Macrolide and Clindamycin Resistance in *Streptococcus milleri* Group Isolates from the Airways of Cystic Fibrosis Patients[▽]

Margot E. Grinwis,¹ Christopher D. Sibley,¹ Michael D. Parkins,^{1,2,4} Christina S. Eshaghurshan,¹ Harvey R. Rabin,^{1,2,4} and Michael G. Surette^{1,3*}

Department of Microbiology and Infectious Diseases,¹ Department of Medicine,² Department of Biochemistry and Molecular Biology,³ and Adult Cystic Fibrosis Clinic,⁴ University of Calgary, Calgary, Alberta T2N 4N1, Canada

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Organisms belonging to the *Streptococcus milleri* group (SMG) are known for their role in pyogenic infections but have recently been implicated as etiological agents of pulmonary exacerbation in adult patients with cystic fibrosis (CF). The prolonged exposure of CF patients to antibiotics prompted us to investigate the susceptibility profiles of 118 SMG isolates from the airways of CF patients to 12 antibiotics compared to 43 SMG isolates from patients with invasive infections. We found that ~60% of all isolates failed to grow using the standard medium for disc diffusion, Mueller-Hinton blood agar (MHBA), so we explored the usefulness of brain heart infusion (BHI) agar for susceptibility testing. Zone-of-inhibition comparisons between BHI and MHBA showed strong correlations for six antibiotics, and interpretations were similar for both medium types. For ceftriaxone and cefepime, both groups of isolates were highly susceptible. Tetracycline resistance levels were comparable between the two groups (22% in CF isolates and 17.4% in invasive isolates). However, more than half of the CF isolates were not susceptible to azithromycin, erythromycin, and clindamycin, compared to 11%, 13%, and 6.5% of invasive isolates, respectively. There were 5-fold and 8-fold increased risks of azithromycin and clindamycin resistance, respectively, for the isolates from the airways of CF patients relative to the invasive isolates. Macrolide resistance was strongly linked to chronic azithromycin therapy in CF patients. This study shows that BHI agar is a suitable alternative for antimicrobial susceptibility testing for the SMG and that SMG isolates from the airways of CF patients are more resistant to macrolides and clindamycin than strains isolated from patients with invasive infections.

The members of the *Streptococcus milleri* group (SMG; also known as the *Streptococcus anginosus* group), consisting of *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*, are considered commensal organisms notable for their tendency to cause pyogenic infections (33). In a recent population-based epidemiological study it was observed that members of the SMG were responsible for as many invasive streptococcal diseases as all other streptococci combined (25). The members of the SMG are also the most common cause of brain (10, 13) and liver (12, 28) abscesses. Furthermore, members of the SMG are recognized respiratory pathogens. The members of the SMG are commonly associated with pleural empyema (1, 24, 31), especially as a complication of community-acquired pneumonia (1, 35, 45, 48), and there is evidence that the SMG members are a cause of “culture-negative” community-acquired pneumonia (21). Recent studies have shown that the SMG may be an intrinsic component of the cystic fibrosis (CF) airway microbiome, with a high carriage rate observed for the sputum of adult CF patients (20, 36, 37). Moreover, members of the SMG were implicated in a large proportion of pulmonary exacerbations requiring hospitalization of adults with CF (29, 36, 37), and SMG-directed therapy in each case led to clinical resolution, correlating with a decrease in the SMG load.

The development and implementation of various suppressive antibiotic strategies have been integral in treating CF (16). A number of strategies are routinely employed as chronic therapies, including suppressive antistaphylococcal beta-lactam therapy (39, 41), aerosolized antibiotics targeting *Pseudomonas aeruginosa*, including aminoglycosides (32) and colistin (23), and chronic suppressive macrolide therapy (15, 34, 47). In addition, pulmonary exacerbations necessitating acute parenteral antibiotic therapies further increase antibiotic exposure. The impact of these practices has been studied only to a limited extent with organisms of the CF microbiome, aside from classic CF pathogens. Studies have shown that the long-term exposure of CF patients to azithromycin leads to significantly increased macrolide resistance in CF pathogens, such as *Staphylococcus aureus* and *Haemophilus* spp. (19, 30, 44), as well as members of the oral flora. Tazumi et al. found that nearly 90% of viridans group streptococci from patients receiving oral azithromycin therapy were not susceptible to erythromycin and postulated that these organisms may act as reservoirs of antibiotic resistance determinants (42).

