* ΔL1 ΔL2 MDR). The rate of hydrolysis of nitrocefin by wild-type *S. maltophilia* strain ULA-511 ($L1^+$ $L2^+$) of 47 nmol g^{-1} min^{-1} was reduced to 0.023 nmol g^{-1} min^{-1} in *S. maltophilia* strain K1784 (Δ*smeC*), suggesting that the presence of SmeC is linked to the expression of the L2 β-lactamase (196).

Integrons, common regions (329), and integron-like elements have been reported for *S. maltophilia* isolates worldwide. Integrons are not self-mobilizable elements but contain an integrase-encoding gene that permits the insertion of antibiotic resistance gene cassettes in between highly conserved nucleotide sequences (330). Transposons and plasmids can facilitate the movement of integrons between bacterial cells (46). Class 1 integrons have been found within transposons, which can be transferred to plasmids/chromosomal DNA by transposition events. Class 1 integrons can capture gene cassettes by using a specific attachment site (*att1*), resulting in composite elements. The 5′-conserved end of the integron contains the integrase gene (*intl1*) and a promoter needed for the expression of the gene cassette integrated into the *att1* site (64, 137). The 3′-conserved end of the class 1 integron contains the *sul1* gene, encoding resistance to sulfonamides, and the *qacE*Δ*1* gene, encoding tolerance to antiseptics containing quaternary ammonium compounds (137, 321). Class 1 integrons have been found in *S. maltophilia* isolates in North and South America, Australia, Asia, and Europe (27, 55, 198, 328). Clonal expansion plays an important role in drug resistance dissemination.

The distribution of class 1 and 2 integrons has been examined for their associations with the presence of SmeABC and SmeDEF pumps and antibiotic resistance in 93 *S. maltophilia* clinical isolates recovered from Kkaohsiung Medical University Hospital in Taiwan during January to December 2002 (54). As determined by PCR, 22% of the isolates harbored class 1 integrons, whereas class 2 integrons were not detected. Resistances to aminoglycosides (*aacA4*) and to trimethoprim (*dfrIIa*) and the small multidrug resistance gene *smr* were found in association with the class 1 integrons. The isolates harboring *smr* demonstrated a 4-fold increase in MICs of ciprofloxacin compared to isolates lacking this gene. Only 1 out of the 93 isolates contained a plasmid carrying a class 1 integron (this carried an *aacA4* gene cassette), suggesting that integrons and plasmids together may not be the major mechanism used for the dissemination of antibiotic resistance among strains of *S. maltophilia*. Real-time PCR, used to measure the gene expression of the *sme* efflux pumps, demonstrated that the SmeABC and SmeDEF efflux pumps contribute to the resistance of *S. maltophilia* isolates to ciprofloxacin and meropenem, respectively (54).

Upstream of the *smeDEF* operon is *smeT*, a proposed transcriptional repressor of the *smeDEF* operon in *S. maltophilia* (301). SmeT was suggested to bind to an operator sequence in the intergenic sequence of *smeT-smeD* containing the promoters for *smeT* and *smeDEF* (301). The overexpression of *smeDEF* in MDR *S. maltophilia* strains that harbor wild-type *smeT* and SmeT binding sites appeared to be influenced by additional mechanisms other than SmeT (302). A comparison of the 16S rRNA sequence and the β-lactamase gene sequence showed them to be linked, result-

ing in three distinct rRNA groups of *S. maltophilia* (17). Sequence data showed that the intergenic sequence of *smeT-smeD* from 10 clinical *S. maltophilia* isolates representing the three 16S rRNA groups is more conserved in isolates of the same rRNA group than in isolates of different rRNA groups (129). These data suggest that the grouping of *S. maltophilia* clinical isolates based on genotypic properties is feasible.

In a recent study of 40 MDR and 30 non-MDR *S. maltophilia* clinical isolates, high-level expression of SmeD and SmeA was observed more for the MDR isolates (85% and 60% for SmeD and SmeA, respectively) than for the non-MDR isolates (33% and 17% for SmeD and SmeA, respectively) (198). The high level of expression of *smeA* or *smeD* correlated with resistance to gentamicin, ciprofloxacin, levofloxacin, ceftazidime, cefepime, ticarcillin-clavulanic acid, piperacillin-tazobactam, aztreonam, and meropenem (198).

The *qnr* gene present in *S. maltophilia* chromosomal DNA (Sm*qnr*) contributes intrinsic resistance to quinolones (300). This resistance appears to be present in limited amounts in wild-type *S. maltophilia*, as providing the *qnr* gene on a plasmid in both the wild type and an ΔSm*qnr* mutant results in increased resistance to quinolones in both the wild type and the mutant. A gene dosage effect of SmQnr was observed when the gene was plasmid borne, in contrast to the relatively low-level expression of chromosomally carried Sm*qnr*. The expression of the Sm*qnr* gene has been reported to result in low-level resistance to quinolones in a heterologous host (299, 313). Resistance to quinolones in *S. maltophilia* can occur as a result of mutations of the bacterial topoisomerase and gyrase genes (126, 339) and may also arise due to the overexpression of the efflux pump SmeDEF (11, 12). The expression of several new variants of the Sm*qnr* genes in *E. coli* resulted in the increased resistance of *E. coli* to quinolones (126). It was proposed that *S. maltophilia* isolates harboring Sm*qnr* genes may act as a reservoir for the transfer of these genes into *Enterobacteriaceae* (126). Two new Sm*qnr* genes have recently been identified in *S. maltophilia* clinical isolates. The cloning and expression of these genes in *E. coli* increased resistance to quinolone antibiotics, including ciprofloxacin, ofloxacin, gatifloxacin, moxifloxacin, gemifloxacin, and enoxacin (358). The *qnr* genes were proposed to originate in the chromosomal DNA of environmental aquatic bacteria, and horizontal transfer to other bacteria appears possible through the genes' presence in conjugative plasmids (322).

Class 1 integrons have been reported to be responsible for increased TMP-SMX MIC values for *S. maltophilia* clinical isolates (27). The *sul* genes that contribute to resistance to TMP-SMX (328) have been reported to be associated with class 1 integrons (27) and insertion element common region (IS*CR*) elements. The *sul1* gene has been found as part of the class 1 integron in TMP-SMX-resistant *S. maltophilia* isolates recovered from Taiwan, Spain, Turkey, Italy, Germany, North America, and South America (27, 328). The *sul2* gene has been found on plasmid DNA (~120 kb) and as part of chromosomal DNA in *S. maltophilia* TMP-SMX-resistant isolates (328). Some *sul2* genes have been linked to IS*CR2*, with the element linked to Δ*glmM*, a deletion of a phosphoglucosamine mutase gene; this molecular arrangement has also been observed for other bacterial species, including *Vibrio salmonicida*, *Shigella flexneri*, and *E. coli* (328).

The *dfrA* gene, encoding the dihydrofolate reductase enzyme, has been reported to contribute to the trimethoprim resistance of *S. maltophilia* (146). Of 102 *S. maltophilia* isolates recovered from hospitals in China, 16 carried *dfrA* genes, and each *dfrA*-positive isolate carried a class 1 integron. The class 1 integrons contained gene cassettes, including *dfrA17-aadA5*, *dfrA12-aadA2*, *aacA4-catB8-aadA1*, *aadB-aadA4*, *aacA4*, *aadA5*, *aadA1*, *aadB–aac(6′)-II–bla*$_{\text{CARB-8}}$, *arr-3–aacA4*, and *cmlA1*. That study revealed that the *sul2* and *dfrA* genes were present on a 7.3-kb plasmid. Together, the *sul2*, *dfrA*, and *sul1* genes contribute to resistance to TMP-SMX (146).

Liaw et al. (198) reported that of 40 MDR and 30 non-MDR *S. maltophilia* isolates, 42 (60%) harbored class 1 integrons with drug resistance genes, most commonly against aminoglycosides, with isolates showing resistance to ciprofloxacin, ceftazidime, cefepime, ticarcillin-clavulanic acid, piperacillin-tazobactam, TMP-SMX, meropenem, and gentamicin. Of the MDR isolates, 83% carried class 1 integrons, while 30% of the non-MDR isolates carried these integrons. Gene cassettes within the class 1 integrons included *aacA4*, *aadB*, *aacC4*, *aacA6′-1b*, *smr*, *smr/aacA4*, *qac*, *cmlA*, *catB2*, and *bla*$_{\text{IMP-8}}$*/aac6-II/aadA5* (198).

A TEM-2 β-lactamase on a Tn*1*-like transposon in the genome of *S. maltophilia* clinical isolate J675Ia was reported (18). The transposon was able to be mobilized onto the broad-host-range conjugative plasmid R388 and moved into *E. coli* UB1832, demonstrating the ability of *S. maltophilia* to harbor and exchange DNA with other bacteria. This observation has clinical significance when considering the potential for the spread of antibiotic resistance in the clinical setting.

The deletion of the *aac(6′)-Iz* acetyltransferase gene in wild-type *S. maltophilia* K1449 resulted in mutant strain K1669, which demonstrated increased susceptibility to 2-deoxystreptamine aminoglycoside antibiotics, including netilmicin, sisomicin, tobramycin, neomycin, and gentamicin (all MICs of $<$4 mg/liter) (195). Resistance to these antibiotics was restored (all MICs of $>$8 mg/liter) in complemented *S. maltophilia* K1669 mutants containing the *aac(6′)-Iz* gene, and *E. coli* transformants expressing this *S. maltophilia* gene demonstrated increased (up to 8-fold) MICs of tobramycin, netilmicin, and sisomicin.

The ease of acquisition and spread of these antibiotic resistance genes in *S. maltophilia* emphasizes the need for antibiotic susceptibility testing of isolates from patients. The monitoring of clinical isolates may identify sources of transmission of *S. maltophilia*.

Hydrolytic Enzymes

The *S. maltophilia* K279a genome encodes extracellular enzymes including proteases, lipases, esterase, DNase, RNase, and fibrolysin (67). Clinical *S. maltophilia* isolates have been reported to demonstrate cytotoxicity activity (111). Supernatants of some clinical *S. maltophilia* isolates recovered from liver and trachea exhibited hemolytic and enzymatic activities. The exposure of Vero (African green monkey) and HeLa (human cervix) cells to *S. maltophilia* culture supernatant filtrates resulted in endocytosis, cell aggregation, and cytotoxicity effects on HEp-2 (human larynx epidermoid carcinoma) cells. These effects included rounding, membrane blebbing, a loss of intercellular junctions, and cell death after 24 h. The tested protease inhibitors failed to inhibit the cytotoxic activity of the *S. maltophilia* isolates. In addition to the hemolytic and cytotoxic activities, these *S. maltophilia* isolates demonstrated additional virulence factors, including protease, lipase, and lecithinase activities, while isolates recovered from

blood did not demonstrate any of these virulence factors or hemolytic and cytotoxic activities (111).

