

Surveillance of Wisconsin Organisms for Trends in Antimicrobial Resistance and Epidemiology: Introduction to the Program and Summary of 2016 Geographic Variation

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ABSTRACT

Background: Antimicrobial resistance merits surveillance because of its impact on quality health care. Past surveillance efforts in Wisconsin involved generation of a statewide antibiogram on the basis of antibiogram compilation. However, this modality of surveillance possesses limitations.

Methods: To characterize Wisconsin antimicrobial susceptibility patterns and elucidate geographic variation in antimicrobial resistance, a statewide surveillance network was created. Clinical microbiology laboratories submitted clinically significant bacterial isolates to a centralized testing facility for performance of standardized broth microdilution testing. Analyzed data included organism-specific susceptible, intermediate, and resistant percentages, along with median and 90th percentile minimum inhibitory concentration values.

Results: In comparison of 378 isolates of *Escherichia coli* (*E coli*) and 279 isolates of *Proteus mirabilis* (*P mirabilis*), susceptibility rates of *E coli* were generally lower than *P mirabilis*, particularly in areas of Wisconsin bordering Lake Winnebago. *P mirabilis* resistance rates were generally higher in northern Wisconsin. From a 211-isolate collection of *Pseudomonas aeruginosa*, it was determined that higher rates of antimicrobial resistance were found in Southeast Wisconsin. On a geographic basis, susceptibility rates within a 212-isolate collection of *Streptococcus pneumoniae* were fairly consistent. However, Southcentral Wisconsin experienced increased rates of erythromycin resistance with this organism, as well as increased aminoglycoside resistance trending with other organisms. Antimicrobial agents with generally lower susceptibility rates statewide included fluoroquinolones and trimethoprim-sulfamethoxazole.

Conclusions: A surveillance program has been initiated in Wisconsin that not only summarizes susceptibility patterns but also has the capacity to indicate potential emerging resistance trends. Future annual studies can begin to characterize antimicrobial resistance in Wisconsin on a temporal basis.

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INTRODUCTION

Considerable attention has been given to the issue of antimicrobial resistance throughout the United States, both in peer-reviewed literature and the popular press. Specific vigilance has been granted by the Centers for Disease Control and Prevention (CDC) to a number of clinical scenarios, stratified by degree of threat.¹ Included in the category of urgent threat are *Clostridium difficile* disease and carbapenem-resistant *Enterobacteriaceae* (CRE). Drug-resistant *Streptococcus pneumoniae* (*S pneumoniae*), multi drug-resistant *Pseudomonas aeruginosa* (*P aeruginosa*), and extended-spectrum β -lactamase-producing *Enterobacteriaceae* constitute examples within a dozen scenarios of serious threat. Predisposing factors for antimicrobial resistance are not localized to inpatient or long-term care facilities. Hicks et al² investigated antibiotic prescription burden within outpatient settings in the United States and reported that over 260 million oral courses were prescribed by clinicians in 2011. Agents within 7 antimicrobial classes accounted for 94% of total outpatient prescriptions. These ranged from

penicillins and macrolides (each accounting for approximately 23% of outpatient utilizations) to tetracyclines and trimethoprim-sulfamethoxazole (each at 8% of outpatient utilizations).

The CDC has advocated a 4-tiered approach to combat the continued emergence of antimicrobial resistance.¹ In addition to strategies advocating research and development, initiation and maintenance of antimicrobial stewardship programs, and infection prevention practices, the CDC promotes the concept of antimicrobial resistance

tracking. Means to accomplish this include data collection and subsequent studies of disease epidemiology. On the basis of the aforementioned outpatient prescription data,² antimicrobial resistance tracking may become increasingly necessary in the Midwest. It was reported that an average of 897 antibiotic prescriptions per 1,000 persons was issued in this 12-state region in 2011, second only to the southern United States (931 prescriptions per 1,000 persons).

