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Antimicrobial susceptibility of hospital acquired *Stenotrophomonas maltophilia* isolate biofilms

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ABSTRACT

Aims: We sought to characterize the antibiotic susceptibility of strains of *Stenotrophomonas maltophilia* isolated from clinical samples, and the role of *Stenotrophomonas maltophilia* biofilm in antibiotic resistance.

Methods: Fifty-one clinical *Stenotrophomonas maltophilia* isolates were obtained from patients with nosocomial infection in the surgical wards and ICUs of six general hospitals in Tianjin, China. *In vitro* models of *Stenotrophomonas maltophilia* biofilms were established and confirmed by scanning electron microscopy and fluorescence microscopy with silver staining. The minimal inhibitory concentrations and biofilm inhibitory concentrations of commonly used antibiotics were determined.

Results: 47 of 51 strains were resistant to three or more antibiotics. 42 of 51 strains formed *Stenotrophomonas maltophilia* biofilms *in vitro*. *Stenotrophomonas maltophilia* biofilm formation greatly reduced sensitivity to most tested antibiotics, but not to levofloxacin. However, in the presence of erythromycin scanning electron microscopy revealed that levofloxacin inhibited *Stenotrophomonas maltophilia* biofilm formation. Factorial ANOVA revealed that erythromycin enhanced susceptibility to levofloxacin, cefoperazone/sulbactam, and piperacillin ($p < 0.05$), and an ΔE model revealed that levofloxacin and erythromycin acted synergistically in biofilms, suggesting specific use of combined macrolide therapy may represent an effective treatment for *Stenotrophomonas maltophilia* infection.

Conclusions: Antibiotics could act synergistically to combat the protection conferred to clinical isolates of *Stenotrophomonas maltophilia* by biofilms. Macrolide antibiotics may be effective where used in combination.

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Introduction

Stenotrophomonas maltophilia (SMA) is an environmental pathogen and opportunistic Gram-negative bacterium that can infect immunocompromised patients or otherwise healthy patients when introduced by contaminated invasive medical devices.[1] Dialysis technology, intubation, artificial implants and other widely employed medical materials can be colonized by bacteria, and SMA has been observed to form bacterial biofilms (BBF) on this equipment. In surgical departments, device-related contamination by potentially pathogenic bacteria can serve as a source for cross-infection,[2] and nosocomial SMA infections have received increased attention in recent years.[3–11] SMA bacteremia has been associated with mortality rates ranging from 14 to 69% in immunocompromised patients.[12–14]

Treatment of SMA infection is complicated by its natural resistance to many antimicrobial drugs, including carbapenems, and the rapid adaptation to the pulmonary environment.[15] SMA can form BBF on host tissues, dramatically enhancing the resistance of SMA to therapeutically important antibiotics including aminoglycosides, fluoroquinolones, and tetracycline.[16–20] Thus, biofilm formation represents an important mechanism of bacterial antibiotic resistance, and presents unique challenges in surgical medicine, complicating therapeutic management of such BBF.[21,22]

SMA biofilm formation was previously reported to be associated with resistance to ceftazidime, cefepime, ticarcillin/clavulanic acid, piperacillin/tazobactam, aztreonam, and gentamicin, but not to ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole (TMP/SMX), or meropenem.[23] The fluoroquinolone moxifloxacin was reported to interfere with SMA BBF formation[24,25]; however antibiotic resistance of clinical isolates has also been widely reported,[26] mostly involving the study of strains isolated from cystic fibrosis (CF) patients.

In this study we sought to investigate the antibiotic-susceptibility of SMA strains isolated from invasive infections in non-CF patients. Using a methodology previously reported[27,28] we established an *in vitro* model of SMA BBF, and investigated the antibiotic-susceptibility of SMA biofilms and planktonic bacteria. We assessed the capacity of antibiotics, applied individually and in combination, to reduce growth and biofilm formation of clinical isolates of SMA, in order to guide future clinical treatment of these patients.

Materials and methods

Antibiotic susceptibility of SMA isolates

Clinical SMA strains were obtained from hospitalized patients with invasive infections that had originated from medical manipulation in the surgical wards and surgical ICUs of six general hospitals in Tianjin, China, between 2006 and 2012 (Table 1). The MICs of SMA to 12 antibiotics commonly used for Gram-negative bacilli were determined by microbroth dilution, analyzed according to the American National Clinical and Laboratory Standards Institute (CLSI) guidelines.[29] The following strains were assessed in parallel for quality control: ATCC27853, ATCC25922 and ATCC25923, preserved in the Infectious Disease Institute of the Second Hospital of Tianjin Medical University, China.

In vitro model of SMA BBF

Using a methodology previously reported by Ceri[27,28] we established an *in vitro* model of SMA BBF, in a Mueller–Hinton broth (MHB)-silica film system, as previously described.[30,31] Cryopreserved SMA was recovered in sheep blood agar plates incubated aerobically overnight. A fresh single colony was transferred to fresh MHB and incubated for 8 h at 35 °C, from which a 200 μL suspension of 0.5 McFarland was prepared and transferred to a 12-well flat-bottom plate, in which sterile silica film (1 cm × 1 cm × 1 mm, $L \times W \times T$) and 1.8 mL MHB were co-cultured at 35 °C for 12 and 24 h. After washing three times with 0.9% sodium chloride to remove planktonic bacteria, the BBF on the silica films was prepared. The culture medium was regarded as the negative control. Morphology was observed by scanning electron microscopy (SEM) and fluorescence microscopy (FSM) as described below.

Biofilm formation assessed using fluorescence microscopy with silver staining

As previously described,[32,33] the biofilm was fixed in 2.5% (v/v) glutaraldehyde in PBS (0.1 M, pH 7.4) for 24 h, then immersed in saturated calcium chloride solution for 15 min, and rinsed with ddH_2O between each step. The film was immersed in 5% silver nitrate solution for 15 min, immediately stained with 1% hydroquinone for 2 min, then rinsed with ddH_2O. The film was fixed in 5% sodium thiosulfate solution for 2 min, then rinsed in ddH_2O and analyzed by FSM.

Biofilm formation assessed using scanning electron microscopy

As previously described,[25] the silica biofilm was fixed in 2.5% (v/v) glutaraldehyde in PBS (0.1 M, pH 7.4) at 4 °C for 2 h, fixed again with 1% osmic acid for 1 h, then rinsed with PBS, dehydrated through a series of ethanol dilutions, then treated with isoamyl acetate. The specimen was dried in a vacuum, then coated with platinum–palladium and analyzed by SEM at 5–10 kV.

Table 1 – Samples from which SMA was isolated.

Tissues	*n* (%)
Pus	7 (13.7)
Intravascular catheter	7 (13.7)
Postoperative and burn wound	7 (13.7)
Bronchial secretions/lavage	6 (11.8)
Urinary catheter	6 (11.8)
Urine	5 (9.8)
Sputum	4 (7.8)
Bile	4 (7.8)
Blood	3 (5.9)
Ascitic fluid	2 (3.9)