The rhizosphere is an adverse environment, and the ability of *S. maltophilia* to express proteolytic activity provides an advantage for the survival, growth, and spread of this organism. A rhizosphere *S. maltophilia* isolate harbored serine protease activity against the free-living nematode *Panagrellus redivivus* and a plant-parasitic nematode, *Bursaphelenchus xylophilus* (152). It is plausible, therefore, to suggest that nosocomial isolates of *S. maltophilia* may have already acquired the genes for these enzymes from the environment outside the hospital. Extracellular serine proteases in nosocomial *S. maltophilia* isolates have been reported. These proteases contribute to the pathogen's ability to degrade connective tissues (collagen and fibronectin) (368).

The protease (elastase) production of *S. maltophilia* has been reported to exacerbate influenza A virus infection of human, equine, and pig host cells (213). Of the 13 samples confirmed to contain influenza virus from animals, including pigs, horses, and humans working in close contact with the animals, 21.11% of them were coinfected with *S. maltophilia*. It was suggested that the elastase produced by *S. maltophilia* cleaves and activates the hemagglutinin glycoprotein spike of influenza A virus, enabling the virus to enter host cells and resulting in cytopathic effects on the infected cells. Following the treatment of the samples with sulfadiazine, protease production by *S. maltophilia* and the cytopathic effects of influenza virus on the host cells decreased (213).

The gene encoding the extracellular protease StmPr1 was found in only 2 of 11 *S. maltophilia* clinical isolates (strains OBGTC9 and OBGTC10, recovered from persistent infections in CF patients), suggesting that the gene may be found in isolates that have been able to cause chronic infections in these patients (88).

Lipopolysaccharide

S. maltophilia has lipopolysaccharide (LPS) that contains lipid A, core oligosaccharide, and O-antigen. The lipid A structure of *S. maltophilia* strain NCTC 10257 contains phosphorylated glucosamine residues with *N*-fatty acyl and *O*-fatty acyl components (240). Components of the core oligosaccharide have included D-glucose, D-mannose, D-galactose, D-galactosamine, D-galacturonic acid, 3-deoxyoctulosonic acid, and L-glycero-α-D-mannoheptose (225, 240). O-antigen components have included rhamnose, fucose, xylose, and glucose (166, 225, 382).

Charged lipopolysaccharides have been reported to influence bacterial cell adhesion to surfaces by covering charges present in deeper cell wall layers (212). The positively charged cell surface of *S. maltophilia* strain 70401 was reported to be important for adherence to glass and Teflon (166). The absence of outer membrane proteins in this strain was suggested to have resulted in the positive charge of the cell surface, and this combined with a noncharged lipopolysaccharide resulted in an increased ability of *S. maltophilia* to adhere to Teflon and glass in comparison to that of a *P. aeruginosa* isolate with a negatively charged cell surface.

Incomplete LPS can alter the biofilm production of *S. maltophilia* (41, 151). Mutagenesis of *S. maltophilia* identified two operons, *rmlBACD* and *xanAB*, that are important for the production of LPS (151). SDS-PAGE analysis of purified LPS from *S. maltophilia rmlA*, *rmlC*, and *xanB* mutants revealed that *rmlC* and *rmlA* are needed for O-antigen biosynthesis and that *xanB* is needed for the biosynthesis of O-antigen and the core region of LPS (151). The *rmlB*, *rmlA*, *rmlC*, and *rmlD* genes encode dTDP-glucose 4,6-dehydratase, glucose-1-phosphate thymidylyltransferase, dTDP-dehydrorhamnose 3,5-epimerase, and dTDP-4-dehydrorhamnose reductase, respectively. The *xanA* and *xanB* genes encode phosphomannomutase and phosphomannose isomerase/GDP-mannose pyrophophorylase, respectively. Biofilm production was assessed for *rmlA*, *rmlC*, and *xanB* transposon insertion mutants after growth in polystyrene microtiter plate wells containing Trypticase broth at 30°C at 50 rpm for 2 days. The *rmlA*, *rmlC*, and *xanB S. maltophilia* mutants displayed a significant decrease ($P < 0.05$) in biofilm production on polystyrene in comparison to the parental isolate. The *rmlA* and *rmlC* mutants produced significantly more ($P < 0.05$) biofilm on glass than that produced by the wild type and the *xanB* mutant (151). In my laboratory, analyses of an *S. maltophilia rmlA* transposon insertion mutant grown for 30 h at 37°C revealed that it formed more ($P < 0.05$) biofilm on polyvinyl chloride than that formed by its parental wild-type strain (334). In contrast to the growth of the parental wild-type isolate, the mutant also demonstrated sensitivity to growth on Luria-Bertani agar containing 0.1% SDS (334).

The *spgM* gene encodes a bifunctional enzyme that has both phosphoglucomutase and phosphomannomutase activities, which are important for O-polysaccharide chain assembly (41, 225). A knockout mutation of the *spgM* gene results in reduced levels of phosphoglucomutase and phosphomannomutase activities. Complementation experiments in which the cloned *S. maltophilia spgM* gene was provided on a plasmid to the knockout mutant restored the activities of both enzymes (225).

S. maltophilia spgM mutant strains display a lower yield of high-molecular-weight O-antigen than that of their parental strains (41, 225, 348). The structure of the core region of LPS is not altered by the knockout mutation of the *spgM* gene (225). A comparison of the monosaccharide composition of O-antigen of *spgM* chromosomal knockout mutant strain K2049 and that of its parental wild-type strain, K1014, revealed that they harbored similar rhamnose/fucose/glucose ratios, indicating that the *spgM* knockout mutant was able to synthesize and assemble an O-antigen with the same chemical structure as that of the wild-type strain (225).

In my laboratory, an analysis of *S. maltophilia spgM* transposon insertion mutant strain JB12-23 showed that it formed more biofilm ($P < 0.001$) than that formed by its parental wild-type strain, X26332, on polyvinyl chloride microtiter wells (41, 348). The *spgM* mutant formed more ($P < 0.05$) biofilm than that formed by the parental strain on polystyrene microtiter wells and on borosilicate glass (41, 348). The doubling times of the parental and mutant strains in the microtiter plates were 96.8 and 93.5 min, respectively, suggesting that differences in biofilm production were not due to increased growth rates. No significant difference ($P > 0.05$) in hydrophobicity between the *spgM* mutant strain and the parental strain was observed. It is possible that the sensitivity of the hydrophobicity assay may not have been able to distinguish between the subtle differences in LPS present on the wild-type and mutant cell surfaces. In contrast to the growth of the wild-type strain, the *spgM* mutant was unable to grow on LB agar containing 0.1% SDS. *spgM* mutant JB12-23 colonies failed to absorb Congo red stain, in contrast to the parental X26332 colonies, which appeared dark red when grown on Congo red agar. Together, these observations suggested that the incomplete LPS expressed by the mutant unmasked cell surface components otherwise concealed

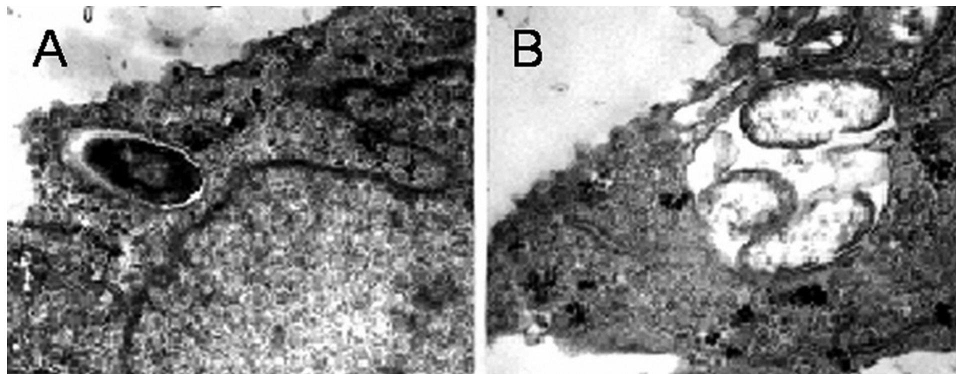

FIG 5 Transmission electron micrographs of epithelial respiratory cells exposed to *S. maltophilia* CF 1 (A) and NCF 13 (B) for 3 h. Note the presence of intracellular bacteria in membrane-bound endocytic vacuoles. Magnifications, ×30,000 (A) and ×35,000 (B). (Panel A courtesy of M.-C. Plotkowski; panel B reprinted from reference 73 with permission of Wiley-Blackwell.)

by LPS, thereby enabling the cells to more easily adhere to the selected plastic or glass surface (41, 348).

Deficits of LPS reduced *S. maltophilia* virulence in a rat lung model of infection (225). *S. maltophilia spgM* chromosomal knockout mutant strain K2049 was unable to colonize rat lungs, in contrast to its parental strain, K1014, which was recovered from rat lungs at 7 days postinfection. The complementation of the mutant with the introduction of the *spgM* gene on plasmid pGAM03 restored the ability to colonize rat lungs, providing evidence that full-length LPS is important for colonization. Rat lung tissues inoculated with *spgM* mutant strain K2049 showed no histopathological changes, in contrast to wild-type strain K1014 or the complemented mutant. *spgM* mutant strain K2049 was susceptible to complement-mediated cell killing, unlike parental strain K1014 or the complemented mutant (225). These observations emphasize the importance of LPS as a virulence factor involved in *S. maltophilia* infection.

Alterations in LPS may change the bacterial cell's susceptibility to particular antimicrobial compounds, e.g., cationic peptides and aminoglycosides. *S. maltophilia spgM* chromosomal knockout mutant strains K2048 and K2049 exhibited sensitivity to polymyxin B, polymyxin E, nalidixic acid, gentamicin, and vancomycin, in contrast to their parent strains (225). In a recent study of 40 MDR and 30 non-MDR *S. maltophilia* clinical isolates, the expression of *spgM* was weakly correlated ($P < 0.05$) with multidrug resistance, with high levels of SpgM expression being associated with only three lactams (ceftazidime, ticarcillin-clavulanic acid, and piperacillin-tazobactam) (198).

