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A Decline in Mupirocin Resistance in Methicillin-Resistant *Staphylococcus aureus* Accompanied Administrative Control of Prescriptions

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Susceptibility to mupirocin was assessed in methicillin-resistant *Staphylococcus aureus* isolates selected from eras corresponding to differences in usage rate and prescription policies at a Veterans Affairs medical center. The eras studied encompassed from the time of introduction of the drug to its widespread use, through recommended judicious use, and finally to subsequent stringent administrative control. Prescriptions declined from 3.0 to 0.1 per 1,000 patient days. Precipitous declines first in the numbers of isolates with high-level resistance (from 31% to 4%) and then in those with low-level resistance (from 26% to 10%) accompanied prescription control.

Mupirocin is a topical antimicrobial agent utilized in eradication and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) (4, 6, 7, 12, 18). The emergence of mupirocin resistance has led to cautions against long-term and widespread institutional usage (11, 14, 15). Moreover, contradictory reports of the ability of mupirocin to eradicate mupirocin-resistant MRSA and reported differences in the rate of emergence of resistance may have contributed to variability in usage patterns among facilities (7, 21, 22).

Successful reduction of mupirocin resistance may depend in part on regional variation in the genetic basis of resistance, a factor implicated in underlying differential rates of emergence (7). High-level mupirocin resistance in *S. aureus* is associated with a gene (*mupA*) that is plasmid borne in most strains and encodes an isoleucyl-tRNA synthetase (10). Strains with a chromosomal *mupA* gene express either low-level mupirocin resistance (17; S. Fujimura, A. Watanabe, and D. Beighton, Letter, Antimicrob. Agents Chemother. **45:**641-642, 2001) or nontransferable high-level resistance (20). More commonly, low-level resistance is caused by mutations in the native, chromosomally encoded isoleucyl-tRNA synthetase (*ileS*) (3).

Because mupirocin can be effective in treating mupirocin-sensitive MRSA, we examined long-term patterns of change in mupirocin susceptibility to determine whether the frequency of occurrence and magnitude of endemic mupirocin resistance would decline in response to an altered prescription policy. We report that mupirocin resistance attained a high frequency in an MRSA population during a period of widespread usage, followed by steep declines following implementation of stringent prescription control. The study is unique in tracking both antibiotic usage and resistance from the time of local introduction of a relatively new antibiotic through unrestricted high usage and into an era of antibiotic control.

The study was conducted at the James H. Quillen Veterans Affairs Medical Center (VAMC) at Mountain Home, Tenn., a site that includes a domiciliary, nursing home, and acute care facilities. Beginning in August 1990, all MRSA isolates were archived at −70°C. From August 1990 until February 1999, the infection control program performed surveillance for nasal carriage of MRSA and, when present, routinely attempted eradication with mupirocin ointment (2% mupirocin calcium cream, Bactroban nasal; SmithKline Beecham, King of Prussia, Pa.). Mupirocin ointment was applied intranasally with a swab, twice daily for 5 days. With increasing awareness of mupirocin resistance, in 1996 infection control efforts continued and a recommendation for more judicious use was issued. Subsequent to February 1999, the routine usage of mupirocin for nasal carriers of MRSA was discontinued and permission was required from infectious disease professionals to prescribe mupirocin.

To examine the population dynamics of mupirocin resistance phenotypes, MRSA isolates (50 to 100/era) were randomly selected from five eras, functionally delimited by differences in mupirocin usage or prescription policy as follows: era 1 (August 1990 to August 1993), "introduction" of the drug; era 2 (September 1993 to December 1995), continued "unrestricted use"; era 3 (January 1996 to February 1999), "judicious use" recommended; eras 4 (March 1999 to April 2000) and 5 (May 2000 to May 2001), early and later eras of "administrative control," respectively. Sample isolates were selected by using random numbers corresponding to isolate identifier numbers. Samples were from nursing home patients and inpatients; with duplicate isolates from individual patients excluded. Because a limited number of nasal MRSA isolates were available post-February 1999, only nonnasal MRSA isolates were included to provide consistency across eras. The mean number of unique patients per year with nonnasal

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TABLE 1. Indicators of mupirocin usage across functional eras at the VAMC[a]

Era	No. of prescriptions per:			No. of MRSA isolates[b]
	1,000 Patient days	Yr	Individual	
1	2.9	400	2.24	1.13
2	3.0	400	2.30	0.85
3	2.6	256	1.00	0.32
4	0.7	53	0.20	0.04
5	0.1	9	0.04	0.02

[a] Era 1, introduction of mupirocin; era 2, unrestricted use; era 3, judicious use recommended; eras 4 and 5, early and later periods of administrative control. Numbers of individuals were tallied as unique occurrences of individuals with MRSA within eras, but individuals may recur between eras. Regression coefficients (r values) are as follows: for number of prescriptions per 1,000 patient days, −0.92; per year, −0.96; and per individual, −0.95; and for number of MRSA isolates, −0.96. Associated probabilities (P values) are as follows: for number of prescriptions per 1,000 patient days, 0.03; per year, 0.01; and per individual, 0.01; and for number of MRSA isolates, <0.01.

