

Sulfonamide Resistance in Isolates of *Nocardia* spp. from a U.S. Multicenter Survey

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Recent reports of increasing *in vitro* sulfonamide resistance in *Nocardia* prompted us to investigate the findings. Despite the reports, there is a paucity of clinical reports of sulfonamide failure in treatment of nocardia disease. We reviewed 552 recent susceptibilities of clinical isolates of *Nocardia* from six major laboratories in the United States, and only 2% of the isolates were found to have resistant MICs of trimethoprim-sulfamethoxazole and/or sulfamethoxazole. We hypothesize that the discrepancies in the apparent sulfonamide resistance between our study and the previous findings may be associated with difficulty in the laboratory interpretation of *in vitro* MICs for trimethoprim-sulfamethoxazole and sulfamethoxazole and the lack of quality controls for *Nocardia* for these agents.

Sulfonamides have remained the treatment of choice for most *Nocardia* infections since the first recorded treatment use by Benbow et al. in 1944 (1). The subsequent introduction of trimethoprim (TMP) in combination with sulfamethoxazole (SMX) in the 1970s created improved treatment possibilities for *Nocardia* (21). Early treatment results with TMP-SMX were more favorable than with the use of sulfonamides alone, and TMP-SMX remains the most widely available and prescribed sulfonamide in the United States (3).

Recently, Uhde et al. reported that of 765 isolates of *Nocardia* submitted to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, from 1995 to 2004, 61% were resistant to SMX and 42% were resistant to TMP-SMX (20). In a 2011 study of 186 *Nocardia* species isolated from patients in Spain, Larruskain et al. described 16.1% resistance to TMP-SMX (11). Another 2011 report from Canada described 43% of 157 *Nocardia* isolates recovered from Quebec from 1988 to 2008 as resistant to TMP-SMX (19). However, it should be noted that the antimicrobial susceptibility testing of the Quebec isolates was performed in the same laboratory as the Uhde et al. study, and the TMP-SMX resistance rates for *Nocardia cyriacigeorgica*, *N. farcinica*, and *N. nova* complex and the overall prevalence of resistance to TMP-SMX were quite similar in the three studies. The authors of the Quebec study noted that isolates reported as resistant were nonetheless successfully treated with the agent (19).

Despite these reports of *in vitro* sulfonamide resistance, there have been only rare recent clinical reports describing treatment failure of *Nocardia* with TMP/SMX (14).

Because this incidence of resistance appeared much higher than that experienced in our laboratories and because of clinical concern that this perceived level of resistance markedly changes how patients with nocardiosis are treated, we reviewed the susceptibility results for isolates of *Nocardia* collected in 2005 to 2011 from six major U.S. medical or referral centers experienced in *Nocardia* identification and susceptibility testing.

MATERIALS AND METHODS

TMP-SMX and/or SMX susceptibilities of 552 isolates of *Nocardia* were retrospectively reviewed among six U.S. laboratories, i.e., Banner

Good Samaritan Medical Center, Phoenix, AZ; the Warren Grant Magnuson Clinical Center of the National Institutes of Health, Bethesda, MD; Creighton University Medical Center, Omaha, NE; Associated and Regional University Pathologists (ARUP), Salt Lake City, UT; Mayo Clinic, Rochester, MN; and the University of Texas Health Science Center, Tyler, TX.

Five of the six laboratories identified *Nocardia* isolates to the species or complex level by 16S rRNA or *secA* gene sequencing, PCR restriction fragment enzyme analysis (PRA), and/or drug susceptibility patterns (2, 8). One laboratory (Arizona) identified isolates to the species/complex level using a combination of biochemicals, growth characteristics, and antimicrobial susceptibility patterns (2, 3, 6, 7, 12, 16).

Broth microdilution of TMP-SMX and/or SMX was performed in all six laboratories according to the current recommendations of the Clinical and Laboratory Standards Institute (CLSI) on 552 isolates of *Nocardia* (100 consecutive isolates submitted for testing to five laboratories from 2010 to 2011 and 52 isolates from one laboratory from February 2005 to 2011) (4, 15). The MICs were retrospectively reviewed. All six laboratories tested TMP-SMX. One laboratory (Mayo) had 78 isolates tested against SMX and 22 isolates tested against TMP-SMX, and another laboratory (NIH) had 45 isolates tested against both SMX and TMP-SMX and 7 isolates against TMP-SMX alone. The species examined, number of MICs performed, and number resistant to TMP-SMX and SMX are shown in Table 1. One laboratory tested three patients with two isolates each (collected on different dates), and another laboratory tested isolates from five patients with multiple cultures. Four of the five patient isolates were collected more than 1 year apart (one with a different species of *Nocardia*), and the fifth patient had three samples with two isolates collected 6 months apart and the third isolate 2 years later. None of the other four laboratories reported more than one isolate per patient.