Temperature has been reported to alter the chemical composition of LPS, resulting in changes in susceptibility to aminoglycosides (275). The growth of 33 clinical *S. maltophilia* isolates and five reference strains (NCTC 10257, NCTC 10258, NCTC 10259, NCTC 10498, and NCTC 10499) at 37°C and 30°C revealed that 23 out of the 38 strains demonstrated a >4-fold difference in MICs of gentamicin and other aminoglycosides, showing increased sensitivity at 37°C. Chemical composition analysis of the LPS of these 23 strains showed that LPS had a significant increase ($P < 0.001$) in the phosphate content at 37°C compared to that of LPS when strains were grown at 30°C; there was no significant difference in the 3-deoxy-D-manno-octusolonic acid (KDO) contents at the two temperatures. Data from fluorescence-activated cell sorter (FACS) analysis of fluorescently labeled gentamicin binding to the cell membranes of these strains revealed that significantly larger amounts ($P < 0.01$) of gentamicin were bound at 37°C than at 30°C (275). That study suggested that when *S. maltophilia* strains are grown at 37°C, increased numbers of aminoglycoside binding sites may be available as a result of the greater number of negatively charged phosphate groups. These experimental results may therefore explain why it is common to see resistance of *S. maltophilia* clinical isolates to aminoglycosides at 30°C and susceptibility at 37°C. These observations can have clinical significance when taking into consideration antimicrobial treatment at various sites of *S. maltophilia* infection in the host.

Adherence to and Invasion of Host Cells

S. maltophilia can adhere to and form biofilms on human bronchial epithelial cells and is able to invade them (73, 90, 268). Transmission electron microscopy indicates that both cystic fibrosis (CF) and non-cystic fibrosis (NCF) *S. maltophilia* isolates adhere to and are able to invade transformed human bronchial epithelial 16 HBE14o− cells (Fig. 5) (73). No significant difference between the adherences of CF and NCF isolates to host bronchial cells was observed.

Flagella have been reported to mediate the adherence of *S. maltophilia* isolates to mouse tracheal mucus. Flagella are highly immunogenic structures and are conserved among clinical isolates of *S. maltophilia* (84, 360). *S. maltophilia* cells preexposed to antiflagellin decreased the adhesion of the bacteria to mucus, and the decrease corresponded to the concentration of antiflagellin. The pretreatment of the mouse tracheal mucus with pure flagellin resulted in a decrease in bacterial adhesion. Deflagellated bacteria also demonstrated a reduced adherence to mouse tracheal mucus (381).

In contrast to their parental wild-type isolates, two flagellum-deficient *S. maltophilia fliI* CF mutants demonstrated decreased adherence to CF-derived bronchial epithelial IB3-1 cells and were defective in swimming motility (268). The *fliI* mutants caused IB3-1 monolayer cell disruption after 6 h, suggesting that the mutants have increased virulence; further research is needed to explain this altered virulence. Swimming and twitching motilities of *S. maltophilia* CF isolates did not correlate with adherence or biofilm formation on bronchial cells (88, 268). In another study, *S. maltophilia* CF isolates and two nonrespiratory *S. maltophilia* reference strains (environmental strain LMG959 and blood-isolated strain K279a) were examined for swimming and twitching motil-

ity and biofilm formation; no correlation was observed for biofilm formation and motility (88).

In my laboratory, *S. maltophilia fliF* transposon insertion mutant strain JB5-39 is flagellum defective and motility defective, as determined by negative-staining TEM and motility agar assays (37). Under the culture conditions used, there was no major difference in the adherences and amounts of biofilm formed on PVC surfaces by the *fliF* mutant and its parental wild-type isolate (37). Taken together, studies of flagella and the adherence of *S. maltophilia* to lung cells and PVC surfaces suggest that flagella may play an important role in the early stages of adherence but do not significantly influence biofilm formation.

S. maltophilia isolates have demonstrated a limited ability to invade human respiratory cells. Tested *S. maltophilia* CF clinical isolates have been reported to adhere to A549 cells, along intercellular junctions. The adherence of *S. maltophilia* to A549 cells was not dependent on the ability of the bacterial strain to form biofilm or demonstrate motility. The CF *S. maltophilia* isolates were able to invade these cells with a reported range of 0.002% to 0.005% (88). The rates of invasion of bronchial epithelial 16 HBE14o− cells by isolates CF 1 and NCF 13 of *S. maltophilia* were reported to be 0.45% and 0.40%, respectively (73). Observations of CF and NCF isolates within membrane-bound endocytic vacuoles suggest that microbial division can occur in the intracellular compartment of host epithelial cells. *S. maltophilia* CF isolates were limited in their abilities to invade IB3-1 bronchial cells, with rates of invasion ranging from 0.01 to 4.94% (268). The limited invasiveness of *S. maltophilia* has been reported for the transient low-level presence of the bacteria in the spleens of DBA/2 mice infected with an *S. maltophilia* CF isolate through the use of an aerosol delivery system (87). Limited invasion and rapid clearance of the bacterium from the lungs were reported for *S. maltophilia* introduced intranasally into mice (380). The invasion of host cells and subsequent protection from host immune defense provide one explanation for the ability of *S. maltophilia* to persist in chronic lung infections.

Diffusible Signal Factor System

S. maltophilia has a diffusible signal factor (DSF) system that was first identified in *Xanthomonas campestris* pv. *campestris* (113, 149). The DSF activity of *S. maltophilia* strain WR-C is due to *cis*-Δ2-11-methyl-dodecenoic acid and seven structural derivatives (150). *rpfF*, part of the *rpf* (*r*egulation of *p*athogenicity *f*actors) gene cluster of *S. maltophilia* K279a, complemented the *rpfF* mutant of *X. campestris*, resulting in DSF production (113). The *rpfF* mutant of *S. maltophilia* K279a demonstrated reduced motility, reduced extracellular protease production, altered LPS, and reduced tolerance to select antibiotics and heavy metals. In contrast to wild-type *S. maltophilia*, the *rpfF S. maltophilia* mutant is unable to form microcolonies in artificial sputum medium. The exogenous addition of DSF (1 μM or from *S. maltophilia* extracts) restored the ability of the *rpfF S. maltophilia* mutant to form microcolonies and restored motility and extracellular protease production. In a nematode model, the *rpfF S. maltophilia* mutant demonstrated reduced killing activity, in contrast to wild-type *S. maltophilia* (113). The *rpfF* gene regulates the expression of FecA, an outer membrane receptor used for ferric citrate uptake (149). The cyclic AMP receptor protein (CRP) positively regulates *rpfF* transcripts; complementation studies and the presence of two potential CRP binding sites upstream of the *rpfF* promoter suggest that CRP is a transcriptional activator of *rpfF*. Transposon mutants in *crp* of *S. maltophilia* were defective in proteolysis and hemolysis, in contrast to wild-type *S. maltophilia* (149). Together, these observations suggest that *rpfF* and *crp* are important for the virulence of *S. maltophilia*.

Providing *rpfF* in *trans* in wild-type *S. maltophilia* and in *S. maltophilia* Δ*rpfB* and Δ*rpfBF* mutants resulted in swimming and radial translocation of these strains (150). The ability of the wild type and a flagellum-defective *S. maltophilia xanB* mutant to demonstrate radial translocation in the presence of an Δ*rpfB/prpfF* (plasmid *prpfF* contains the 975-bp *rpfF* native promoter and coding sequences in pBBR1MCS5) *S. maltophilia* strain suggested that the Δ*rpfB/prpfF* strain secreted molecules that enabled flagellum-independent translocation. High-performance liquid chromatography, electrospray ionization mass spectrometry, and gas chromatography-mass spectrometry analyses of these extracellular compounds have shown them to be derivatives of *cis*-Δ2-11-methyl-dodecenoic acid. Synthetic *cis*-Δ2-11-methyl-dodecenoic acid or 11-methyl-dodecenoic acid enabled the surface translocation of wild-type *S. maltophilia* carrying pBBR1MCS5 (150).

The DSF activity of *S. maltophilia* is recognized by *P. aeruginosa*, alters susceptibility to polymyxin, and influences biofilms of *P. aeruginosa* (Fig. 6) (291). An *rpfF* mutant of *S. maltophilia* does not synthesize DSF, and biofilms of the mutant are not as filamentous as those produced by wild-type *S. maltophilia* (113, 291). The complementation of the *S. maltophilia rpfF* mutant with the cloned *rpfF* gene or the supplementation of the mutant with DSF (10 or 50 μM) restores the filamentous structure of the biofilm (291). *P. aeruginosa* formed flat biofilms when grown in monoculture or in coculture with the *S. maltophilia rpfF* mutant. In cocultures with DSF-producing *S. maltophilia* and *P. aeruginosa*, the biofilm of *P. aeruginosa* changed from a flat to a filamentous biofilm. A filamentous biofilm was also observed in monocultures of *P. aeruginosa* supplemented with 10 or 50 μM DSF. The PA1396 protein of *P. aeruginosa* was identified as a two-component sensor of DSF (291). The addition of DSF or the mutation of *PA1396* resulted in increased resistance to polymyxins B and E. Mutations of *PA1396* also resulted in the increased expression of a number of proteins involved in stress tolerance (291). The recent identification of *cis*-2-decenoic acid as a fatty acid that induces the dispersal of *P. aeruginosa* PAO1 biofilms provides further evidence for the role of select fatty acids as cell-cell signaling molecules that influence biofilm architecture (72). Together, these observations have clinical significance for the treatment of polymicrobial infections of *S. maltophilia* and *P. aeruginosa*. The DSF system may be a target for pharmacological therapy.

S. maltophilia and the Cystic Fibrosis Lung Environment

The role of *S. maltophilia* in the pathogenesis of CF lung disease is not clear. It was reported that approximately 11% of CF patients are colonized by *S. maltophilia* (69). Determining if *S. maltophilia* is colonizing or causing infection can be challenging (245). *S. maltophilia* has been reported to impair lung function in CF patients, but several studies have reported no difference in lung function in *S. maltophilia*-positive CF patients, and one study demonstrated that the presence of *S. maltophilia* did not reduce the survival of CF patients (76, 128, 168, 217, 320).