Antimicrobial susceptibility profiles of clinical isolates of the SMG typically show these organisms to display low levels of resistance to beta-lactams, fluoroquinolones, and clindamycin (2–4, 6, 43, 49). The frequent use of antibiotics for the treatment of classical CF pathogens, such as *S. aureus* and *P. aeruginosa*, during pulmonary exacerbation and need for chronic suppressive antimicrobial therapy may result in vastly different resistance profiles of the CF-related SMG. In this study, we determined the antibiotic susceptibility profiles of SMG isolates from the sputum of 46 adult CF patients. Twelve antibiotics were chosen for study; eight

* Corresponding author. Mailing address: Department of Microbiology and Infectious Diseases, University of Calgary, Faculty of Medicine, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada. Phone: (403) 220-2744. Fax: (403) 270-2772. E-mail: surette@ucalgary.ca.

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of them, including, ceftazidime, cefepime, ceftriaxone, meropenem, azithromycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and rifampin, are commonly employed for CF. Given the fastidious nature of the members of the SMG, many of which fail to grow using standard protocols (5, 7, 8, 37), we also sought to determine if susceptibility testing on brain heart infusion (BHI) agar correlated with the Clinical and Laboratory Standards Institute (CLSI) approved methods. Furthermore, we determined antimicrobial susceptibility profiles of SMG isolates cultured from sites of invasive infections (blood and abscesses) for comparison.

MATERIALS AND METHODS

Bacterial strains and growth conditions. SMG isolates initially included in this study were 128 airway isolates from 47 CF patients, 45 invasive infection isolates, and three reference strains (*S. anginosus* ATCC 33397, *S. constellatus* ATCC 27823, and *S. intermedius* ATCC 27335). Methods of isolation and identification have previously been described (18, 37). CF isolates were cultured at times of both clinical stability (106/128) and pulmonary exacerbation (22/128). Invasive infection isolates were cultured from sterile sites, including blood and abscesses (pleural empyema, brain, liver, and soft tissue). Generally, invasive infection isolates were obtained from non-CF patients, although two of the invasive infection isolates were obtained from a CF patient with invasive *S. intermedius* infection coincident with a pulmonary exacerbation. Ten CF isolates and two invasive isolates were excluded due to poor recovery from frozen stocks or fastidious growth (requiring more than 24 h to form a lawn on agar plates), which is unsuitable for the determination of antibiotic susceptibilities by disc diffusion. Strains were inoculated from frozen skim milk stocks to Columbia blood agar (CBA; Difco) containing 5% defibrinated sheep blood (Med-Ox) and incubated anaerobically for 24 h at 37°C prior to setting up disc diffusion assays. The quality control strain *Streptococcus pneumoniae* ATCC 49619 was maintained on CBA at 37°C with 5% CO₂.

Antimicrobial disc diffusion assay. Mueller-Hinton blood agar (MHBA; Difco) containing 5% defibrinated sheep blood (Med-Ox) was prepared according to CLSI guidelines (11). BHI agar (Difco) was prepared according to the manufacturer's instructions. Isolated colonies from 24-hour anaerobic cultures on CBA were suspended in sterile 0.85% saline to a 0.5 McFarland standard. MHBA plates were inoculated as described in the CLSI guidelines, and BHI plates were inoculated in the same way. The following antimicrobial discs were stamped with a disc dispenser or manually placed: penicillin G (P; 10 U), ceftriaxone (CRO; 30 µg), cefepime (FEP; 30 µg), ceftazidime (CAZ; 30 µg), meropenem (MEM; 10 µg), azithromycin (AZM; 15 µg), erythromycin (E; 15 µg), clindamycin (DA; 2 µg), rifampin (RD; 5 µg), tetracycline (TE; 30 µg), ciprofloxacin (CIP; 5 µg), and trimethoprim-sulfamethoxazole (SXT; 25 µg). All discs were purchased from Oxoid (Nepean, Ontario). Inducible clindamycin resistance was determined by the disc diffusion D-zone test as described in the CLSI guidelines for streptococci (11). Plates were incubated for 24 h at 37°C with 5% CO₂, after which zone sizes were determined. MHBA plates were quality controlled with the reference strain *S. pneumoniae* ATCC 49619, and BHI plates were tested for batch-to-batch reproducibility using an *S. intermedius* isolate from this study. Zone diameters (mm) used to define susceptible (S), intermediate (I), and resistant (R) breakpoints for viridans group streptococci as stated in the CLSI guidelines (11) were as follows: ceftriaxone, S ≥ 27, I = 25 or 26, R ≤ 24; cefepime, S ≥ 24, I = 22 or 23, R ≤ 21; azithromycin, S ≥ 18, I = 14 to 17, R ≤ 13; erythromycin, S ≥ 21, I = 16 to 20, R ≤ 15; clindamycin, S ≥ 19, I = 16 to 18, R ≤ 15; and tetracycline, S ≥ 23, I = 19 to 22, R ≤ 18. BHI values were first converted to MHBA equivalents using linear regression analysis from data presented in Fig. 2. Strains that had been classified as having resistance or intermediate resistance were grouped together.