[b] Number of MRSA isolates isolated by the hospital laboratory.

MRSA increased across eras (number per year within eras: era 1 = 78, era 2 = 116, era 3 = 163, era 4 = 142, and era 5 = 176). Samples represented 19 to 30% of the isolates per era.

Susceptibility to mupirocin was determined by Etest (AB Biodisk, Solna, Sweden), using the following resistance breakpoints: susceptible, MIC of <4 mg/liter; low-level resistance, MIC of ≥4 and <256 mg/liter; and high-level resistance, MIC of ≥256 mg/liter. Apparent heteroresistant isolates, detected as pure cultures reproducibly displaying double inhibition zones on Etest (2), were categorized as low- or high-level resistant based on the most resistant subpopulation. Chi-square tests for heterogeneity were used to test for differences in the frequencies of the three resistance categories between pairs of eras.

The numbers of prescriptions per 1,000 patient days and prescriptions per year were used as standard measures of the mupirocin usage rate. Because the mupirocin target molecule is unique among antibiotics and mupirocin was used almost exclusively to eradicate a specific organism (MRSA) at a particular body site (nares), two additional, perhaps more relevant, usage rate indicators were defined: (i) number of prescriptions per MRSA-carrying patient and (ii) number of prescriptions per MRSA isolate. Linear regressions were used to determine whether each of the indicators showed a significant decline over time.

A sample of isolates from each resistance category within each era was chosen for PCR analysis. DNA was extracted from 1-ml aliquots of overnight cultures by a boiling method (16). Primers for the *mupA* gene (8) were M1 (5′-GTTTATC TTCTGATGCTGAG-3′) and M2 (5′-CCCCAGTTACACCG ATATAA-3′). A PCR-based assay for the chromosomally encoded native isoleucyl tRNA synthetase gene (*ileS*) served as a positive control, using primers ileS1601U (5′-AAAGAGAAG CGAAAGACTTACTACCAG-3′) and ileS2365L (5′-AAGA TTGGTGCTAACAACTTCGTCATA-3′). PCRs consisted of 200 μM deoxynucleotide triphosphates, 1× reaction buffer, 1 μM each primer, 1.5 mM $MgCl_2$, and 1 U of AmpliTaq Gold (Applied Biosystems), with 2 μl of DNA per 50-μl reaction mixture. The PCR cycling protocol consisted of 10 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 42°C, and 30 s at 72°C; followed by 10 min at 72°C in a Perkin-Elmer GeneAmp 9600 thermocycler.

Mupirocin usage declined significantly across eras as assessed from the perspective of the pool of human hosts or the

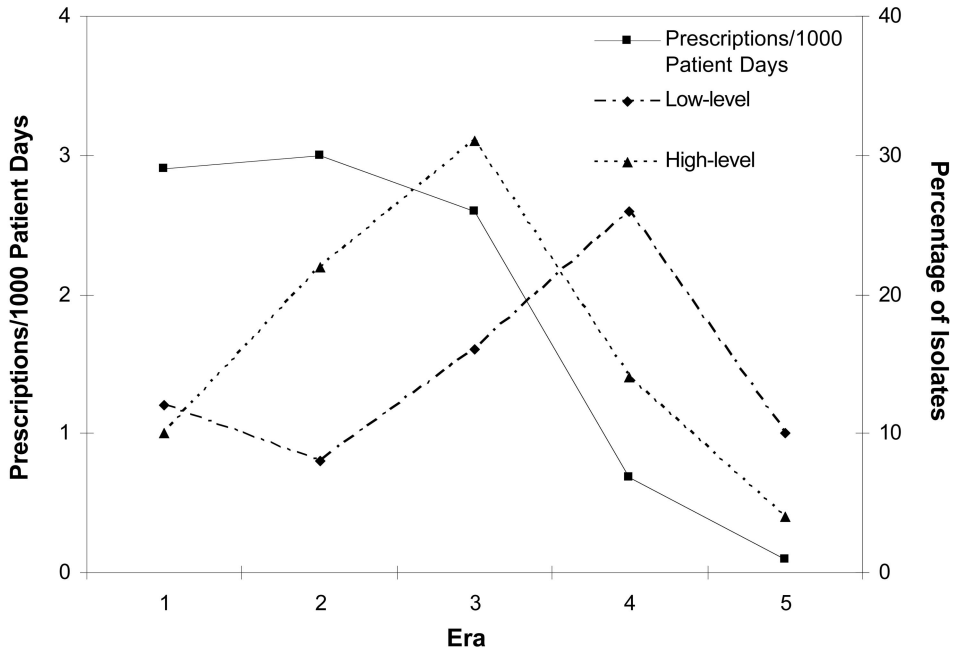

FIG. 1. Number of mupirocin prescriptions per 1,000 patient days and percentage of isolates showing low- and high-level mupirocin resistance per era.