One initial surveillance undertaking in Wisconsin was orchestrated by the Wisconsin Clinical Laboratory Network (WCLN) Laboratory Technical Advisory Group.³ The basis for that 2013 investigation was voluntary submission of local antibiogram data from 72 health care entities, with compilation of those data stratified by 7 geographic regions demarcated by WCLN. However, limitations exist with the practice of antibiogram compilation. These apply to both the procurement of primary data for the antibiogram (particularly as it relates to variability in local susceptibility testing),⁴⁻⁷ as well as generation of the antibiogram itself.⁸⁻¹⁰ In contrast, a program by which a centralized laboratory assesses representative organisms using a standardized antimicrobial susceptibility testing method would advance the paradigm of resistance surveillance. Moreover, discreet data associated with each tested organism may provide an additional means for identifying emerging patterns of antimicrobial resistance and begin to elucidate epidemiologic trends relative to antimicrobial resistance. Herein we describe creation of the Surveillance of Wisconsin Organisms for Trends in Antimicrobial Resistance and Epidemiology (SWOTARE) program and present selected statewide findings from the first year of surveillance.

MATERIALS AND METHODS

Selection of Study Sites

The 7 Bioterrorism Preparedness Team regions of the WCLN³ provided the basis for geographic demarcations of the SWOTARE program; 21 clinical microbiology laboratories participated in the program. In general, to prevent potential bias provided by facilities in urban areas, 2 laboratories per region were set in more rural areas, with the 3rd participant from a larger population center. This strategy was executed less efficiently in regions with increased population density and fewer rural microbiology laboratories (Southeast, Lake Winnebago regions).

Isolates and Demographic Data

Study sites were requested to submit consecutive isolates of *E coli*, (18), *P mirabilis* (15), *P aeruginosa* (10), and *S pneumoniae* (14) identified from in-house culture of clinically-significant infection. Duplicate isolates were excluded. Because of the lack of direct involvement in the collection of specimens and because of the utilization of deidentified isolates from routine clinical care, the SWOTARE program was not considered to be actively engaged in human subjects research by the Marquette University Institutional Review Board.

Antimicrobial Susceptibility Testing

Broth microdilution antimicrobial susceptibility testing was executed¹¹ and interpreted¹² using standards published by Clinical and Laboratory Standards Institute (CLSI). Panels consisted of antimicrobials described in Tables 1 and 2 using customized dilution ranges that extended beyond individual CLSI breakpoints.

Data Analysis

Percentage susceptible, intermediate (susceptible-dose dependent, when indicated), and resistant values, as well as median minimum inhibitory concentration (MIC₅₀) and 90th percentile (MIC₉₀) determinations were made on a statewide or geographic basis. To characterize geographic variation, the statewide (mean) susceptibility percentage for a given organism/antimicrobial combination established a baseline value. An interval of 5% on either side of that mean represented normal distribution. Region-specific values $\geq 5\%$ less than the state mean indicated areas with increased resistance. Region-specific values $\geq 5\%$ greater than the state mean indicated less resistance potential.

RESULTS

Distribution of Isolates

In 2016, 1,080 isolates were submitted and tested. *E coli*, *P mirabilis*, and *P aeruginosa* per-region contribution percentages ranged from 12.2% to 16.1%. In contrast, individual region contribution percentages of *S pneumoniae* ranged from 7.5% (Southeast) to 20.8% (Lake Winnebago).

Statewide Assessment of Gram-Negative Bacilli

Agents demonstrating greatest potency against Wisconsin *E coli* isolates included carbapenems (100% susceptibility), nitrofurantoin, piperacillin-tazobactam, and aminoglycosides (93.1%; Table 1). β -lactam agents other than carbapenems demonstrated greater variability, ranging from 56.3% susceptibility (ampicillin) to greater than 92% susceptibility (3rd- and 4th-generation cepheims and aztreonam). Other agents with less potency included fluoroquinolones (less than 80% susceptibility), trimethoprim-sulfamethoxazole (80.7%), and ampicillin-sulbactam (62.7%). Susceptibility of *P mirabilis* isolates to several agents was generally increased when compared to *E coli* (greater than 91% susceptibility to 12 of 16 agents tested, Table 1). Exceptions included ampicillin and trimethoprim-sulfamethoxazole. Interestingly, significant fluoroquinolone resistance was documented throughout Wisconsin, with the *in vitro* ciprofloxacin susceptibility rate lower than that for levofloxacin. Statewide *P aeruginosa* isolates demonstrated highest rates of susceptibility to aminoglycosides and less susceptibility to aztreonam and fluoroquinolone agents (Table 1). Most isolates were susceptible to 3rd- and 4th-generation cephem agents.