Statistical methods. BHI zone sizes for CF and invasive infection isolates were compared using a simple linear regression model. Analysis of the risk of antimicrobial nonsusceptibility in CF isolates relative to invasive infection isolates was performed using Stata 10.0 (Stata Corp., College Station, TX). Differences in proportions among categorical data were assessed using Fisher's exact test for pairwise comparisons and the chi-square test for multiple groups. Data not conforming to a Gaussian distribution (as determined by the D'Agostino and Pearson omnibus normality test) were analyzed with the nonparametric Mann-Whitney U test, and a two-tailed *P* value of <0.05 was considered statistically significant.

FIG. 1. (A) Percentage of *S. anginosus*, *S. constellatus*, and *S. intermedius* isolates used in this study. (B) Percentage of species cultivated on the standard medium for susceptibility testing, MHBA, and BHI.

RESULTS

BHI as an alternative medium for antibiotic susceptibility testing of the SMG. We assessed 118 isolates from adult CF sputum (46 *S. anginosus*, 40 *S. constellatus*, and 32 *S. intermedius* isolates) and 46 clinical isolates from patients with invasive diseases (9 *S. anginosus*, 13 *S. constellatus*, 24 *S. intermedius* isolates), including three reference strains for antimicrobial susceptibility. Each of the three species was equally represented in the complete strain collection (Fig. 1A). All isolates were tested against a panel of 12 antibiotics using the disc diffusion method on the standard medium recommended for susceptibility testing, MHBA, and test medium, BHI. While all nonfastidious organisms grew on BHI, only 42.7% (70/164) grew on MHBA (Fig. 1B). Of those that grew on MHBA, 70% (49/70) were *S. anginosus*, 11.4% (8/70) were *S. constellatus*, and 18.6% (13/70) were *S. intermedius* (Fig. 1B). Thus, 89% (49/55) of *S. anginosus* isolates were cultivated on MHBA, but only 15% (8/53) of *S. constellatus* and 23% (13/56) of *S. intermedius* isolates were cultivable on this medium. Despite these growth differences, susceptibility profiles for the three species combined were comparable between BHI and MHBA for both strain collections (Tables 1 and 2).

Due to the inability of MHBA to adequately support SMG growth, we investigated the usefulness of BHI for antibiotic susceptibility testing because it has been reported that this medium is well suited for recovery of SMG from clinical specimens (37). Figure 2 shows the relationships between zone sizes on BHI versus MHBA for the 70 organisms that grew on both medium types. The coefficient of determination, *R*², was used to indicate the correlation between the measured zones of inhibition on the two medium types for each antibiotic. Six of twelve antibiotics showed strong correlations between the two medium types, with *R*² values ranging from 0.71 to 0.95. These were ciprofloxacin, tetracycline, rifampin, clindamycin, erythromycin, and azithromycin (Fig. 2). Meropenem, cefepime, penicillin, ceftriaxone, ceftazidime, and trimethoprim-sulfamethoxazole had *R*² values ranging from 0.36 to 0.58, indicative of modest correlations.