In a retrospective cohort study of the period from 1997 to 2008 using the Toronto (Canada) CF database, CF patients with chronic *S. maltophilia* infection had a lower mean percent predicted FEV_1 (47.06%) than patients with intermittent *S. maltophilia* infection (78.6%) or patients never infected with *S. malto-*

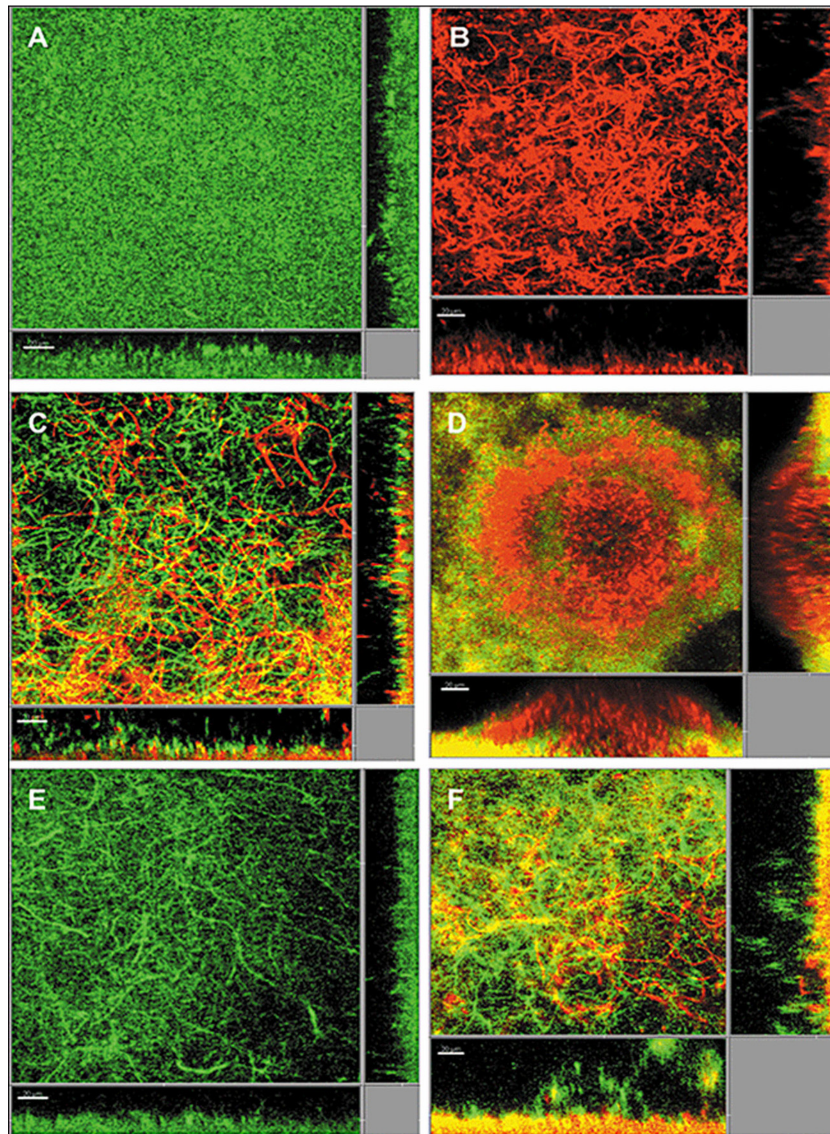

FIG 6 The biofilm architecture of *P. aeruginosa* is influenced by *S. maltophilia* and DSF. Images are of 4-day-old biofilms in flow cells in FABL medium. (A) *P. aeruginosa* PAO1; (B) *S. maltophilia* K279a; (C) coculture of *P. aeruginosa* PAO1 and *S. maltophilia* K279a; (D) coculture of *P. aeruginosa* PAO1 and *S. maltophilia* K279a*rpfF*; (E) *P. aeruginosa* PAO1 with 50 μM exogenous DSF; (F) coculture of *P. aeruginosa* PAO1 and the complemented *S. maltophilia* K279a*rpfF* mutant. *P. aeruginosa* was tagged with mini-Tn*7gfp*, and *S. maltophilia* was visualized with Syto62. Scale bars, 20 μm. The confocal scanning laser microscopy images shown are representative of 12 images from three independent experiments. (Reprinted from reference 291 with permission of John Wiley & Sons.)

philia (73.40%) ($P < 0.0001$) (359). Chronic *S. maltophilia* infection was identified as an independent risk factor for pulmonary exacerbation requiring hospitalization and antibiotic therapy. Using a model adjusted for patient age, pancreatic insufficiency, *P. aeruginosa*, body mass index, and percent predicted baseline FEV_1, patients with chronic *S. maltophilia* infection had a significantly higher risk of pulmonary exacerbation ($P = 0.0002$) than patients without *S. maltophilia* infection. The rate of decline in the percent predicted FEV_1 for patients with chronic *S. maltophilia* was −1.02% predicted per year, that for patients with intermittent *S. maltophilia* was −0.94% predicted per year, and that for patients never infected with *S. maltophilia* was −1.06% predicted per year. The lack of an association of chronic *S. maltophilia* infection with an increased rate of decline in the percent predicted FEV_1 reported in that study agrees with the results obtained by Goss et al. (127). In a cohort study, CF patients aged ≥6 years in the CF Foundation National Patient Registry from 1994 to 1999 demonstrated a negative correlation of *S. maltophilia* with FEV_1 ($P \leq 0.0001$); however, *S. maltophilia* did not appear to have an effect on lung function decline (127).

The sputum of CF patients contains glycoproteins and high-molecular-weight DNA at high concentrations, resulting in a highly viscous physical barrier that surrounds and protects bacterial inhabitants from the antimicrobial activities of pharmaceutical treatments (206). The ability of these macromolecules to bind to antimicrobial drugs and interfere with the drugs' ability to enter bacterial cells and the relatively low pH of CF sputum can all reduce the activity of antimicrobial drugs (206). As mentioned

above, under nutrient limitation conditions, *S. maltophilia* forms UMC (316). It is interesting to speculate whether in artificial sputum medium, *S. maltophilia* UMC form and assemble into biofilms.

Molecular biology strategies have been developed to improve the ability to detect *S. maltophilia* in CF patient sputum samples. PFGE, ERIC-PCR, *gyrB* restriction fragment length polymorphism (RFLP) analysis, and ribotyping have been used to analyze *S. maltophilia* isolates recovered from sputum samples (45, 57, 63, 78, 222, 246, 287, 338, 364). Multiplex PCR has been used to identify *S. maltophilia*, *P. aeruginosa*, and *B. cepacia* complex isolates in respiratory samples from CF patients (71). Primers designed to amplify a 149-bp fragment of the chitinase A gene of *S. maltophilia* were included in a multiplex procedure with universal primers to detect 16S rRNA genes and primers specific for *P. aeruginosa* and 16S rRNA genes of the *B. cepacia* complex. The method successfully detected 50 pg of *S. maltophilia* DNA, 5 pg of *P. aeruginosa* DNA, and 250 pg of *B. cepacia* genomovar I DNA. This multiplex protocol demonstrated high negative predictive values ($>$90%) for the identification of the three pathogens, in contrast to the relatively low positive predictive values. It was proposed that the genomic heterogeneity observed among *S. maltophilia* strains may provide a reason for the low sensitivity of the method for the detection of *S. maltophilia* (71).

Cough-generated aerosols from CF patients have been reported to contain respiratory particles of $\leq$3.3 μm from which viable *S. maltophilia*, *P. aeruginosa*, *Burkholderia cenocepacia*, and *Achromobacter xylosoxidans* could be cultured (351). A cough aerosol sampling system was used to capture aerosolized droplets generated from CF patients. *S. maltophilia* was cultured from 4 patients, 2 of the patients did not produce sputum, and the other patients were sputum culture negative for *S. maltophilia*. These observations indicate that these aerosols can be a potential source of transmission of *S. maltophilia*. Future work is needed to determine the concentration and size of respiratory particles needed to cause *S. maltophilia* infection in susceptible individuals.

The accumulation of mucous in the CF lung provides a favorable growth environment for *P. aeruginosa* and *S. maltophilia*. Iron is restricted in the human lung by lactoferrin, which sequesters iron and reduces its accessibility by microbial pathogens. High concentrations (100 μM) of ferric chloride inhibit the biofilm development of *P. aeruginosa* PAO1, suggesting that the aerosolized delivery of ferric chloride may provide an effective nonantibiotic treatment for CF patients (373). Recent work in my laboratory indicated that this high concentration of ferric chloride did not prevent biofilm production by *S. maltophilia* clinical isolate X26332 (219). Future studies are needed to examine the simultaneous incubation of *S. maltophilia* with *P. aeruginosa* and test the hypothesis that during the course of infection and disease, *S. maltophilia* protects *P. aeruginosa* against the inhibitory effects of this relatively high concentration of iron. Lactoferrin has demonstrated efficacy at inhibiting biofilm formation by *P. aeruginosa* (319). It is interesting to speculate about the influence of lactoferrin on biofilm production by *S. maltophilia*.

In vitro cell viability and antibiotic susceptibility assays indicated that β-lactamases produced by *S. maltophilia* clinical isolates increase the growth of *P. aeruginosa* exposed to imipenem (4 or 16 μg/ml) or 32 μg/ml of ceftazidime (169). These data are important, as they suggest that *S. maltophilia* may indirectly contribute to disease development by providing a favorable growth environment for *P. aeruginosa* in the CF lung.

Panresistant bacteria are a concern for lung transplant CF patients. A recent study reported that CF patients harboring panresistant bacteria (defined as resistant bacteria demonstrating intermediate resistance to an antibiotic from each class of antibiotics) other than *B. cepacia* have slightly decreased survival following lung transplantation. Compared with CF patients harboring susceptible bacteria, the survival rates of CF patients with panresistant bacteria other than *B. cepacia* are excellent (136).

S. maltophilia and *P. aeruginosa* have been coisolated from the human lung environment. It may be that in the CF lung environment, *P. aeruginosa* infection is followed by *S. maltophilia* infection. The cell adherence and invasion of *S. maltophilia* in the presence of *P. aeruginosa* have been studied. The incubation of transformed host human bronchial epithelial 16 HBE14o$-$ cells with *P. aeruginosa* strains 1412, 1440, and PAK prior to incubation with *S. maltophilia* isolate CF 1 did not alter the adhesion of *S. maltophilia* to the host cells, suggesting that the two bacteria are not competing for the same host cell receptors (73). These data are in contrast to data from a study reported by Pompilio et al. (268), in which the preexposure of human bronchial CF-derived epithelial IB3-1 cell monolayers to *P. aeruginosa* increased the adhesion of *S. maltophilia* to lung cells.

When *P. aeruginosa* was incubated simultaneously with *S. maltophilia*, the adherence of *P. aeruginosa* to human bronchial epithelial 16 HBE14o$-$ cells was not significantly altered, whereas the adherence of *S. maltophilia* to the host cells was reduced to approximately 50% (73). A heat-labile substance of *P. aeruginosa* appears to inhibit the adherence of *S. maltophilia* to the respiratory cells (73). It is of interest that the preincubation of the epithelial cell monolayers with *S. maltophilia* decreased the adherence of *P. aeruginosa* to bronchial epithelial IB3-1 cells (268). Future work is needed to determine if the differences in the adhesion of *S. maltophilia* to the host cells reflected differences in the *P. aeruginosa* and *S. maltophilia* strains used in the two studies.

Cell adherence and biofilm studies and the discovery of the diffusible signal factor of *S. maltophilia* suggest a relationship between these two bacterial species in the human lung environment. Both organisms have the ability to form biofilms on lung cells *in vitro* (291). *P. aeruginosa* may provide a more hospitable environment for the adherence, invasion, and persistence of *S. maltophilia* in the CF lung.