Statewide Assessment of *S pneumoniae*

Approximately 70% of *S pneumoniae* isolates yielded penicillin

Table 1. Characterization of *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* Isolates on the Basis of Susceptibility to Clinically Relevant Antimicrobial Agents, Wisconsin 2016

Organism	n	Percentage Susceptible																	
		Penicillin Derivatives			Cepheids					Monobactam	Carbapenems		Fluoroquinolones		Aminoglycosides		Others		
		AMP	A/S	P/T	CFZ	FOX	CAX	CAZ	FEP	AZT	MER	ERT	LEV	CIP	GEN	TOB	T/S	NIT	
<i>E coli</i>	378	56.3	62.7	97.6	87.3	91.5	92.6	93.4	94.7	92.9	100	100	79.9	79.1	93.1	93.1	80.7	97.9	
<i>P mirabilis</i>	279	84.6	93.9	100	96.1	98.6	98.6	99.6	99.3	99.6	100	99.6	81.0	75.6	91.4	92.1	82.4		
<i>P aeruginosa</i>	211			93.4				94.8	96.7	81.0	92.9		88.2	88.2	99.1	99.5			

Abbreviations: AMP, ampicillin; A/S, ampicillin-sulbactam; P/T, piperacillin-tazobactam; CFZ, cefazolin; FOX, ceftoxitin; CAX, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; AZT, aztreonam; MER, meropenem; ERT, ertapenem; LEV, levofloxacin; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; T/S, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin.

Table 2. Characterization of 212 Isolates of *Streptococcus pneumoniae* on the Basis of Susceptibility to Clinically Relevant Antimicrobial Agents, Wisconsin 2016

Antimicrobial Agent	Percentage Susceptible
Penicillin	70.3*
Ceftriaxone	93.9**
Cefepime	95.3
Meropenem	87.7
Levofloxacin	98.6
Moxifloxacin	99.1
Erythromycin	54.2
Clindamycin	87.3
Tetracycline	84.4
Trimethoprim-sulfamethoxazole	75.9
Chloramphenicol	97.6
Linezolid	100
Vancomycin	100

*Penicillin susceptibility (MIC ≤ 0.06 µg/mL) percentage listed in Table is based on Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for parenteral delivery vs meningial *S pneumoniae* isolates.

**Ceftriaxone susceptibility (MIC ≤ 0.5 µg/mL) percentage listed in Table is based on CLSI interpretive criteria for parenteral delivery versus meningial *S pneumoniae* isolates.

MIC ≤ 0.06 µg/mL (Table 2). Nearly 94% of statewide isolates exhibited ceftriaxone MIC ≤ 0.5 µg/mL. Fluoroquinolone susceptibility rates approximated 99%. Decreased rates of susceptibility were noted with erythromycin (54.2%), trimethoprim-sulfamethoxazole, tetracycline, and clindamycin.

Geographic Variation in Gram-negative Bacilli Susceptibility

With respect to *E coli*, the Lake Winnebago region demonstrated susceptibility rates lower than the state mean for 12 of 17 antimicrobials tested. In contrast, Northwest and Southwest regions yielded susceptibility rates greater than the state mean for 13 and 12 antimicrobials tested, respectively. Regional levofloxacin susceptibility distribution (with corresponding MIC₅₀ and MIC₉₀ values) is presented in Figure 1A as a representative summary of *E coli* resistance throughout the state. In addition to the decreased susceptibility rate demonstrated in the Lake Winnebago region,

this region and the Southcentral region also exhibited increased MIC₉₀ values. The continuing and potentially emerging trends of increased resistance for the Lake Winnebago and Southcentral regions, respectively, were also noted for tobramycin (Figure 1B) and trimethoprim-sulfamethoxazole (Figure 1C).