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TABLE 1 Species of *Nocardia* recovered from six major U.S. laboratories, number tested, and number resistant to TMP-SMX and/or SMX^a

Species	UTHSCT		Creighton		ARUP		Arizona		Mayo ^b			NIH ^c		
	No. tested	No. resistant	No. tested	No. resistant	No. tested	No. resistant	No. tested	No. resistant	No. tested	No. resistant		No. tested	No. resistant	
										(TMP-SMX)	(SMX)		(TMP-SMX)	(SMX)
<i>N. abscessus</i> complex	2	0	1	0	4	0	3	0	9	0	0	1	0	0
<i>N. aobensis</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>N. asiatica</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>N. asteroides</i> complex	0	0	0	0	4	0	12	0	9	0	0	0	0	0
<i>N. beijingensis</i>	0	0	0	0	1	0	0	0	1	0	0	0	0	0
<i>N. brasiliensis</i>	7	0	6	0	19	0	19	0	13	0	0	0	0	0
<i>N. brevicatena</i>	0	0	3	0	0	0	0	0	0	0	0	0	0	0
<i>N. cyriaci-georgica</i>	8	0	51	0	20	0	30	0	16	0	0	11	0	1
<i>N. farcinica</i>	10	0	18	0	8	0	16	0	13	0	0	8	1	3
<i>N. niigatensis</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>N. nova</i> complex	33	0	18	0	14	0	1	0	22	0	0	20	0	4
<i>N. otitidiscaviarum</i>	0	0	1	0	3	0	4	0	4	0	1	0	0	0
<i>N. paucivorans</i>	0	0	0	0	2	0	0	0	0	0	0	0	0	0
<i>N. pseudobrasiliensis</i>	0	0	1	0	0	0	0	0	2	0	1	1	0	0
<i>N. transvalensis</i> complex	10	0	1	0	10	0	14	1	1	0	0	4	1	1
<i>Nocardia</i> sp.	30	0	0	0	13	0	0	0	10	0	0	6	0	0

^a Four laboratories (University of Texas Health Science Center at Tyler [UTHSCT], Creighton, ARUP, and Arizona) tested only TMP-SMX.

^b This laboratory performed MIC tests with TMP-SMX on 22 isolates and with SMX on 78 isolates.

^c This laboratory performed MIC tests with a total of only 52 isolates (including 7 isolates with only TMP-SMX); 1 isolate each of *N. farcinica* and the *N. transvalensis* complex was resistant to both SMX and TMP-SMX.

RESULTS

Of 552 susceptibility results submitted for review, only 14 isolates (2.5%) were identified as sulfonamide resistant, with three isolates resistant to TMP-SMX and 11 resistant to SMX. One laboratory (NIH) had eight isolates initially reported as sulfonamide resistant (SMX only) and one isolate resistant to both SMX and TMP-SMX. However, repeat MIC testing with TMP-SMX (currently recommended by the CLSI) showed only 2 of those 9 isolates to be resistant to TMP-SMX. Panels with SMX were not available for retesting.

DISCUSSION

Our data differ from the previous three reports describing high levels of sulfonamide resistance but are strikingly similar to the low incidence (2%) of sulfonamide resistance seen by Lai et al. (9, 10) in a study of *Nocardia* isolates in Taiwan and an earlier study in South Africa with 0% sulfonamide resistance in 39 isolates (12). Similarly, in a recent study of 1,641 U.S. *Nocardia* isolates, the investigators found only 2% of the isolates studied to be resistant to TMP-SMX. *N. pseudobrasiliensis* and *N. transvalensis* complex isolates were sulfonamide resistant, while no other *Nocardia* species in their study showed resistance (17).

The disparity between the data is of interest, since all of the reporting laboratories in this study are geographically diverse. This fact suggests that some factor other than geographic location may play a major role in the susceptibility patterns that are reported, with the most probable factor being a methodological or interpretational difference.

The MICs of sulfonamides, including TMP-SMX, are based on an 80% inhibition endpoint of growth compared to the growth control without drug rather than no growth or 100% inhibition, as is standard for other antimicrobials (4, 15). In a recent CLSI mul-

ticenter study among six laboratories using the same strains of *Nocardia* and the same lot numbers of susceptibility panels, media, etc., multiple laboratories reported discrepancies in MIC interpretations for sulfonamides (5). This multicenter study showed that careful training and close scrutiny of the 80% inhibition endpoint were necessary to produce accurate MIC results. We believe the differences in resistance to sulfonamides reported in the current and prior studies may reflect similar issues of reading the 80% endpoint.

The current study and the CLSI multicenter study emphasized the necessity for laboratory proficiency testing with unusual organisms, such as *Nocardia* (5). Unfortunately, there is no standardized proficiency testing program currently available, including from the College of American Pathologists (CAP). Thus, the exchange of organisms between two or more accredited laboratories is the best current means to meet this criterion. Another recommendation to help in training laboratory personnel to better interpret MICs, would be the establishment of a number of control strains of *Nocardia* with known (and consensus) susceptibility patterns.

Significant concerns about recent reports detailing the significant increase in sulfonamide resistance among numerous *Nocardia* spp. prompted this study. It is generally accepted that the incidence of nocardial disease is increasing (2, 13), and TMP-SMX remains the drug of choice for treatment (2, 18). We suggest that future studies with the currently approved CLSI susceptibility testing method involve a representative set of the previously identified sulfonamide-susceptible and -resistant strains of *N. cyriaci-georgica*, *N. farcinica*, and *N. nova*. We consider this to be necessary to preserve the traditional approach to empirical therapy of significant infections involving *Nocardia* spp., which almost always includes a sulfonamide preparation (TMP-SMX). If signifi-

cant levels of sulfonamide resistance are detected in these previous or future isolates, further investigation of the mechanism(s) of resistance would be indicated. CLSI advocates that all clinically significant isolates of *Nocardia* be identified by currently accepted molecular methods and that antimicrobial susceptibility testing be performed as a guide to therapy (4). In conclusion, our study strongly suggests that reporting of resistance to TMP-SMX must take note of the inherent difficulties of reading MIC wells and should be supported by one or more of the above recommendations.

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