An animal model of acute respiratory infection has been used to study lung infection of DBA/2 mice with a CF *S. maltophilia* isolate (87). An aerosol delivery system was used to inoculate female and male mice with 8 ml of 1.0×10^{10} to 3.0×10^{10} CFU/ml *S. maltophilia* CF isolate OBGTC9. Almost all ($>$99%) of the bacteria were killed within the first week postinoculation. The invasiveness of *S. maltophilia* was assessed by using an analysis of spleen homogenates, with the day 1 data showing the highest percentage of *S. maltophilia*-positive spleens and no bacteria being recovered from spleens on day 14. A major inflammatory response against the bacterial pathogen was observed in the mice. Cytokine and chemokine levels were elevated in infected mice. On day 1, the following cytokines were observed at higher levels than in control uninfected mice: interleukin-1β (IL-1β), IL-6, IL-12, gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α). On day 3, only the IFN-γ level was higher in infected than in control mice. The higher levels of TNF-α, IL-1β, and IL-6 in infected mice

than in control mice are in agreement with observations of lung secretions from CF patients versus healthy individuals (36). On day 1, the following chemokines were expressed at higher levels in infected than in control mice: keratinocyte-derived cytokine (GROα/KC), monocyte chemotactic protein 1 (MCP-1/JE), macrophage chemoattractant protein 5 (MCP-5), macrophage inflammatory protein 1α (MIP-1α), MIP-2, and thymus- and activation-regulated chemokine (TARC). After day 3, no chemokines were observed at higher levels in infected mice than in control mice. These data indicate an immune response defined by a Th1-type response with the recruitment of polymorphonuclear leukocytes (PMNs) and monocytes. Severe weight loss in the infected mice occurred immediately after the increased expression of TNF-α, supporting the role of this cytokine in inducing the excessive inflammatory response and CF-like systemic effects. Additional research is needed to determine if this inflammatory response is induced by *S. maltophilia* strains in general or if it is bacterial strain specific. One recent study (380) of respiratory tract infection in BALB/c mice inoculated with 1×10^9 CFU of clinical *S. maltophilia* isolate Sm2 reported similar results of early (4 h postinfection) increased levels of IL-1β and TNF-α compared with control mice and IL-10 levels in lung homogenates at a maximum of 2 days postinfection. By day 5, levels of IL-1β, TNF-α, and IL-10 were normal compared to those observed for the control mice. These data provide evidence to support the hypothesis that this induced inflammatory response is common across different strains of *S. maltophilia*.

Sodium chloride concentrations are relatively high in the CF lung environment. Hypertonic saline has been proposed by several studies to have therapeutic potential in CF patients (23, 92, 96, 99, 280). The inhalation of hypertonic saline improves mucociliary clearance, with dose dependency reported for treatments of 0.9%, 3.0%, 7.0%, and 12% NaCl (280). It was suggested that hypertonic saline increases the hydration of airway surfaces, resulting in improved forced expiratory volume and increased mucus clearance (280). Increased airway inflammation assessed through the monitoring of sputum cytokines such as IL-8 has not been observed for hypertonic saline, and no increase in the severity of bacterial infection has been observed for CF patients treated with hypertonic saline (96). Bronchodilator use prior to treatment with hypertonic saline reduces airway constriction (96).

In my laboratory, the growth and motility of an *S. maltophilia* clinical isolate exposed to 4% sodium chloride were inhibited, while culture growth was still observed with 2% sodium chloride (219). These observations suggest that hypertonic saline should be considered for the treatment of *S. maltophilia* infections in CF patients. Hypertonic saline (7%) was reported to abolish the growth and motility of *P. aeruginosa* (139). These observations suggest that at this concentration, sodium chloride may reduce *P. aeruginosa* colonization of CF patient lungs. Future studies are needed to test the hypothesis that salt inhibits the biofilm formation of binary cultures of *S. maltophilia* and *P. aeruginosa*. If shown to be effective at inhibiting the development of biofilms *in vitro*, the aerosolized delivery of salt solutions to the lungs of CF patients may help delay biofilm development in the lung.

Regular treatment with recombinant human DNase (rhDNase) has been shown to reduce sputum viscoelasticity and respiratory tract infections and improve lung function in CF patients (114, 277). In a randomized crossover pilot study of 14 CF patients treated with either inhaled hypertonic saline (5.85% NaCl given as 10 ml twice a day) or rhDNase (2.5 mg/day), improved forced expiratory volume was observed for hypertonic saline (mean = 7.7%; standard deviation [SD] = 14%) and for rhDNase (mean = 9.3%; SD = 11.7%), with no significant difference observed between the hypertonic saline and rhDNase treatments (23). In this small study, patients reported a higher acceptance of the rhDNase than the hypertonic saline, possibly due to the shorter time required for its inhalation (23).

Several studies have investigated the use of combination antibiotics to treat CF patients infected with MDR Gram-negative bacteria (1, 294, 304). The macrolides azithromycin and clarithromycin paired with ceftazidime, quinolones, chloramphenicol, tetracycline, or tobramycin have demonstrated modest synergistic and additive effects against *S. maltophilia* isolates from CF patients (294). Antibiotic susceptibility testing was performed on *S. maltophilia* isolates recovered from 673 CF patients during 1996 to 2001 (304). The study isolates reflected approximately 7 to 23% of CF patients in the United States who were colonized with *S. maltophilia* annually. Synergistic or additive effects were demonstrated for TMP-SMX and ticarcillin-clavulanate, ciprofloxacin and ticarcillin-clavulanate, ciprofloxacin and piperacillin-tazobactam, TMP-SMX and piperacillin-tazobactam, and doxycycline and ticarcillin-clavulanate (304). A clinical trial of CF patients with chronic Gram-negative bacterial infections reported no better outcomes for patients with an exacerbation of pulmonary disease who had received two blinded intravenous antibiotics chosen based on sputum culture testing or results of multiple-combination bactericidal antibiotic testing (MCBT) in comparison with antibiotic therapy chosen based on standard conventional culture testing (1). The suggested explanations for these results included the possibility that *in vitro* bacterial antibiotic susceptibility may not correlate with the clinical response to antibiotic therapy; that the antibiotic resistance of bacterial strains can change over time, leading to ineffective treatment outcomes; and that biofilms may inhibit the clinical response to antimicrobial therapy (1). Those studies together underscore the need to continue to monitor the emergence and presence of MDROs in CF patients, with the intent of developing more effective antimicrobial therapies for these patients.

A recent study of *S. maltophilia* K279a demonstrated that the expression of GroEL, a member of the group I chaperonins, is influenced by changes in temperature (74). Immunoblot analyses demonstrated that sera from CF patients chronically infected with *S. maltophilia* reacted with recombinant GroEL, whereas no reactivity was observed with sera obtained from patients infected sporadically or not infected with *S. maltophilia*. No cross-reactivity was observed between GroEL of *P. aeruginosa* and GroEL of *S. maltophilia*. The results of this study suggest that GroEL may serve as a useful indicator of *S. maltophilia* in CF patients chronically infected with this organism.

MICROSCOPY OF *S. MALTOPHILIA*

Electron microscopy has been used to examine the ultrastructure of *S. maltophilia* cells. Transmission electron microscopy (TEM) and SEM have identified flagellum-like filaments (approximately 40 to 50 nm in width) and thin fibrillar structures (5 to 7 nm in width) resembling fimbriae of *S. maltophilia* SMDP92 as being important for adherence to plastic and glass surfaces and HEp-2 cells (Fig. 2 and 3) (83, 84). TEM studies have revealed that *S.*

maltophilia is able to adhere to and invade respiratory host cells (Fig. 5) (73).

There are some major limitations of using electron microscopy to examine the interaction of *S. maltophilia* clinical isolates with host cells. The specimens are killed during specimen preparation, and the bacterial cell contact with host cells may potentially be altered or distorted. The dehydration of the specimen during SEM preparation causes a distortion of biofilm matrices. In contrast to electron microscopy, confocal microscopy offers the opportunity to examine living *S. maltophilia* cells within hydrated biofilms.

Confocal microscopy has revealed that biofilm formation by *P. aeruginosa* is altered by the diffusion signal factor expressed by *S. maltophilia* (Fig. 6) (291). The data reported in that study suggest that cell-cell signaling between these two pathogens may offer new target sites for pharmaceutical intervention and the inhibition of polymicrobial biofilm formation in patients with compromised lung function. More research is needed to identify and develop target inhibitors suitable for testing in animal model systems.

Confocal microscopy and flow chamber studies with *P. aeruginosa* containing green fluorescent protein and *rhlA-gfp* reporter fusions have shown that iron limitation induces rhamnolipid synthesis, promotes twitching motility, and alters biofilm structure, resulting in the formation of thin flat biofilms (124). Rhamnolipids may be important for maintaining water channels in biofilms (124). Further studies are needed to establish if the biofilms of *S. maltophilia* are similarly altered by iron limitation.

Recently, fluorescence recovery after photobleaching analysis and confocal laser scanning microscopy have been used to measure the diffusion of fluorescent dextran inside the biofilm of *S. maltophilia* (350). A mean diffusion coefficient value of 10 ± 5 $\mu m^2/s$ was obtained for fluorescent dextran in the *S. maltophilia* biofilm. This nondestructive method has the advantage of using confocal laser scanning microscopy to analyze the diffusion of molecules *in situ* within the spatial architecture of the biofilms. This technique has potential use for the study of the movement of antimicrobial agents and their antimicrobial effects on *S. maltophilia* biofilms.

There is a need for further studies using confocal microscopy to look at the adherence and development of biofilms of *S. maltophilia* on clinically relevant surfaces (e.g., plastic, respiratory tubing, blood transfusion equipment surfaces, and plumbing surfaces) that can come into contact with susceptible individuals. Future studies using confocal microscopy are needed to look at the development of *S. maltophilia* biofilms under different environmental conditions (e.g., disinfection treatments). The Live/Dead BacLight kit (Invitrogen) has been used in combination with flow cytometry to assess bacterial viability (32), and the Film Tracer Dead/Live biofilm viability kit (Invitrogen) has been used with confocal microscopy to determine the effects of biocides on the biofilm formation of other bacterial pathogens.