Using the equations of the least-squares lines from Fig. 2, BHI zone sizes were converted to MHBA equivalents and then

TABLE 1. Susceptibilities of SMG isolates from airways of CF patients to six antimicrobials determined by disc diffusion using the standard medium, Mueller-Hinton blood agar, and test medium, brain heart infusion agar

Antimicrobial and medium ^b	No. of nonsusceptible isolates/no. of isolates tested (%) ^a			
	<i>S. anginosus</i>	<i>S. constellatus</i>	<i>S. intermedius</i>	All strains
Ceftriaxone				
MHBA	0/40 (0)	0/5 (0)	0/6 (0)	0/51 (0)
BHI	0/46 (0)	0/40 (0)	0/32 (0)	0/118 (0)
Cefepime				
MHBA	1/40 (2.5)	0/5 (0)	0/6 (0)	1/51 (2)
BHI	0/46 (0)	0/40 (0)	1/32 (3.1)	1/118 (0.8)
Tetracycline				
MHBA	11/40 (27.5)	0/5 (0)	0/6 (0)	11/51 (21.6)
BHI	16/46 (34.8)	1/40 (2.5)	9/32 (28.1)	26/118 (22)
Azithromycin				
MHBA	24/40 (60)	1/5 (20)	3/6 (50)	28/51 (54.9)
BHI	26/46 (56.5)	26/40 (65)	11/32 (34.4)	63/118 (53.4)
Erythromycin				
MHBA	23/40 (57.5)	1/5 (20)	3/6 (50)	27/51 (52.9)
BHI	24/46 (52.2)	26/40 (65)	11/32 (34.4)	61/118 (51.7)
Clindamycin				
MHBA	21/40 (52.5)	1/5 (20)	3/6 (50)	25/51 (49)
BHI	26/46 (56.5) ^c	25/40 (62.5)	9/32 (28.1)	60/118 (50.8)

^a All 118 isolates were tested on both medium types. The denominator reflects the number of isolates that grew on the given medium, which is lower for MHBA since many isolates failed to grow on this medium.

^b MHBA, Mueller-Hinton blood agar; BHI, brain heart infusion agar.

^c Six *S. anginosus* isolates had the inducible resistance phenotype as determined by the D-zone test.

interpreted as resistant, intermediate-resistant, or susceptible for the six antibiotics with interpretable CLSI guidelines for viridans group streptococci. For strains that grew on both medium types ($n = 70$), we compared interpretations for all six antibiotics and determined the discordance percentage, which is the percentage of strains for which the BHI and MHBA interpretations were not the same for a given antibiotic. In each case, the discordance percentage was less than 10% (data not shown). The discordance percentages were not significantly different when original BHI zone sizes were used instead of MHBA equivalents (data not shown).

Comparison of antimicrobial susceptibilities between CF airway and invasive SMG isolates reveals increased macrolide and clindamycin resistance in CF isolates. In order to compare the complete collections of CF airway and invasive SMG isolates, only interpretations determined on BHI were used (Tables 1 and 2) to avoid disproportionately representing a species in the analysis (Fig. 1). Resistance to the beta-lactams was nearly absent in the SMG, with only one CF *S. intermedius* isolate identified as having intermediate resistance to cefepime. Tetracycline resistance levels were comparable between the CF (22%) and invasive (17.4%) isolates ($P = 0.67$; Table 3). All exacerbation-associated CF *S. anginosus* isolates ($n = 3$) were resistant to tetracycline.

A substantial difference in macrolide and clindamycin resistance levels was observed between the CF airway and invasive isolates. More than half of the CF isolates were resistant to

TABLE 2. Susceptibilities of invasive SMG isolates to six antimicrobials determined by disc diffusion using the standard medium, Mueller-Hinton blood agar, and test medium, brain heart infusion agar

Antimicrobial and medium	No. of nonsusceptible isolates/no. of isolates tested (%) ^a			
	<i>S. anginosus</i>	<i>S. constellatus</i>	<i>S. intermedius</i>	All strains
Ceftriaxone				
MHBA	0/9 (0)	0/3 (0)	0/7 (0)	0/19 (0)
BHI	0/9 (0)	0/13 (0)	0/24 (0)	0/46 (0)
Cefepime				
MHBA	0/9 (0)	0/3 (0)	0/7 (0)	0/19 (0)
BHI	0/9 (0)	0/13 (0)	0/24 (0)	0/46 (0)
Tetracycline				
MHBA	1/9 (11.1)	1/3 (33.3)	0/7 (0)	2/19 (10.5)
BHI	1/9 (11.1)	4/13 (30.8)	3/24 (12.5)	8/46 (17.4)
Azithromycin				
MHBA	1/9 (11.1)	0/3 (0)	2/7 (28.6)	3/19 (15.8)
BHI	0/9 (0)	1/13 (7.7)	4/24 (16.7)	5/46 (10.9)
Erythromycin				
MHBA	1/9 (11.1)	0/3 (0)	2/7 (28.6)	3/19 (15.8)
BHI	1/9 (11.1)	1/13 (7.7)	4/24 (16.7)	6/46 (13)
Clindamycin				
MHBA	0/9 (0)	0/3 (0)	2/7 (28.6)	2/19 (10.5)
BHI	0/9 (0)	0/13 (0)	3/24 (12.5)	3/46 (6.5)