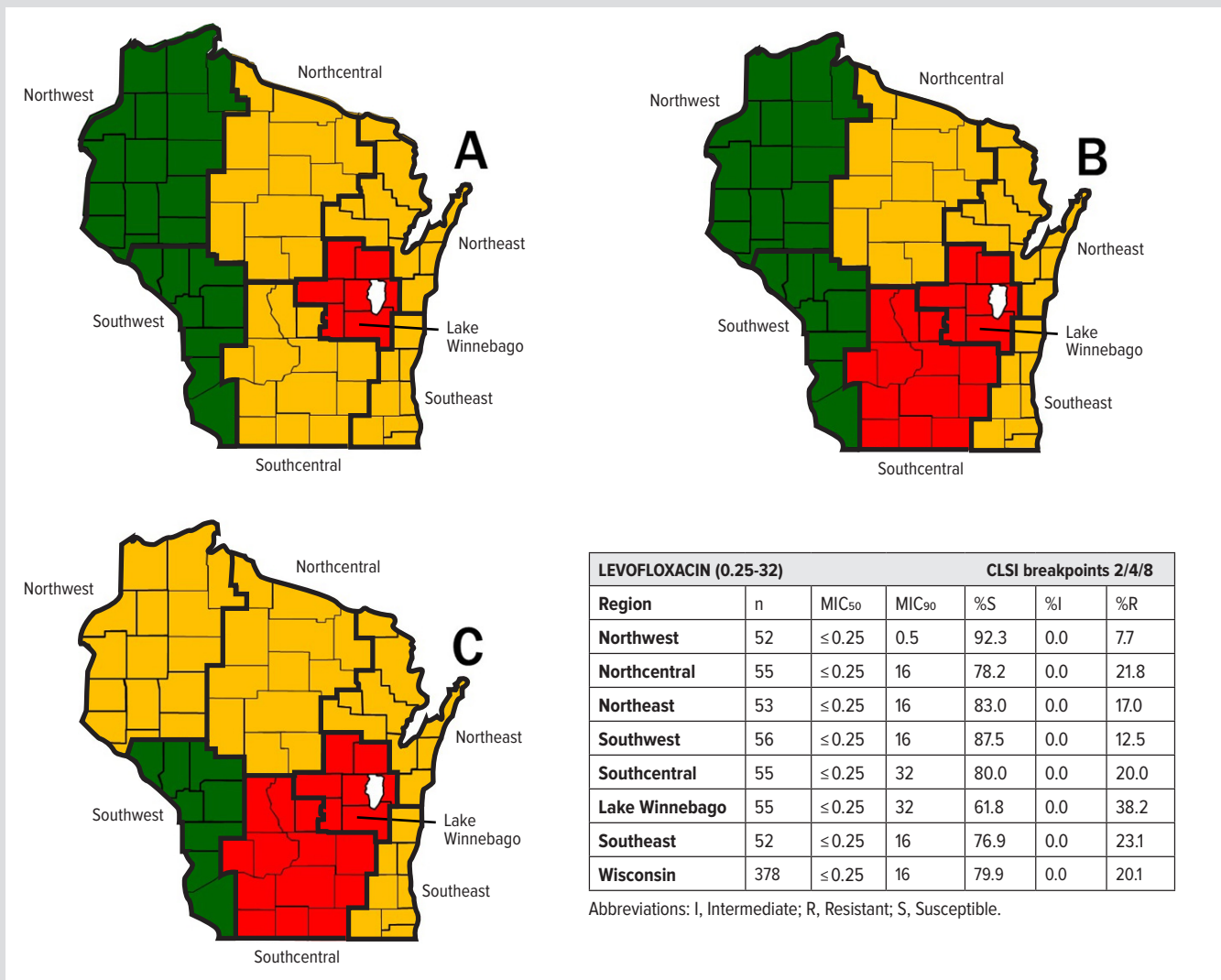
Region-specific *P mirabilis* susceptibility rates mirrored or exceeded the state mean for 9 of 16 agents tested. Susceptibility rates for fluoroquinolone agents, trimethoprim-sulfamethoxazole, ampicillin, ampicillin-sulbactam, and aminoglycoside agents were decreased in the Northcentral region (Table 3) when compared to state mean data (Table 1). Additional evidence of decreased aminoglycoside susceptibility in the Southcentral region was observed via increased *P mirabilis* MIC₉₀ values.