COMPARING CLINICAL AND ENVIRONMENTAL *S. MALTOPHILIA* ISOLATES

Genome Sequencing and Molecular Diversity of *S. maltophilia* Strains

Recent studies have explored the differences between environmental and clinical isolates of *S. maltophilia* to try to determine what mechanisms are responsible for the bacterium's pathogenicity in humans. It is important that the genome sequencing of two *S. maltophilia* strains has been accomplished. One strain is a clinical isolate (*S. maltophilia* K279A) from a cystic fibrosis patient who was undergoing chemotherapy in 1998 (67), and the other strain is an environmental isolate (*S. maltophilia* R551-3) from the poplar *Populus trichocarpa* (EMBL/GenBank/DDBJ database accession no. NC_011071). The genome sequence of clinical isolate K279a harbors genes that are not found in the genome of environmental isolate R551-3. The genome sequence of *S. maltophilia* K279A contains 4,851,126 bp and a G+C content of 66.7% (67). *S. maltophilia* K279A harbors 9 antimicrobial RND transporters and several genes important for drug resistance mechanisms. The genome sequence of isolate R551-3 contains 4,573,969 bp (accession no. NC_011071). Both *S. maltophilia* genomes (from K279A and R551-3) contain genes for the mismatch repair (MMR) system, the guanine oxidation system, the nucleotide excision system, the recombination repair system, and the SOS system (335). The MMR genes of both isolates shared high sequence identity, ranging from 90 to 95%. A closer examination of the MMR genes *mutS*, *mutL*, and *uvrD* in these isolates and their corresponding amino acid sequences revealed polymorphisms in MutS, MutL, and UvrD. Defects of the MutS protein were suggested to result in the emergence of hypermutable strains from patients (335).

The availability of the genome sequence of *S. maltophilia* K279a has enabled efforts to develop a methodology for effectively and rapidly typing *S. maltophilia* isolates (287). PCR has been used to amplify multilocus variable-number repeats from the genomes of *S. maltophilia* isolates. Palindromic elements called SMAG (*S. maltophilia* GTAG) elements that contain the sequence GTAG at one terminus have been observed for the genome of *S. maltophilia* K279A (282, 287). These repetitive extragenic palindromic sequences that flank *S. maltophilia* genes were suggested to control gene expression by their folding within the mRNA and either stabilizing or facilitating the degradation of gene transcripts. The chromosome of K279A carries 1,650 SMAG sequences assigned to five major subfamilies based on their consensus sequences. The stem sequences were complementary in most of the SMAGs within the subfamilies, suggesting that the secondary folding of the repeat sequences can occur at the DNA and RNA levels of organization. Gene transcription analyses of *S. maltophilia* genomes from strains K279A, STM2, 545, and 1029 have provided evidence to support the hypothesis that SMAG repeats influence mRNA stability (281). A comparison of the SMAG sequences in K279A with the genomes of environmental isolates *S. maltophilia* R551-3 and SKA14 revealed them to contain SMAG sequences of all 5 subfamilies but with different sizes of these subfamilies. The subfamily SMAG-3 is dominant in strain K279A, and it was proposed that molecular analysis of this subfamily may be helpful for epidemiological and genotyping studies (282). Future research is needed for an in-depth analysis of the quantity and identification of genes whose expressions may be controlled by SMAG sequences.

Sequencing data for *S. maltophilia* isolates K279a and R551-3 demonstrated the highly variable content of genomic islands (281). In K279a, 41 genomic islands (constituting 12.1% of the genome and 597 open reading frames [ORFs]) have been identified, with the majority being >15 kb in size, while R551-3 harbors 36 islands (constituting 6.6% of the genome and 249 open reading frames), and just four islands are >15 kb. The presence or absence of genomic islands is a major source of the heterogeneity observed between the two isolates. Genomic island gene products identified

to have a role in interactions with the environment have included metal resistance genes, type I and IV secretion systems, LPS genes, and filamentous hemagglutinin genes (281). The two *S. maltophilia* isolates do not share common genomic islands but do contain genes with the same function. No correlation between the presence of specific genomic island gene products and the pathogenic life-style of *S. maltophilia* K279a has been discovered. Strain-specific ORFs also contribute to the genomic heterogeneity between the two isolates, constituting ~17.5% and 10.1% of the potential gene products for K279a and R551-3, respectively. PCR and slot blot analyses have demonstrated that strains of *S. maltophilia* can harbor genomic islands at locations that differ from those of isolate KI279a.

A study of the codon usage by *S. maltophilia* isolate R553-1 revealed that highly expressed genes are asymmetrically distributed and found mostly on the lagging strand of the genome, in contrast to low-level-expressed genes, which are evenly distributed between the lagging and leading strands (24). Nine of 14 genes encoding antibiotic resistance are expressed at low levels, and four of these genes were reported to have been acquired through horizontal transfer. That study proposed that these acquired genes are needed for the pathogenic mode of living of the *S. maltophilia* isolate (24). It was also hypothesized that the predominance of highly expressed genes on the lagging strand of the genome provides an advantage for the relatively slow growth of *S. maltophilia*. Further studies are needed to determine if this asymmetric distribution of highly expressed genes is common across clinical and environmental isolates of *S. maltophilia*.

The genetic heterogeneity of *S. maltophilia* has been identified through the use of several molecular biology methods (71, 246, 308, 339). The use of restriction fragment length polymorphism (RFLP) analysis of the gyrase B gene (*gyrB*) after HaeIII digestion demonstrated considerable diversity among *S. maltophilia* isolates (63). Cluster analysis of the *gyrB* RFLP patterns of 183 *Stenotrophomonas* isolates (including *S. maltophilia*, *S. africana*, *S. nitritireducens*, *S. acidaminiphila*, and *S. rhizophila*) placed the majority (36 out of 40) of the *S. maltophilia* isolates from CF patients into two clusters, clusters B and C. Future research is needed to determine if these two clusters contain *S. maltophilia* isolates with traits advantageous for the establishment and persistence of infection in CF patients.

PFGE and ERIC-PCR have both been used to type *S. maltophilia* isolates. Although PFGE is generally accepted as the more reliable method of typing, ERIC-PCR has the advantages of ease and lower cost than PFGE. Both methods have demonstrated heterogeneity among *S. maltophilia* strains.

PFGE and RFLP analysis of the *gyrB* gene and sequencing of the hypervariable regions of the 16S rRNA gene were used in a 2011 study to compare *S. maltophilia* strains recovered from CF patients and environmental sources (246). In addition, selected virulence factors were tested for their presence in *S. maltophilia* strains and compared for their expressions using cell assays and virulence testing in larvae of the greater wax moth, *Galleria mellonella*. A high degree of genetic diversity was observed among the 52 tested strains (41 from CF patients and the remainder from environmental samples), with 47 different pulsotypes and a similarity of 78 to 100%. Nine different *gyrB* RFLP profiles and 6 different 16S rRNA groups were observed, with 7 strains exhibiting highly divergent 16S rRNA signature sequences, making it impossible to assign them to a group. PCR detected the presence of SMF-1 fimbriae only in the clinically derived strains, providing support for the hypothesis that these structures are important for the colonization of CF patients (83). All strains exhibited only swimming motility, and no swarming motility was detected. All tested strains demonstrated the expected PCR product corresponding to the StmPr2 gene, encoding an extracellular protease (67). Of the 52 tested strains, 38 exhibited a 1,621-bp PCR product, and 11 exhibited an 868-bp PCR product, corresponding to the StmPr1 gene, encoding an alkaline serine protease (368). The putative esterase encoded by the Smlt3773 locus was detected by PCR in 49 isolates, but esterase activity was absent from 11 isolates; sequencing and BLAST analysis revealed premature stop codons resulting in a significant number of spontaneous nonfunctional variants. The major protease StmPr1 appeared to contribute more than the minor protease StmPr2 and the esterase to the virulence of *S. maltophilia*, as indicated by 50% lethal dose (LD_{50}) values observed for the killing of wax moth larvae; however, it was suggested that protease activity is not solely responsible for virulence, as two environmental protease-positive strains exhibited poor killing activity. Further studies using mammalian models of infection or CF-derived cell assays of strains with defined mutations in these virulence factor-encoding genes will be helpful to elucidate the individual contributions of these genes to the virulence of *S. maltophilia*.

A relatively new method, melting-curve analysis of RAPD-generated DNA fragments (McRAPD), has shown promise for the analysis of small numbers of *S. maltophilia* isolates at a time (86). The method demonstrated a sensitivity comparable to that of RAPD analysis followed by agarose gel electrophoresis in its ability to discriminate between *S. maltophilia* isolates and group them into genotypes. The McRAPD method is advantageous over traditional methods used to differentiate DNA sequences, as it does not require electrophoresis. The McRAPD method is, however, currently restricted, as it is not able to analyze large numbers of isolates due to differences observed in DNA fingerprints obtained between thermal cycling runs. Further optimization studies may improve the sensitivity and comparability between runs.

Linking Clinical Isolates to Sources

Several molecular biology methods have been used to compare and link clinical isolates to environmental sources. RAPD-PCR used to examine the epidemiology of *S. maltophilia* isolates from CF patients demonstrated that patients can harbor one or more persistent isolates and/or become colonized with new isolates (183). The identification of sources of *S. maltophilia* isolates can suggest preventative measures to be designed and implemented to decrease the possibility of infections. PFGE and random primer PCR fingerprinting have been used to determine if outbreaks of a particular strain of *S. maltophilia* have occurred in a hospital (308). In a 1-year study, PFGE with SpeI enzyme digestion of chromosomal DNA from 96 *S. maltophilia* clinical isolates obtained from patients in a tertiary care university hospital and random primer PCR fingerprinting were used to identify possible clonality among the isolates. No outbreak was confirmed in that study. The particular enzyme chosen for the digestion of chromosomal DNA in the PFGE method is important, as it can determine the level of sensitivity of differentiation between *S. maltophilia* isolates. The SpeI enzyme has been successfully used to identify different *S. maltophilia* isolates (189). Several studies have used

the XbaI enzyme with PFGE analysis to distinguish between *S. maltophilia* isolates (222, 227).

ERIC-PCR and PFGE have been used to compare clinical *S. maltophilia* isolates from CF patients with isolates from environmental samples (hospital ward, outpatient clinic, and patient homes) (81). During September 1993 to December 1995, 41 out of 163 patients demonstrated colonization by *S. maltophilia*, an incidence of 25%. Sampling of environmental sites resulted in the recovery of 82 *S. maltophilia* isolates from 67 positive sites. ERIC-PCR analysis revealed only 1 patient with multiple strains of *S. maltophilia*. The 45 clinical isolates were characterized by 10 different biotypes, 13 different antibiograms, and 41 different genotypes by ERIC-PCR. Unique ERIC-PCR types were found in 32 patients, four pairs of patients had the same ERIC-PCR type, and one patient was revealed to have 5 different strains. Twenty-one *S. maltophilia* isolates were recovered from the CF ward environment, and DNA analysis revealed 12 different ERIC-PCR types. Three environmental isolates (one from a sink drain, one from a faucet, and one from a water sample) shared an ERIC-PCR type identical to that of a pair of clinical isolates. Six *S. maltophilia* isolates (three from sink drains, one from a faucet, one from a water sample, and one from a toothbrush) had the same ERIC-PCR type and were identical to the ERIC-PCR type of 2 clinical isolates. No home environmental *S. maltophilia* isolates shared the same ERIC-PCR types as those of the clinical isolates from patients. PFGE after digestion with XbaI could distinguish all of the clinical isolates that shared the same or similar ERIC-PCR types. No patient-to-patient transmission was detected. A few patients may have acquired *S. maltophilia* from the hospital setting, but the repeated sampling of sites revealed the isolation of genetically different strains, suggesting that genetic drift may have occurred over time in *S. maltophilia*. The results of that study made it very challenging to identify environmental sources of clinical isolates acquired over time (81). That study highlights the importance of environmental sampling immediately following the diagnosis of nosocomial *S. maltophilia* infection. The sampling of moist environments that come into direct or indirect contact with patients may lead to the identification of the source of the *S. maltophilia* infection.