^a All 46 isolates (including three reference strains) were tested on both medium types. The denominator reflects the number of isolates that grew on the given medium, which is often lower for MHBA since many isolates failed to grow on this medium.

azithromycin (53.4%), erythromycin (51.7%), and clindamycin (50.8%), while only 10.9%, 13%, and 6.5% of invasive isolates were resistant, respectively. Statistical analyses revealed that the risk of nonsusceptibility to azithromycin, erythromycin, and clindamycin in CF airway isolates was 5-fold, 4-fold, and 8-fold higher relative to invasive isolates, respectively ($P < 0.0001$; Table 3). Of the CF airway isolates, macrolide and clindamycin resistance was highest in *S. constellatus* (65% and 62.5%, respectively). However, for the invasive isolates, macrolide and clindamycin resistance was highest in *S. intermedius* (16.7% and 12.5%, respectively). There was a strong correlation between macrolide and clindamycin resistance. Of the invasive isolates, clindamycin resistance was detected only in *S. intermedius* and was concomitant with macrolide resistance in all cases ($n = 3$). Of the 60 CF airway isolates resistant to clindamycin, all but two also showed resistance to one of the macrolides. All of the macrolide and clindamycin coresistant strains were constitutively resistant to clindamycin as determined by the D-zone test, except for six CF *S. anginosus* isolates, which required erythromycin to induce clindamycin resistance. Interestingly, 73.7% (14/19) of exacerbation-associated CF isolates were both macrolide and clindamycin resistant. However, the risk of increased macrolide and clindamycin resistance in exacerbation-associated strains relative to strains isolated during periods of clinical stability was not statistically significant ($P = 0.07$ and $P = 0.08$, respectively).

Although breakpoints were available only for six of the 12 antibiotics studied, we compared BHI zone sizes for each an-

FIG. 2. The relationship of the zone-of-inhibition sizes for SMG isolates that were cultivated on MHBA and BHI is illustrated with a simple linear regression model. For each antibiotic (indicated on the bottom right of each panel), the coefficient of determination (R^2) and the equation of the least-squares line are shown within the corresponding panel. Antibiotics on the right have predicted MHBA breakpoints.

antibiotic between the CF and invasive isolates (Fig. 3). For four beta-lactams, there was a slight tendency for invasive isolates to have somewhat smaller median zone sizes than CF isolates ($P < 0.05$; Fig. 3). Macrolide and clindamycin zone sizes were significantly smaller for CF isolates ($P < 0.0001$), reflective of the larger number of resistant strains observed and shown in Table 1. For trimethoprim-sulfamethoxazole, zones of inhibition for the CF isolates were distributed over a larger range; however, there was not a statistical significance between the median zone sizes compared to those of the invasive isolates

TABLE 3. Risk of antimicrobial nonsusceptibility in CF isolates relative to invasive isolates of the SMG

Antibiotic	No. of nonsusceptible isolates/no. of isolates tested (%)		RR (95% CI) ^a	P value
	CF	Invasive		
Ceftriaxone	0/118 (0)	0/46 (0)		
Cefepime	1/118 (1)	0/46 (0)		
Tetracycline	26/118 (22.0)	8/46 (17.4)	1.26 (0.62–2.59)	0.67
Azithromycin	63/118 (53.4)	5/46 (10.8)	4.91 (2.11–11.4)	<0.0001
Erythromycin	61/118 (51.6)	6/46 (13.0)	3.96 (1.84–8.53)	<0.0001
Clindamycin	60/118 (50.8)	3/46 (6.5)	7.80 (2.57–23.62)	<0.0001

^a RR, relative risk; CI, confidence interval.