With respect to *P aeruginosa*, the Southeast region demonstrated susceptibility rates lower than the state mean for aztreonam, ceftazidime, and fluoroquinolone agents (Table 3). Increased MIC₉₀ values were noted for piperacillin-tazobactam in this region. Susceptibility rates of *P aeruginosa* to aztreonam were also decreased in the Northeast and Southcentral regions. Despite high values of aminoglycoside potency statewide (Table 1), the Southcentral region was the only region to submit *P aeruginosa* that demonstrated resistance to both gentamicin and tobramycin.

Geographic Variation in *S pneumoniae* Susceptibility

Region-specific susceptibility rates for 8 of 13 agents tested against *S pneumoniae* isolates approximated or exceeded the statewide average. One noteworthy exception was erythromycin in the Southcentral region. In addition to the 40% susceptibility rate characterized by these isolates (Table 3), this region exhibited an MIC₅₀ value exceeding those from all other regions. The Southcentral region also exhibited a trimethoprim-sulfamethoxazole susceptibility rate that was 13.4% less than the state average. *S pneumoniae* susceptibility to clindamycin was decreased in the Southwest region when compared to the state mean. The Northwest region yielded a ceftriaxone susceptibility rate that was > 5% less than the state mean. MIC₉₀ values for this agent, as well as penicillin (data not illustrated), suggested a potential trend toward increased resistance.

Figure. Geographic Variation With Respect to *E coli* Susceptibility to Levofloxacin (A, also presented with median and 90th percentile MIC data), Tobramycin (B), and Trimethoprim-sulfamethoxazole (C), Wisconsin 2016



Regions outlined in gold represent percentage susceptible rates $\pm 5\%$ of the Wisconsin mean rate for the antimicrobial agent. Regions outlined in red represent percentage susceptible rates $\geq 5\%$ less than the state mean rate for the antimicrobial agent. Regions outlined in green represent percentage susceptible rates $\geq 5\%$ greater than the state mean rate for the antimicrobial agent.

Abbreviations: MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute.

DISCUSSION

Limitations of an antibiogram compilation method for antimicrobial resistance surveillance have been described. Beyond assumptions that laboratories that procure these data are properly utilizing FDA-cleared and laboratory-validated susceptibility testing formats on clinically significant isolates,⁶ CLSI provides additional specifications regarding preparation of the antibiogram document itself.¹³ One tenet involves the inclusion of species with an *n* value of at least 30 isolates per annum. It is therefore probable that smaller participating institutions would not be contributing data for certain organisms to a statewide antibiogram survey; as such, clusters of certain resistance patterns may be overlooked. Furthermore, due to variable configurations of susceptibility testing panels used by local

microbiology laboratories, a statewide antibiogram may not have consistent antimicrobial agent representation within each organism group from all laboratories. In addition, antimicrobial susceptibility testing practices can impact final antibiogram data by way of selective reporting,⁴ particularly with organism groupings in which cephem cascading is an advocated practice.¹²

An alternative paradigm in which a single facility conducts standardized testing and analysis will advance the cause of antimicrobial resistance surveillance. Because all antimicrobial agents are simultaneously tested on a single panel, categorical interpretations are recorded without the influence of selective reporting or laboratory information system collation. The demarcation of SWOTARE geographic regions paralleled those described in a pre-

Table 3. Selected foci of decreased susceptibility of *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* to selected antimicrobial agents, Wisconsin 2016

