Rapidly growing mycobacteria in Singapore, 2006–2011

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Abstract

Nontuberculous mycobacteria infection is a growing global concern, but data from Asia are limited. This study aimed to describe the distribution and antibiotic susceptibility profiles of rapidly growing mycobacterium (RGM) isolates in Singapore. Clinical RGM isolates with antibiotic susceptibility tests performed between 2006 and 2011 were identified using microbiology laboratory databases and minimum inhibitory concentrations of amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, imipenem, linezolid, moxifloxacin, sulfamethoxazole or trimethoprim-sulfamethoxazole, tigecycline and tobramycin were recorded. Regression analysis was performed to detect changes in antibiotic susceptibility patterns over time. A total of 427 isolates were included. Of these, 277 (65%) were from respiratory specimens, 42 (10%) were related to skin and soft tissue infections and 36 (8%) were recovered from blood specimens. The two most common species identified were *Mycobacterium abscessus* (73%) and *Mycobacterium fortuitum* group (22%), with amikacin and clarithromycin being most active against the former, and quinolones and trimethoprim-sulfamethoxazole against the latter. Decreases in susceptibility of *M. abscessus* to linezolid by 8.8% per year (p 0.001), *M. fortuitum* group to imipenem by 9.5% per year (p 0.023) and clarithromycin by 4.7% per year (p 0.033) were observed. *M. abscessus* in respiratory specimens is the most common RGM identified in Singapore. Antibiotic options for treatment of RGM infections are increasingly limited.

Keywords: Antimicrobial susceptibility, *Mycobacterium abscessus*, *Mycobacterium fortuitum* group, Nontuberculous mycobacteria, Rapidly growing mycobacteria

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous organisms commonly found in the environment. Although they are traditionally not considered a major public health issue, as with tuberculosis, NTM is of emerging global interest and concern as its pathogenic potential becomes more apparent and diseases caused by NTM are increasingly prevalent. Incidence rates of 7.2 to 13.6 cases per 100 000 persons were recently reported [1,2]. Rapidly growing mycobacteria (RGM) are important causes of NTM infections, especially in Asia [3]. The proportion of NTM contributed by RGM has increased more than 2-fold to 35% in 2001 vs. 14% for the period 1992 through 1996 [4]. Among the RGM, *Mycobacterium abscessus*, *Mycobacterium fortuitum* group and *Mycobacterium chelonae* are most common.

Clinical presentations of RGM disease are highly varied and include infections of the respiratory tract (most common), skin and soft tissue structures, bone and joint, lymphadenitis, ophthalmic infections, otitis media and infection of the central nervous system. In addition, infective complications caused by RGM after surgical procedures and catheter use, as well as disseminated infections, especially in immunocompromised hosts, have been widely documented [5]. Treatment of serious RGM infections is challenging; drug therapy typically involves a multidrug regimen for a lengthy duration, is costly and is

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associated with drug-related toxicities. Moreover, response rates are highly variable, particularly in pulmonary RGM infections, with cure rates of only 30% to 50% [6].

The choice of antimicrobial therapy for RGM infections are primarily based on *in vitro* antimicrobial susceptibility testing, which in turn varies with the RGM species involved [7]. However, published reports on the epidemiology of NTM infections to date are derived mainly from continents other than Asia, and the possible geographical variation in the distribution of this group of environmental bacteria suggest that their data may not be directly relevant to the local context. Furthermore, we have observed possible increase in antimicrobial resistance among RGM species. We investigated the epidemiology and *in vitro* antibiotic susceptibility profiles of RGM species isolated in Singapore.

Methods

This observational cohort study was conducted involving RGM isolates recovered from patients at three major hospitals in Singapore: National University Health System (NUHS), Singapore General Hospital (SGH) and Tan Tock Seng Hospital (TTSH). The Central Tuberculosis Laboratory at SGH and the NUHS mycobacteriology laboratory are involved in the identification and antimicrobial susceptibility testing of all mycobacterial specimens in Singapore, and they provided the database of clinical RGM isolates for this study. Institutional review board approval was obtained at all study sites.