($P < 0.052$). There was no significant difference in the zone sizes between CF and invasive isolates for ceftazidime, tetracycline, rifampin, and ciprofloxacin.

Macrolide resistance in CF isolates is associated with exposure to azithromycin. Given the chronic use of azithromycin with CF patients, we investigated whether increased azithromycin resistance was associated with exposure to the antibiotic. Figure 4 shows the distribution of resistant, intermediate-resistant, and susceptible CF strains as determined by susceptibility testing on BHI and marked according to the type of azithromycin exposure (chronic prophylaxis or acute infectious complication). Clearly, chronic exposure was associated with resistance in the SMG (Fig. 4; $P < 0.0001$). Of the 57 isolates from patients who had been on chronic azithromycin therapy, 48 (84.2%) were not susceptible to azithromycin. In contrast, only 24.6% (15/61) of isolates from patients who had not been on chronic azithromycin therapy were resistant. It is noteworthy to mention that two of the four macrolide-resistant invasive *S. intermedius* isolates were from a CF patient who had received oral azithromycin therapy.

FIG. 3. A box-and-whisker plot depicting the comparison of the spread in sizes of the zones of inhibitions for SMG isolates recovered from CF airways and invasive infection. Boxes delineate the first (75%) and third (25%) quartiles, with the median shown as an internal black line; whiskers represent the 95th and 5th percentiles. Statistically significant differences between the median zone sizes of CF airway and invasive isolates are indicated with an asterisk ($P < 0.05$ and > 0.0001) and double asterisk ($P < 0.0001$).

FIG. 4. Chronic macrolide exposure results in high-level resistance in SMG isolates recovered from the airways of CF patients treated with prolonged azithromycin therapy. The vast majority of azithromycin-resistant SMG isolates, as determined by the size of the zone of inhibition on BHI, were isolated from patients that were chronically exposed to the antibiotic during suppressive therapy. Likewise, the majority of sensitive isolates were recovered from naive patients. Acute exposure to azithromycin does not appear to be associated with increased resistance.

DISCUSSION

Since the first description of the SMG several decades ago, clinicians have been well aware of its clinical relevance in pyogenic infections. Although members of the SMG have been implicated as etiological agents in pneumonia and empyema (1, 21, 48), only recently have the SMG members been implicated as respiratory pathogens in CF (9, 29, 36, 37). Several studies have assessed the antimicrobial susceptibilities of clinical isolates of the SMG from invasive community and nosocomial infections. Owing to the unique nature of the CF host, with both chronic suppressive antimicrobial therapies and frequent acute treatments, we postulated that the published antibiograms of invasive isolates might underestimate the degree of drug resistance in CF isolates. This study profiled the responses of 118 CF SMG isolates to 12 antibiotics in comparison to 43 SMG isolates from invasive infections and three reference strains.

In keeping with published reports, more than half of all isolates failed to grow using recommended culture conditions (5, 7, 8). Our results revealed a species bias in which 77% of *S. intermedius* and 85% of *S. constellatus* isolates failed to grow adequately on MHBA, the standard susceptibility medium used for streptococci. Accordingly, we addressed this concern by exploring BHI as an alternative medium for susceptibility testing and found that zone sizes were comparable to those obtained on MHBA. For antibiotics with large zone sizes and poor correlation between the two medium types, the measurements were often difficult to obtain due to poor growth and thinning of the lawn near the boundary of growth, particularly on MHBA. Although ceftriaxone and cefepime fell into this category, the observed zone sizes were generally well beyond the susceptibility breakpoint and in more than 90% of cases, the zone sizes led to the same interpretations. For antibiotics with smaller zone sizes and a greater degree of resistance, such as the macrolides, clindamycin, and tetracycline, the correlations were strong, and as with the beta-lactams, more than 90% of the interpretations were the same on either medium for each antibiotic. Our findings demonstrate that BHI is a suit-

able alternative for disc diffusion with the SMG when MHBA fails to support adequate growth of the strains.