TABLE 2. Pairs of eras showing significant differences in frequencies among mupirocin resistance categories[a]

Eras compared	χ^2	P	Trend	
			High	Low
Introduction vs judicious use	9.89	0.01	↑	—
Unrestricted use vs administrative control I	6.04	0.05	—	↑
Unrestricted use vs administrative control II	7.16	0.03	↓	—
Judicious use vs administrative control I	5.82	0.05	↓	—
Judicious use vs administrative control II	17.57	<0.001	↓	↓
Administrative control I vs administrative control II	8.65	0.01	↓	↓

[a] Significance was based on the χ^2 test for heterogeneity. df = 2 for each comparison (3 resistance categories; 2 eras). Between-era trends for high- and low-level resistance are shown by up and down arrows to indicate significant increases and decreases, respectively. —, no significant change.

incidence of bacterial isolates (Table 1). Although the prescription rate decreased with the judicious use policy (era 3), this era was characterized by a significant increase in high-level resistance (Table 2). In contrast, both eras of administrative control were accompanied by significant declines in high-level resistance relative to the incidence during judicious use. Low-level resistance showed a similar but lagging pattern of increase and decline (Fig. 1). There was no significant difference in the resistance category frequency spectrum between the beginning and end of the study, a pattern that indicates a return to conditions prior to unrestricted high usage. In fact, by the end of the study, the rate of high-level resistance was the lowest recorded at the VAMC (Fig. 1).

The *mupA* gene was exclusively associated with high-level mupirocin resistance, having been detected in all 37 isolates with phenotypic high-level resistance but absent from the 15 susceptible isolates and the 14 isolates with low-level resistance. The lag in the response of the low-level resistance phenotype to prescription changes relative to the high-level phenotype may be a consequence of the expected difference in the population dynamics of plasmid- and chromosomally encoded factors. Once acquired, chromosomal mutations that confer low-level resistance may be less likely to be lost. In contrast, plasmid-borne high-level resistance is more labile, as evidenced by in vitro conjugative transfer in filter matings and loss of the high-level phenotype following culture at elevated temperatures (data not shown). However, occasional reports of high-level mupirocin resistance encoded by a chromosomal copy of the *mupA* (20) gene suggest a potential for the *mupA* gene to achieve a more stable state within *S. aureus*.

Factors other than prescriptions may have influenced the prevalence of mupirocin resistance during the study. Educational efforts designed to reinforce appropriate infection control practices, such as frequent hand washing and cohorting of MRSA carriers, coincided with increased education on judicious prescription practice. Furthermore, the judicious use era coincided with the implementation of system-wide reforms of Department of Veterans Affairs health care policies that decreased the number of admissions and reduced length of stay for inpatients (5). Moreover, the number of acute-care beds at the VAMC declined threefold from 1990 to 2001. Each of these changes may have been expected to contribute to a decline in antibiotic resistance, but instead, both mupirocin resistance and methicillin resistance increased in *S. aureus*—the latter from 24 to 28% between 1990 and 1994 to 67% in 2001. These increases were recorded during a time of relative stability in the numbers of *S. aureus* isolates (numbers per year within eras: era 1 = 470; era 2 = 396; era 3 = 291; era 4 = 396; era 5 = 295). In contrast, the rise and decline in mupirocin resistance that coincided with the transitions from antibiotic introduction to widespread usage to prescription control provide support for the hypothesis that the reduction in the prescription rate was the primary causative factor in reducing resistance.

Antibiotic control as a means of reversing a rise in resistance is based on the premise that resistant cells incur metabolic and fitness costs associated with resistance. The premise is predicated on the idea that removal of the antibiotic restores a selective advantage to sensitive cells that then translates into a decline in resistance. While the efficacy of antibiotic restriction in reducing antibiotic resistance in the community is controversial (1), several hospital-based studies have shown an association between reduced antibiotic usage and decreased resistance (reviewed in references 9 and 13). For example, White et al. (23) used broad-based antibiotic control to counter an outbreak of bacteremia caused by multidrug-resistant *Acinetobacter*. Within a year of adopting antibiotic controls, resistance to a suite of 12 β-lactam agents and quinolines declined significantly, not only in *Acinetobacter*, but also in other sentinel species. In addition, reductions in mupirocin resistance in staphylococci followed administrative control of prescriptions within a single hospital ward in The Netherlands (24) and regionally in western Australia (19). Although we have shown that mupirocin resistance tracked the usage rate, the rate of reemergence will depend upon the remaining pool of resistant *S. aureus*, potential reservoirs in other species, and the future antibiotic usage rate.

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The experiments in this study comply with guidelines of the Institutional Review Board.

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