RGM isolates with antimicrobial susceptibility testing performed between January 2006 and December 2011 were included in this study. The types of clinical specimens from which the isolate was recovered were noted. Specimen site was classified as pulmonary if the RGM was isolated from sputum, lung tissue biopsy sample, pleural fluid or bronchoalveolar lavage; as skin and soft tissue structure if it was a culture of wound discharge or a biopsy specimen of a lesion involving skin, subcutaneous tissue, muscle, synovium or bone [8]; as a Tenckhoff catheter exit site if the specimen was of wound discharge from an exit site without a positive peritoneal dialysate (PD) culture; as PD peritonitis if RGM was isolated from PD fluid culture; and as lymphadenitis if a biopsy specimen or swab of a lymph node yielded a RGM [9].

Minimum inhibitory concentrations (MICs) of all antibiotics tested were recorded; only unique and nonduplicate isolates from the first culture of each patient were analysed. Linear regression analyses were performed by SPSS Statistics software, version 17.0 (IBM, Armonk, NY), to detect if there were any significant changes in antibiotic susceptibility over time. Results with a p value of <0.05 were deemed statistically significant.

RGM isolates at the Central Tuberculosis Laboratory in SGH were identified by negative DNA probe (AccuProbe; Gen-Probe Inc., San Diego, CA) and NAP (ρ-nitro-α-acetylamino-β-hydroxy-propiophenone) tests for *Mycobacterium tuberculosis* complex. Clinically significant isolates (determined by the attending physician in accordance with the criteria set out by the American Thoracic Society (ATS) [7]) were subsequently identified to species level by DNA reverse hybridization (INNO-LiPA MYCOBACTERIA v2, Innogenetics NV, Ghent, Belgium) and high-performance liquid chromatography. Discrepancies, if any, were resolved through 16S ribosomal RNA sequencing using primers 16S-27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 16S-907R (5′-CCG TCA ATT CMT TTR AGT TT-3′). The NUHS mycobacteriology laboratory identified RGM to species level by conventional biochemical methods (3-day arylsulphatase reaction, nitrate reduction, mannitol, inositol, sorbitol, and citrate utilization, tolerance to 5% NaCl, polymyxin susceptibility and presence of pigmentation) [10]. Microbroth dilution method was used in both institutions for antimicrobial susceptibility testing and MICs were determined and interpreted according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [11]. Antibiotics tested included amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, imipenem, linezolid, moxifloxacin, trimethoprim-sulfamethoxazole, tigecycline and tobramycin.

Results

A total of 427 RGM isolates from 392 patients were included in this study. The rate of positive RGM cultures requiring species identification and antibiotic susceptibility testing was fairly stable at an average of 14.6 isolates per 100 000 population each year [12]; there was also little variation in the proportion contributed by each RGM species (Fig. 1). *M. abscessus* was the most frequently recovered species (74%), followed by *M. fortuitum* complex (22%) and *M. chelonae* (3%); *Mycobacterium mucogenicum* was isolated in five cases and *Mycobacterium neoaurum* in one.

Approximately two-thirds of all isolates (n = 277) were from respiratory specimens; clinical samples from skin and soft tissue structures were the next most common but accounted for only 42 of cases (10%). *M. abscessus* was the predominant species identified across all sites, although *M. fortuitum* group was also equally important in lymphadenitis (Table 1). Of 11 *M. chelonae* isolates, six (55%) were recovered from the respiratory tract and three (27%) from the bloodstream. *M. mucogenicum* (n = 3) and *M. neoaurum* (n = 1) were largely implicated in central venous catheter–related bloodstream infections; *M. mucogenicum* was also identified from two respiratory specimens.