Using BHI in parallel with MHBA, we examined the rates of resistance of the CF airway and invasive isolates to antibiotics for which CLSI breakpoints were available. Unlike other viridans group streptococci, which have shown increasing resistance to beta-lactams over time, the SMG has remained largely susceptible to this class of antibiotics (2, 3, 14). Ceftriaxone appears to be highly effective against the SMG *in vitro* and *in vivo* (29, 37), but its widespread use may eventually lead to increased resistance in the SMG and other organisms, which is already as high as 24% for *Streptococcus mitis*, another member of the viridans group streptococci (14).

For antibiotics that may be used empirically or to target other pathogens, namely, ciprofloxacin, tetracycline, ceftazidime, rifampin, and trimethoprim-sulfamethoxazole, resistance profiles and/or zone sizes did not differ significantly between CF and invasive isolates. Zone sizes without breakpoint interpretations do not provide information on resistance but may have some clinical value in comparisons of CF and invasive strains. For rifampin and trimethoprim-sulfamethoxazole, the small zone sizes of outlying strains in the CF collection appeared concurrently with a loss of clinical improvement in the respective patients who had been treated with those antibiotics (37). Tetracycline resistance was comparable between the CF and invasive strains but somewhat lower than the rates of >40% reported elsewhere (17, 27). Ceftazidime is an effective antipseudomonal often used to treat *P. aeruginosa*-related exacerbations but has poor activity against Gram-positive bacteria, including the SMG and other viridans group streptococci (2, 3, 40). The lack of difference observed between CF and invasive zone sizes for ceftazidime is likely reflective of its minimal activity toward these organisms. For antibiotics for which resistance and zone size profiles did not differ significantly between the CF and invasive isolates, we considered three plausible explanations: the antibiotic is ineffective against the SMG, as was likely the case with ceftazidime; antibiotic administration is not frequent enough to select resistance; and resistance is slow to evolve in the SMG despite frequent administration, such as with the beta-lactams.

The rates of resistance of 13% and 6.5% of the invasive strains to macrolides and clindamycin, respectively, are consistent with other studies (17, 27). However, in CF airway isolates of the SMG there were 5- and 8-fold increased prevalences of resistance, respectively, in which more than half of strains were resistant. The heightened macrolide resistance was found to be associated with the chronic use of azithromycin with CF patients (Fig. 4). Azithromycin therapy effectively improves lung function and reduces the frequency of pulmonary exacerbations in CF patients chronically colonized with *P. aeruginosa* (16), but evidence suggests that these effects may be only temporary, especially now that the average CF patient lives well into adulthood (38, 44). We hypothesize that the mechanism of azithromycin therapy failure could be linked to its antimicrobial properties and the development of resistance in organisms resident in the CF airway microbiome (38).

Clindamycin resistance is relevant to the clinical management of CF airway disease (37) and invasive infections (31) and is presumed to be a secondary consequence of chronic azithromycin exposure (26). Only two CF isolates possessed clindamycin resistance that was not associated with macro-

lide resistance. These observations are characteristic of the MLS_B phenotype, encoded by erythromycin methyltransferases (the *erm* genes), which confers either inducible or constitutive cross-resistance to macrolides, lincosamides, and streptogramin B antibiotics (26). However, mutations in ribosomal proteins or rRNA may also confer cross-resistance to these antibiotics (46). In a study investigating the mechanisms of macrolide resistance in the SMG, all 15 SMG isolates with the constitutive MLS_B phenotype harbored the *ermB* gene (22). In contrast, preliminary results show that the presence of *ermB* accounts for only 30% of coresistance to macrolides and clindamycin in our strain collection (data not shown). Further studies are under way to determine the mechanisms of macrolide and clindamycin resistance prevalent in our strain collection.

In conclusion, this study demonstrates that SMG isolated from CF airways are substantially more resistant to macrolides and clindamycin than their invasive counterparts. Such resistance is likely due to chronic azithromycin therapy, a common regimen upheld for its notable benefits to CF patients. The impact of highly resistant SMG in CF is uncertain, although a large proportion of exacerbation-associated strains in this study were macrolide and clindamycin resistant. Prospective studies designed to determine when and where macrolide resistance develops within the CF airway microbiome will be required to determine how changes in the CF airway resistome impact the efficacy of suppressive antibiotic therapies, such as azithromycin. Overall, these results emphasize the deficiencies in standard protocols for susceptibility testing of the SMG and show that BHI agar may be a suitable alternative medium for susceptibility testing for those isolates which fail to grow on MHBA.

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