Nosocomial infections of *S. maltophilia* have been difficult to trace to environmental sources within the hospital. A recent report investigated the association between the prevalence of nonfermentative Gram-negative bacillus (NFGNB) species in hospital tap faucets and the colonization or infection of patients in intensive care units with these bacteria (355). Seven intensive care units, including a neurosurgical ICU, a surgical ICU, a cardiac surgical ICU, a pediatric ICU, two medical ICUs, and a respiratory care unit, were sampled for the presence of NFGNB. PFGE and electrokaryotyping analyses revealed no similarity between the *S. maltophilia*, *P. aeruginosa*, *B. cepacia*, and *A. xylosoxidans* isolates from faucets and the corresponding species of clinical isolates (355). That study also reported a strong positive correlation between the presence of NFGNB in the hospital tap water and the prevalence of waterborne NFGNB in ICU patients (355). The findings of that study suggest that alternatives to the use of hospital tap water should be considered, including disinfection of the water supply, point-of-use water filtration, and the use of sterile water.

The finding of *S. maltophilia* in hospital water does not always correlate with patient infection with this organism. In a study of a regional CF center, no correlation was observed between clinical isolates of *S. maltophilia* and isolates recovered from hospital tap water (222). PFGE with XbaI pattern analyses of the 110 clinical and 24 water isolates resulted in 59 and 14 different phenotypes, respectively. Of the 22 rooms of the CF center, 6 rooms were positive for *S. maltophilia*. Each room was colonized by *S. maltophilia* isolates of a unique PFGE phenotype, and most rooms were persistently colonized with *S. maltophilia*. It was suggested that the high frequency of *S. maltophilia* isolation from tap water presents the possibility of its transmission to CF patients, but the lack of evidence in that study did not suggest that the prevention of water contamination is a necessary infection control measure (222).

Electronic ventilator temperature sensors have been identified as a potential source of respiratory tract colonization with *S. maltophilia* (283). In an epidemiological investigation of a surgical ICU, environmental cultures from case patient room surfaces, including ventilator equipment and taps of sinks; hand-washing sinks in the staff lounge and nurses station; and a quaternary ammonium compound detergent-disinfectant solution were tested for the presence of *S. maltophilia* and compared to *S. maltophilia* isolates from 5 mechanically ventilated patients. Environmental cultures of *S. maltophilia* were recovered from a tracheal tube, traps, ventilator inspiratory and expiratory circuits, patient room surfaces after cleaning, and temperature sensors. No *S. maltophilia* cultures were recovered from water of patient room sinks or hand-washing sinks or from the detergent-disinfectant solution. Three patients shared the same RAPD profile with an environmental culture recovered from an in-use temperature sensor. These results led to a more effective disinfection regimen for the temperature sensors, with high-level disinfection by immersion in glutaraldehyde. The implementation of this new disinfection procedure resulted in a significantly lower incidence of new cases of *S. maltophilia* sputum positivity in ICU patients (283).

S. maltophilia was recently isolated from patients' charts in a surgical intensive care unit (327). That study reported the recovery of pathogenic or potentially pathogenic bacteria on 90.0% of the charts in the surgical ICU and 72.2% of the charts in the surgical ward. Two *S. maltophilia* isolates were recovered from the 81 contaminated charts in the surgical ICU. The *S. maltophilia* isolates from the patients' charts demonstrated the same antibiograms as those of the *S. maltophilia* isolates obtained from the patients. It was suggested that charts are fomites in nosocomial infections, acting as sources of transmission of the bacterium to other patients, and the importance of hand washing in reducing the possibility of transmission is underscored (327). Further research is needed to determine the survivability of *S. maltophilia* on the relatively dry surface of patient charts and assess the risk of infection posed by the microbial contamination of charts.

Adaptation and Evolution of Clinical Isolates

A reservoir of strains of *S. maltophilia* has been proposed to exist in the environment, and this has implications for the possibility of horizontal drug resistance transfer and subsequent spread within the clinical environment (233). Analyses of clinical and environmental isolates of *S. maltophilia* have revealed genomic heterogeneity among isolates (31).

A study by Turrientes et al. (335) compared the mutation frequencies of *S. maltophilia* clinical isolates (48 from 13 CF patients

and 66 from 53 non-CF patients with different infections) with those of 60 isolates recovered from nonclinical environments (rhizospheres of different plants, seawater, and sewage). The tested hypothesis was that upon entry into the host, the *S. maltophilia* isolate adapts to the host environment and that in chronic infections, strong pressures exerted by the host local environment and immune defense systems will increase the recovery of a variety of mutants derived from a single isolate, providing evidence for a high mutation frequency. In that study, higher mutation frequencies were found for clinical isolates than for environmental isolates. Highly variable mutations were present in isolates recovered from the same patient. The recovery of a number of isolates from a single CF patient over a 6-year period demonstrated the persistence of hypermutable strains. These strains were proposed to be the result of a mutation of the *mutS* gene of the mismatch repair system. The data from that study suggested that the cost of hypermutation does not hinder chronic infection of the lung by these hypermutable strains (335). These results are in agreement with those reported for *P. aeruginosa* isolates obtained from the lungs of CF patients (252).

Antimicrobial-producing and antimicrobial-resistant *S. maltophilia* isolates have been recovered from a number of aqueous-associated habitats in nature and in animals. It is interesting to speculate on the clinical significance of these isolates, as they have potential as sources of antimicrobial agents and/or as opportunistic pathogens if they come into direct contact with susceptible humans.

A comparison of the antifungal activities and 16S rRNA sequences of 25 clinical and 25 environmental isolates of *S. maltophilia* was reported (233). Of the clinical isolates, just one demonstrated antifungal activity against tested plant-pathogenic fungi (*Rhizoctonia solani*, *Verticillium dahliae*, and *Sclerotinia sclerotiorum*), and 32% of the clinical isolates demonstrated activity against *C. albicans*. Of the environmental isolates, 62% demonstrated activity against the plant-pathogenic fungi, and just 21% were active against *C. albicans*. The data indicate that the *S. maltophilia* antimicrobial activity against plant-pathogenic fungi and *C. albicans* is not exclusive to either set of clinical and environmental isolates. 16S rRNA gene sequencing of the isolates suggested that the majority of the clinical and environmental isolates could be differentiated (233). A limitation of that study was its inability to answer the question of whether clinical isolates have evolved from environmental *S. maltophilia* isolates.

An environmental, moderately halotolerant (growth in Trypticase soy agar [TSA] medium with 75 g/liter sodium chloride) isolate of *Stenotrophomonas* from sinkholes of the Yucatan peninsula demonstrated limited antimicrobial activity (75). *S. maltophilia* isolate 1X25 produced a bacteriocin-like substance and exhibited inhibitory activity against *Bacillus subtilis* (ATCC 6633) but no activity against the five additional target organisms, including *C. albicans* (ATCC 10231), *S. aureus* (ATCC 6536), *Pseudomonas syringae* pv. *pisi* (ATCC 11043), *X. campestris* pv. *carotae* (ATCC 10547), and *Erwinia carotovora* subsp. *carotovorum* (ATCC 138).

S. maltophilia isolates with antimicrobial activities have been recovered from deep-sea invertebrates (286). Four strains of *S. maltophilia*, KMM349, KMM339, KMM3045, and KMM365, were isolated from sponge, sea urchin, and ophiura specimens from the Philippine Sea, the Fiji Sea, and the Bering Sea. All four isolates are MDROs, with resistance to kanamycin (30 μg/disc), tetracycline (30 μg/disc), and erythromycin (15 μg/disc). All four isolates displayed antagonistic activities against the following fungi: *Aspergillus candidus*, *Aspergillus flavus*, *Beauveria bassiana*, *Epicoccum nigrum*, and *Fusarium oxysporum*. Three out of four isolates displayed antagonistic activities against *C. albicans*. Two out of four isolates exhibited antagonistic activities against the Gram-positive bacteria *Enterococcus faecium*, *S. aureus*, and *B. subtilis*. These observations demonstrate the potential of *S. maltophilia* environmental isolates as sources of antimicrobial metabolites.

In a phylogenetic study of microorganisms, two-primer RAPD analyses and sequencing of 16S rRNA genes were used to identify *S. maltophilia* isolates from superficial water of the saline subterranean Lake Martel in Spain (279). That study offered tourism as an explanation for the recovery of *S. maltophilia* from human infections.

MDR *S. maltophilia* strains have been isolated from domestic and wild animals (117, 138, 140, 141, 163) (Table 2). All 15 *S. maltophilia* isolates recovered from Omani goats with lymphadenitis demonstrated resistance to cephalosporins (ceftazidime [30 μg], cefotaxime [30 μg], cephalothin [30 μg]), β-lactams (penicillin G, ampicillin [10 μg], amoxicillin-clavulanic acid [30 μg]), or ticarcillin but were sensitive to aminoglycosides (kanamycin [30 μg], gentamicin [10 μg], and amikacin [30 μg]), erythromycin (15 μg), tetracycline (30 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), and TMP-SMX (25 μg) (163). That study suggested the potential for *S. maltophilia*-colonized goats living in close proximity to humans to serve as a reservoir of *S. maltophilia* infection of humans.

Mouth swabs of 16 out of 22 species of captive snakes revealed the presence of *S. maltophilia* (140). PFGE profiling revealed the heterogeneity of *S. maltophilia* isolates, as 8 snake species had more than one strain of *S. maltophilia*. In total, 47 isolates of *S. maltophilia* were recovered from 34 (29.6%) individual snakes. Antibiograms demonstrated that the most effective antibiotics against these isolates were TMP-SMX, levofloxacin, ofloxacin, colistin, and gentamicin. It was suggested that the presence of *S. maltophilia* originated in the water dishes of the vivariums (288). In another study, 45 *S. maltophilia* isolates recovered from captive snakes were tested for antibiotic susceptibility (141). After 24 h and 48 h of incubation at 37°C, the percentages of isolates demonstrating resistance were 44.4% and 71.1% against ceftazidime, 28.9% and 51.1% against chloramphenicol, 0% and 8.9% against levofloxacin, and 2.2% and 2.2% against TMP-SMX, respectively (141). The results of the increased effectiveness of the antibiotics at 37°C compared to 30°C are in agreement with results from an earlier study (140). These studies raise the issue of risk assessment for multidrug-resistant *S. maltophilia* infection of snake handlers.