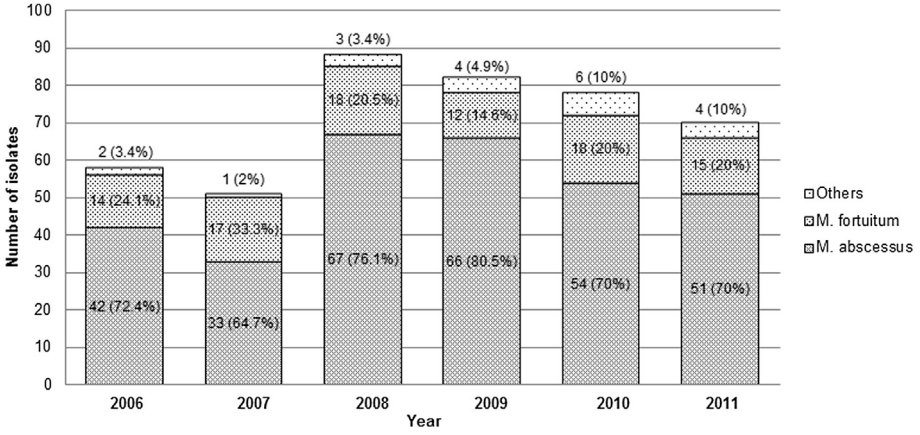

FIG. 1. Species distribution of rapidly growing mycobacteria by year from 2006 to 2011.

Table 2 summarises the antibiotic susceptibility profiles of *M. abscessus* and *M. fortuitum* group. Amikacin was active against more than 90% of all RGM isolates, while tobramycin had poor *in vitro* activity against most species, including *M. chelonae*. Nearly all *M. fortuitum* group isolates were susceptible to ciprofloxacin, moxifloxacin and trimethoprim-sulfamethoxazole; in contrast, virtually all isolates of *M. abscessus* and *M. chelonae* were resistant to these antibiotics (Fig. 2). None of the isolates resistant to ciprofloxacin retained susceptibility to moxifloxacin; similarly, isolates that were susceptible to ciprofloxacin were also susceptible to moxifloxacin. More than 95% of *M. abscessus* and *M. chelonae* isolates were susceptible to clarithromycin, vs. 20% against *M. fortuitum* group. Activities of cefoxitin and imipenem were moderate against *M. abscessus*, *M. chelonae* and *M. fortuitum* group; further analysis revealed that 86% of cefoxitin-resistant *M. fortuitum* group but only 12.5% of cefoxitin-resistant *M. abscessus* isolates retained at least intermediate susceptibility towards imipenem.

Only two-fifths of *M. fortuitum* group and *M. mucogenicum* isolates remained susceptible to doxycycline. On the other hand, tigecycline displayed excellent *in vitro* activity against all RGM isolates, with MIC_{90} not exceeding 0.5 μg/mL. On the basis of the susceptibility breakpoint of MIC ≤8 μg/mL, linezolid was active against 85% of *M. fortuitum* group isolates but exhibited activity against only 42% and 33% of *M. abscessus* and *M. chelonae* isolates, respectively.

Decrease in susceptibility of *M. abscessus* to linezolid by 8.8% per year (68% in 2006 to 24% in 2011) (R^2 = 0.948, p 0.001) was observed. *M. fortuitum* group displayed increasing resistance to imipenem at a rate of approximately 9.5% per year (67% in 2006 to 7% in 2011) (R^2 = 0.761, p 0.023) as well as to clarithromycin at 4.7% per year (29% in 2006 to 7% in 2011) (R^2 = 0.719, p 0.033) (Fig. 3). Interestingly, a slight improvement in susceptibility of *M. abscessus* to clarithromycin by 1.9% per year (93% in 2006 to 98% in 2011) (R^2 = 0.745, p 0.027) was noted. No other secular trends in variation of antimicrobial susceptibility were found.

TABLE 1. Distribution of RGM species by site, regardless of clinical relevance

Specimen site	No. (%) of isolates for:	
	Mycobacterium abscessus	*Mycobacterium fortuitum* group
Pulmonary (*n* = 269)	210 (76)	59 (21)
Skin and soft tissue structure (*n* = 41)	30 (71)	11 (26)
Blood (*n* = 33)	26 (65)	7 (18)
PD (*n* = 25)	21 (84)	4 (16)
Tenckhoff catheter exit site (*n* = 11)	10	1
PD peritonitis (*n* = 14)	11	3
Lymph node (*n* = 11)	5 (45)	6 (55)
Eye (*n* = 9)	9 (100)	0 (0)
Other (*n* = 22)[a]	13 (59)	9 (41)

RGM, rapidly growing mycobacterium; PD, peritoneal dialysis.
[a]Other sites of infection included brain, ear, gastrointestinal tract, liver and urinary tract.