Organism	Region	Selected Antimicrobial Agent	Region-specific Data			Wisconsin Data [†]	
			Percentage susceptible	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
<i>P. mirabilis</i>	Northcentral	Levofloxacin	55.8	≤0.25	>32	≤0.25	16
		Trimethoprim-sulfamethoxazole	72.1	≤1	>16	≤1	>16
		Ampicillin	72.1	≤8	>64	≤8	>64
		Ampicillin-sulbactam	83.7	≤4	16	≤4	8
		Tobramycin	86	≤2	8	≤2	≤2
	Southcentral	Gentamicin	88.4	≤2	8	≤2	≤2
		Tobramycin	88.4	≤2	8	≤2	≤2
<i>P. aeruginosa</i>	Northeast	Aztreonam	74.2	8	16	8	16
	Southcentral	Aztreonam	75.0	8	16	8	16
	Southeast	Aztreonam	65.3	8	32	8	16
		Ciprofloxacin	73.1	≤0.25	16	≤0.25	2
		Levofloxacin	76.9	0.5	32	0.5	4
		Ceftazidime	84.6	≤2	16	≤2	4
		Piperacillin-tazobactam	88.5	≤8	32	≤8	16
<i>S. pneumoniae</i>	Northwest	Ceftriaxone	87.5	≤0.12	1	≤0.12	0.5
	Southwest	Clindamycin	76.2	≤0.06	>4	≤0.06	4
	Southcentral	Erythromycin	40.0	4	>4	≤0.06	>4
		Trimethoprim-sulfamethoxazole	62.5	0.25	4	0.25	4

[†]Corresponding Wisconsin percentage susceptibility values presented in Tables 1 and 2. Abbreviation: MIC, minimum inhibitory concentration.

vious report³ with 2 exceptions. On the basis of hospital microbiology laboratory availability, Grant County was reassigned from the Southcentral to Southwest region to allow participation of a health care facility in Platteville. On the basis of geographic location, Fond du Lac County was reassigned from the Southeast to Lake Winnebago region. These assignments may slightly affect comparisons between 2016 SWOTARE data and those derived from the previous antibiogram compilation.³ As the SWOTARE program progresses on an annual basis, it is anticipated that the same geographic demarcations will be employed, with largely the same health care facilities, for relevant geographic comparisons on a temporal basis.

One additional advantage of the SWOTARE program lies in its extensive inventory of MIC values. When considering antibiogram compilation-based surveillance, the end point of the antibiogram (percentage susceptibility) does not specifically describe frank resistance or increases in rates of intermediate resistance. In certain instances, Farner⁵ related that monitoring of changing MIC values for a given antimicrobial/organism combination can detect local increases in the rate of resistance before such changes can be observed in an antibiogram. In data presented in Figure 1, increased *E. coli* resistance to levofloxacin in the Lake Winnebago region was characterized not only by an overall susceptibility percentage of 61.8%, but also by an MIC₉₀ of 32 µg/mL (MIC breakpoint of ≥ 8

µg/mL for resistance). While the same antimicrobial/organism combination for the Southcentral region appeared to resemble the state mean on a percentage susceptible basis, it was noted that its MIC₉₀ value was also 32 µg/mL. Such data should warrant continued monitoring and vigilance during succeeding annual SWOTARE collections. Moreover, surveillance efforts at the level of the bacterial isolate allow for the collection of demographic and epidemiologic information associated with the isolate.¹⁴

CONCLUSION

In conclusion, a statewide antimicrobial resistance surveillance system has been formulated to characterize individual clinically-significant isolates using a standardized testing system. Results from the program in 2016 indicate geographic differences in Wisconsin for a number of antimicrobial/organism combinations. Median and 90th percentile MIC data derived from the surveillance program may indicate antimicrobial/organism groupings that warrant vigilance for potential emerging resistance prior to the categorical reporting of frank resistance. Annual continuation of this program should allow for trending of antimicrobial resistance patterns on a temporal basis. Timely dissemination of these findings to important stakeholders provides an informed opportunity to impact local clinical and prescription practices.

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