All 6 *S. maltophilia* isolates recovered from yellowtail fish from a marine fish farm exhibited resistance to ampicillin, panipenem, cefotaxime, and ceftazidime (117). In veterinary medicine, *S. maltophilia* is not commonly considered to be a significant pathogen. This assessment of *S. maltophilia* may need to be revised, as future research is needed to establish whether animals harboring *S. maltophilia* are potential sources of infection for humans.

MOLECULAR ECOLOGY AND *STENOTROPHOMONAS* INFECTION

Gene Transfer in the Environment

S. maltophilia can acquire genes from other bacterial species. *S. maltophilia* has been reported to acquire genes involved in antibi-

otic and heavy metal resistance from Gram-positive bacteria (12). *S. maltophilia* can transfer antibiotic resistance to other bacteria (21). *S. maltophilia* has been isolated from the rhizosphere of plants; the rhizosphere was suggested to be a source of antibiotic resistance (30). In the rhizosphere, horizontal gene transfer has been reported for *S. maltophilia* (29).

The acquisition of DNA from other bacterial species has serious implications for gene transfer within microbial communities in environments such as wastewater and biofilms in plumbing, where *S. maltophilia* has been found in association with other MDROs that are members of the genera *Citrobacter*, *Sphingomonas*, *Serratia*, and *Klebsiella*. *S. maltophilia* has been reported to transfer antimicrobial resistance genes to bacteria, including *P. aeruginosa*, members of the *Enterobacteriaceae*, and *Proteus mirabilis* (34, 155). *Sphingomonas paucimobilis* is a persistent nosocomial infectious bacterium that is emerging as an opportunistic pathogen and is able to form biofilms in water-associated environments (39, 263, 290). The reported recovery of *S. paucimobilis* and *S. maltophilia* from the same biofilm (39) suggests that gene transfer is possible between these organisms. A recent report of dental solid waste harboring *S. maltophilia* (347) raises new questions about the viability and persistence of this opportunistic pathogen and whether DNA transfer from this organism can increase the potential pathogenicity and virulence of other microorganisms.

PCR amplification has been used to detect the presence of L1 metallo-β-lactamase (L1) and L2 serine β-lactamase (L2) in six strains of *S. maltophilia* isolated from yellowtail (*Seriola quinqueradiata*) in a fish farm (117). 16S rRNA gene sequencing revealed two clusters, clusters A and B, of the strains. Differences in DNA sequences of the β-lactamase genes within these clusters suggested that horizontal gene transfer of the β-lactamase genes had occurred. The possibility of the horizontal transfer of L1 and L2 β-lactamases is supported by their reported presence on 200-kb plasmids (17).

The conjugation of plasmids has contributed to the spread of antibiotic resistance among different bacterial species. The conjugal transfer of plasmid-bearing genes coding for multiple-drug resistance has been reported for *E. coli* isolates (336). Out of 105 clinical isolates of *E. coli*, 67 (64%) isolates carried plasmids, and 51 (76.1%) were able to transfer their plasmids into recipient cells. A high frequency of the isolates carrying plasmids was resistant to antibiotics, including ampicillin, imipenem, and TMP-SMX (336).

The conjugative transfer of plasmid DNA into *S. maltophilia*-like bacteria in river water has been reported (28). IncP-1 plasmid pJP4 carrying genes for the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) was successfully transferred from the *Pseudomonas putida* SM1443 donor into members of the genus *Stenotrophomonas* that are present in mixed liquor from a wastewater treatment plant (28). The data from that study suggest that as pJP4 is self-transmissible and is a broad-host-range plasmid, it may be useful in genetic engineering for complementation experiments in which wild-type genes are introduced into *S. maltophilia* mutants to restore the wild-type phenotype.

Stenotrophomonas maltophilia IAM12423 (EMBL/GenBank/DDBJ database accession no. AB294553) has been reported to acquire plasmid pCAR1, a conjugative IncP-7 plasmid involved in the degradation of carbazole (CAR) from the donor *P. putida* SM1443 (314). A fluorescent protein reporter gene cloned into plasmid pCAR1 was used to track the plasmid's transfer to bacteria in river water samples supplemented with a CAR-dimethyl sulfoxide (DMSO) solution. Natural pressures such as the presence of CAR appear to have resulted in a higher number of transconjugants in growth medium supplemented with CAR than the number of transconjugants identified in growth medium without CAR. That study suggested that the bacteria harboring the plasmid have an advantage for growth on CAR.

Lightning has also been implicated in DNA transfer between bacterial cells (51). Two *Pseudomonas* sp. strains, N3 and Ee2.2, isolated from soil demonstrated the ability to be transformed in a laboratory-scale lightning experiment. Strains N3 and Ee2.2 demonstrated an electrotransformation frequency for the uptake of pBHC, an 8.1-kb plasmid, of 10^{-4} in the absence of sucrose at 22°C, in comparison with control strains *E. coli* DH10B (frequency of 10^{-4}) and *Pseudomonas fluorescens* C7R12 (frequency of $<10^{-8}$). The electrotransformation frequencies increased for both N3 and Ee2.2 in the presence of 0.5 M sucrose in comparison to *E. coli* DH10B (frequency range of 10^{-6} to 10^{-7}), and *P. fluorescens* C7R12 required 10 mM $MgCl_2$ to achieve electrotransformation (frequency of 10^{-7}). In two lightning-induced transformation experiments at 20°C, strains N3 and Ee2.2 achieved their highest transformation frequencies, 10^{-4} and 10^{-5}, respectively, in comparison to the transformation frequency of $<10^{-9}$ displayed by both control strains *E. coli* DH10B and *P. fluorescens* C7R12. The electroporation efficiencies for the uptake of pBHC in the presence of 0.5 M sucrose by strains N3 and Ee2.2 ranged from 10^4 to 10^5 CFU/μg of plasmid DNA (51). These data suggest that antibiotic resistance gene acquisition by *S. maltophilia* strains can occur through lightning-induced transformation in the environment, and subsequently, when these strains gain entry into the clinical setting, they can retain the antibiotic resistance phenotype (31, 51).

The acquisition of genes from environmental bacteria by *S. maltophilia* emphasizes the importance of monitoring the antibiotic resistance of *S. maltophilia* clinical isolates. Such monitoring can provide insight into the environmental source of antibiotic resistance genes, show how these genes are being spread among clinical isolates, and suggest prevention strategies to reduce the level of antibiotic resistance.

Climate Change

There is a potential impact of climate change on the spread of infection associated with *S. maltophilia* through the transmission of waterborne infectious agents and the importance of sanitation for providing safe drinking water supplies (186, 253, 315). It was predicted that global temperatures will rise 1.8°C to 5.8°C by the end of this century, resulting in changes to the hydrologic cycle and rainfall and drought patterns (315). These temperature changes will likely shift the geographical distribution of waterborne diseases. Alterations in rainfall patterns and water chemical composition from pollution can alter the population diversity of microbes present, and it is expected that these changes will enable the emergence of new opportunistic pathogens. It is expected that some geographical areas will experience more droughts, and this may lead to poor sanitation as the population is forced to work with limited water supplies.

S. maltophilia is a common water inhabitant. An increase in the global temperature is likely to result in an increased growth rate of cells and higher cell concentrations that can come into contact with susceptible individuals and possibly pose an in-

creased risk of infection. Increases in cell concentrations of *S. maltophilia* in aqueous environments may lead to increases in the uptake of foreign DNA and the further acquisition of genes important for drug resistance and pathogenicity. This speculation must also consider the likelihood that the growth of other microorganisms is keeping *S. maltophilia* in check by their competition for space and nutrients.

S. maltophilia colonization or infection of plants and animals in close proximity/contact with humans may pose a risk of infection to human handlers. The concern for the impact of climate change on the distribution of infectious disease must therefore extend to workers in agriculture and aquaculture (186).

More information is needed about the survival and biofilm formation of *S. maltophilia* and its ability to transfer genetic material to and receive genetic material from other emerging pathogens. As noted above in this review, *S. maltophilia* has acquired genes from Gram-positive bacteria. It will be interesting to see if this opportunistic pathogen develops adequate strategies to acquire genes useful for pathogenesis from other cell types, e.g., algal or fungal cells.

FUTURE CHALLENGES

A major challenge facing clinical personnel will be to hinder *S. maltophilia*'s ability to adapt to the local environment of the patient and to alter antimicrobial strategies to keep pace with the evolution of *S. maltophilia*. The development of new treatments needs to take a microbial ecology/community approach to consider the interaction of *S. maltophilia* with host cell surfaces and antimicrobial defenses presented by the host and evaluate any effect on other potential pathogens colocalized with *S. maltophilia*. The use of biocides in clinical/medical settings should be carefully controlled to avoid encouraging the spread of biocide-tolerant *S. maltophilia* strains (e.g., those carrying the *qac* gene cassette).

An increase in the number of immunosuppressed individuals in the global population due to HIV infection, chemotherapy, drug therapies, and genetic disorders has been predicted (253). This anticipated increase underscores the need to continue to monitor worldwide the drug resistance status of emerging opportunistic pathogens such as *S. maltophilia* and identify genetic transfers that are occurring between different bacterial species. These studies may give insights into novel molecular and cellular targets that, when disrupted, result in decreased cooperation between different species and help reduce the incidence of particular polymicrobial infections, such as those found in CF or cancer patients. The identification of novel genetic mechanisms that enable the persistence of opportunistic bacterial pathogens in the community and clinical environments will likely lead to new strategies aimed at weakening or eliminating the survival of these organisms.

To combat the increasing incidence of *S. maltophilia* infections in hospitals and clinics, education to increase awareness of health care personnel is a key step in preventing the transmission and spread of this opportunistic pathogen. The prevention of biofilm formation and a reduction of the risk of infection within the clinical setting necessitate an observation of aqueous-associated environments and regular cleaning and disinfection regimens for surfaces of medical equipment that comes into contact, directly or indirectly, with patients. The hygienic practice of hand washing by health care personnel must continually be reinforced to reduce the possibility of organism transfer from tap water to patients. The avoidance of the use of hospital tap water for bathing and cleaning of wounds is a necessary measure of care for particularly vulnerable populations such as neonatal patients. The discarding of residual antibiotic solutions, residual and possibly contaminated hand soap solutions, and patient body fluids into the hospital plumbing system should be avoided. An increased vigilance for the observation and replacement of worn parts of susceptible surfaces, such as old deteriorating plumbing systems, can help reduce the risk of infection. Steps taken such as these are actions that can help lower the number of fatalities associated with *S. maltophilia* infections.

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