Discussion

Teo and Lo [13] reported the first case series of 23 NTM infections in Singapore in 1992, which was limited to pulmonary manifestations but included slow-growing mycobacteria from 1976 to 1988. Our present study, involving RGM species alone, identified a total of 392 cases over 6 years. Furthermore, we identified *M. abscessus* to be the most important RGM in our setting across all specimen sites; *M. fortuitum* group that was reported to be the most common RGM species in the earlier study was implicated in only 22% of our cases. This finding is consistent with reports from Taiwan [8,14,15] and Japan [16], and it underscores the importance of *M. abscessus* in the

TABLE 2. ***In vitro*** **antimicrobial susceptibilities of RGM isolates**

RGM species and antimicrobial agent (no. of isolates tested)	No. (%) of isolates:			MIC (μg/mL)		
	Susceptible	Intermediate	Resistant	Range	50%	90%
Mycobacterium abscessus						
Amikacin (313)	282 (90)	28 (9)	3 (1)	≤0.5 to >128	16	16
Cefoxitin (300)	12 (4)	280 (93)	8 (3)	4 to >128	32	64
Clarithromycin (313)	299 (96)	3 (1)	11 (3)	≤0.06 to >16	0.25	1
Ciprofloxacin (312)	3 (1)	16 (5)	293 (94)	≤0.12 to >4	>4	>4
Doxycycline (292)	4 (1)	13 (5)	275 (94)	≤0.12 to >32	>32	>32
Imipenem (256)	4 (2)	202 (78)	50 (20)	4 to >64	16	>64
Linezolid (306)	127 (42)	128 (42)	50 (16)	≤0.5 to >64	16	32
Moxifloxacin (124)	4 (3)	5 (4)	115 (93)	0.25 to >8	>8	>8
TMP/SMX (101)	6 (6)	—	95 (94)	1/9 to >8/152	>8/152	>8/152
Tobramycin (288)	2 (1)	4 (1)	282 (98)	≤1 to >32	16	>32
Mycobacterium fortuitum group						
Amikacin (94)	89 (95)	2 (2)	3(3)	≤0.5 to >128	1	16
Cefoxitin (87)	5 (6)	75 (86)	7 (8)	16 to >128	64	64
Clarithromycin (94)	19 (20)	31 (33)	44 (47)	0.12 to >16	4	>16
Ciprofloxacin (92)	85 (92)	4 (5)	3 (3)	≤0.12 to >4	0.12	1
Doxycycline (80)	32 (40)	4 (5)	44 (55)	≤0.12 to >32	16	>32
Imipenem (85)	25 (29)	57 (67)	3 (4)	1 to 32	8	16
Linezolid (87)	74 (85)	7 (8)	6 (7)	1 to >64	8	16
Moxifloxacin (40)	40 (100)	0	0	0.25 to 0.5	≤0.25	≤0.25
TMP/SMX (35)	34 (97)	—	1 (3)	≤0.25/4.75 to >8/152	0.5/9.5	2/38
Tobramycin (79)	4 (5)	2 (3)	73 (92)	≤1 to >32	16	>32

RGM, rapidly growing mycobacterium; TMP/SMX, trimethoprim-sulfamethoxazole.

epidemiology of RGM infections in Asia. Unfortunately, *M. abscessus* is resistant to many currently available antibiotics, with amikacin and clarithromycin as the only two agents with reliable *in vitro* activity.

Indeed, amikacin was the only antibiotic that retained activity against all RGM species. On the other hand, tobramycin had poor activity against most isolates. The CLSI [11] and ATS [7] guidelines have advocated tobramycin as the preferred aminoglycoside for *M. chelonae* infections as a result of its previously demonstrated superior *in vitro* activity. However, our analyses revealed that only 2 (18%) of 11 *M. chelonae* isolates were susceptible to tobramycin, whereas amikacin was active against 86% of them. The recent studies by Fernandez-Roblas *et al.* [17] and Shen *et al.* [18] also reported high susceptibility rates of *M. chelonae* to amikacin, although notably more than 80% of their isolates remained susceptible to

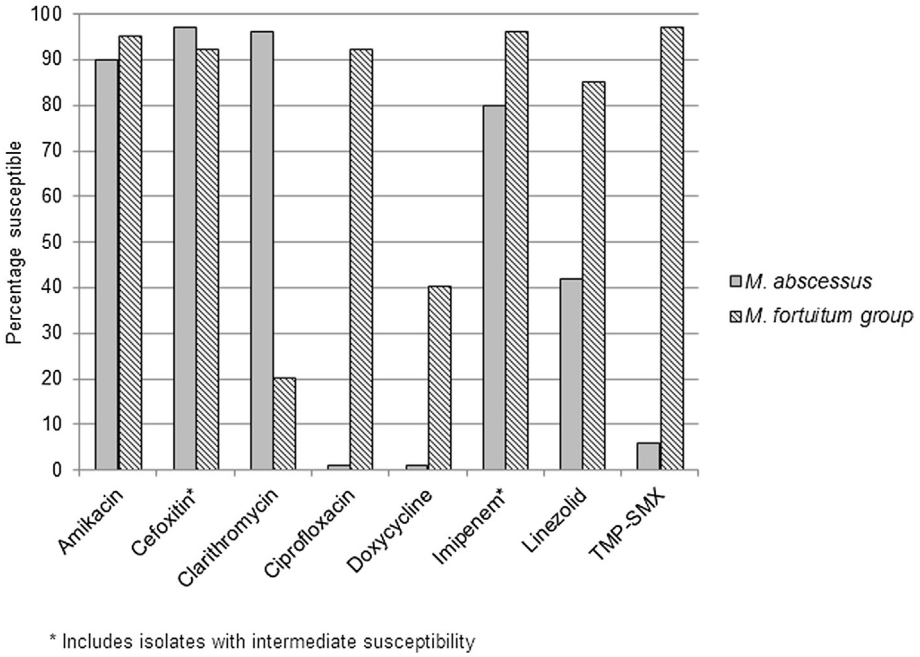

FIG. 2. Antimicrobial susceptibilities of *Mycobacterium abscessus* and *Mycobacterium fortuitum* group.

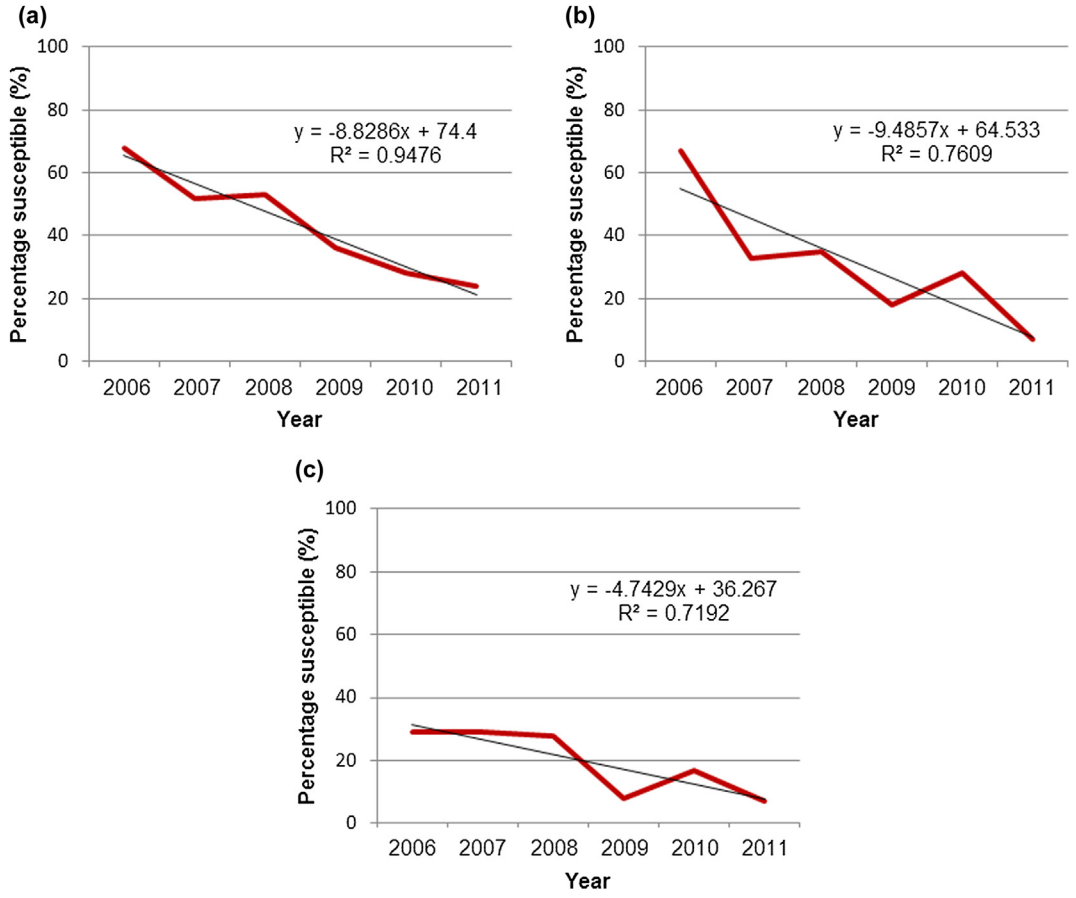

FIG. 3. Secular trend of antimicrobial susceptibilities of (a) linezolid against *Mycobacterium abscessus;* (b) imipenem against *Mycobacterium fortuitum* group; and (c) clarithromycin against *M. fortuitum* group.

tobramycin. The small number of *M. chelonae* isolates in our study precluded definitive conclusions; nonetheless, the observation that few isolates were tobramycin susceptible, coupled with the almost nonexistent use of this aminoglycoside in clinical practice locally, highlighted the need to review the value of routine testing of tobramycin activity against *M. chelonae* (and other RGM) species in our local setting.

Linezolid was one of several newer agents reported to possess antimycobacterial activity. Although mycobacterial resistance to linezolid generally remains a rare occurrence, cases of linezolid-resistant *M. tuberculosis* complex were reported [19]. The molecular basis of linezolid resistance has yet to be fully elucidated but involved mutations in genes encoding for 23S rRNA and ribosomal proteins [20], as well as the presence of efflux pumps [21]. *M. abscessus* was found to be the least susceptible among common RGM species in our study, and its increasing resistance to linezolid over time is of concern. Although the reason for this observed trend is unclear, this phenomenon is unlikely to be explained by variations in laboratory practices (there were no changes in laboratory protocols or susceptibility breakpoints) and/or sampling bias (the number of isolates each year was comparable).

The increasing use of carbapenems in the context of growing gram-negative resistance may have contributed to the observed decreasing susceptibility of *M. fortuitum* group to imipenem. Nevertheless, the *M. fortuitum* group species remained highly susceptible to multiple antibiotics such as the quinolones, trimethoprim-sulfamethoxazole, linezolid and doxycycline, all of which are available in oral formulations and provide an attractive regimen for outpatient therapy. Although *M. fortuitum* group is known to be more resistant than *M. abscessus* to clarithromycin, the MICs observed in our study were significantly higher, with less than 20% of our isolates inhibited by clarithromycin at 2 μg/mL while Yang *et al.* [4] and Fernandez-Roblas *et al.* [17] reported clarithromycin susceptibility in more than half of their isolates.

A major shortfall of this study is the lack of data on antibiotic use in the patient before recovery of the RGM isolate. Previous drug exposure could have influenced susceptibility patterns by exerting selective pressure for more resistant strains.

Nevertheless, the antibiotic susceptibility profiles of the various RGM species presented in this study remain applicable as a broad guide to the choice of empiric antibiotic therapy. Further, this study was dominated by *M. abscessus* species isolated from respiratory specimens; continued study of less common RGM species, such as *M. mucogenicum* and *M. neoaurum,* and of patients with extrapulmonary manifestations of RGM infection is necessary to obtain a more reliable representation of their antimicrobial susceptibility profiles.

In conclusion, in this novel report on the epidemiology and antibiotic susceptibility of RGM species in Singapore, we demonstrated that there was temporal variation in distribution and antimicrobial susceptibility profiles of RGM. Antibiotic options for treatment of RGM infections are increasingly limited and may be particularly challenging in our setting because we observed generally higher MICs in our study cohort.

Transparency Declaration

Part of this work was previously presented as a poster at IDWeek 2012, San Diego, California, 17–21 October 2012. All authors report no conflicts of interest relevant to this article.

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