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Staphylococcus aureus resistance to topical antimicrobials in atopic dermatitis[*]

Giancarlo Rezende Bessa[1,2]
Daiane Corrêa Machado[1]
Magda Blessmann Weber[1]
Pedro Alves D'Azevedo[1]

Vanessa Petry Quinto[1]
Caroline Lipnharski[1]
Renan Rangel Bonamigo[1]

Abstract: BACKGROUND: Topical antimicrobial drugs are indicated for limited superficial pyodermitis treatment, although they are largely used as self-prescribed medication for a variety of inflammatory dermatoses, including atopic dermatitis. Monitoring bacterial susceptibility to these drugs is difficult, given the paucity of laboratory standardization.
OBJECTIVE: To evaluate the prevalence of *Staphylococcus aureus* topical antimicrobial drug resistance in atopic dermatitis patients.
METHODS: We conducted a cross-sectional study of children and adults diagnosed with atopic dermatitis and *S. aureus* colonization. We used miscellaneous literature reported breakpoints to define *S. aureus* resistance to mupirocin, fusidic acid, gentamicin, neomycin and bacitracin.
RESULTS: A total of 91 patients were included and 100 *S. aureus* isolates were analyzed. All strains were methicillin-susceptible *S. aureus*. We found a low prevalence of mupirocin and fusidic acid resistance (1.1% and 5.9%, respectively), but high levels of neomycin and bacitracin resistance (42.6% and 100%, respectively). Fusidic acid resistance was associated with more severe atopic dermatitis, demonstrated by higher EASI scores (median 17.8 vs 5.7, p=.009). Our results also corroborate the literature on the absence of cross-resistance between the aminoglycosides neomycin and gentamicin.
CONCLUSIONS: Our data, in a southern Brazilian sample of AD patients, revealed a low prevalence of mupirocin and fusidic acid resistance of *S. aureus* atopic eczema colonizer strains. However, for neomycin and bacitracin, which are commonly used topical antimicrobial drugs in Brazil, high levels of resistance were identified. Further restrictions on the use of these antimicrobials seem necessary to keep resistance as low as possible.
Keywords: Anti-infective agents, local; Dermatitis, atopic; Drug resistance, bacterial; *Staphylococcus aureus*

INTRODUCTION

Antimicrobial drugs applied topically offer several advantages over systemic administration, including avoidance of systemic toxicity and side effects, the decreased induction of bacterial resistance, high local concentration of antibacterial agents and low costs.[1] Moreover, the broad use of this class of drugs is largely the result of self-prescription of over-the-counter preparations containing antibiotics, including ointments, eye-drops and otological solutions, uses that are supported by little or no scientific evidence.[2]

Staphylococcus aureus is the major causative agent of skin and soft tissue infections and treatment of *S. aureus* infections has become more difficult with time due to the emergence of multidrug-resistant strains.[3] Atopic dermatitis (AD) is a chronic skin condition that has been strongly linked to the presence of *S. aureus*, while cutaneous infection constitutes an important mechanism of worsening disease.[4] Thus, patients are frequently prescribed or self-prescribe antimicrobial medication, often topical preparations of antimicrobials associated with corticosteroids.

However, there is growing concern over resistance to topical antimicrobials.[5] Some of these drugs, such as fusidic acid, can also be used systemically in the treatment of methicillin-resistant *S. aureus* (MRSA) infections.[6,7] Employing topical preparations in superficial infections and a variety of inflammatory dermatosis, including AD, has been deemed responsible for the emergence of fusidic acid-resistant *S. aureus* (FRSA) strains. Some authors have called for its topical use to be restricted or even abolished. [8,9] Nevertheless, mupirocin can only be administered topically and its efficacy on *S. aureus* carriage eradication has disseminated its use for nasal decolonization.[10] High-level mupirocin resistance, defined by minimum inhibitory concentration (MIC) ≥256µg/mL, has been increasingly reported and has emerged as a significant problem.[11,12] It has been associated with multidrug-resistant MRSA isolates, and in a clinical setting, with decolonization failure.[13-15] Finally, despite the wide use in several countries, information in the literature about topical neomycin and bacitracin antimicrobial activity remains limited.

Topical antimicrobial susceptibility testing is not routinely performed at laboratory centers. With the exception of mupirocin and fusidic acid, which have been discussed in many publications concerning bacterial resistance in recent years, data on other topical antimicrobial drugs are scarce. This raises concerns regarding the monitoring of these agents' continued efficacy.

The aims of this study were to: determine the prevalence of *S. aureus* topical antimicrobial resistance in AD patients according to standard procedures (for mupirocin and fusidic acid); verify neomycin and bacitracin activities against *S. aureus* isolates according to historical MIC breakpoints; analyze neomycin cross-resistance with gentamicin; and suggest ways to monitor the activity of commonly used topical antimicrobial drugs in a clinical setting.

METHODS

Patients and isolates

We conducted a cross-sectional study of children and adults diagnosed with AD who consecutively attended two outpatient dermatologic clinics in Porto Alegre, southern Brazil, over a one-year period (December 2009 to December 2010). The diagnosis of AD was made by the treating dermatologist, based on standard criteria.[16] Patients who had at least one dermatitis lesion at the time of their dermatologic visit were invited to participate. Following agreement from patients or children's parents, we collected patient demographics and information on their medical histories. The following data were recorded: self-reported use of topical and systemic antimicrobials over the previous year, skin infection episodes and hospitalization periods. Recurrent cutaneous infections were defined as involving three or more episodes by year. We also performed physical examinations to evaluate eczema severity according to the Eczema Area and Severity Index (EASI) score guidelines.[17]

For microbiological analyses, two sites from every patient were sampled with a sterile swab with transport medium in each case, namely: the anterior nose cavity and an eczema plaque without clinical signs of infection (pustules, serous crusts, purulent exudate). Swabs were sent to the microbiology laboratory and samples were inoculated onto Brain Heart Infusion agar with 5% sheep blood and mannitol agar, and subsequently incubated at 35°C over-night. Each plate was examined for colonial morphology consistent with *S. aureus*. Isolates were identified according to standard procedures.[11] Colonization was defined as positive culture for *S. aureus* on nasal cavities or cutaneous lesions and an absence of clinical signs of infection.

This study was approved by the Institutional Research Ethics Committee of the *Universidade Federal de Ciências da Saúde de Porto Alegre*. Prior to participation, informed consent was obtained from all patients and children's parents or legal guardians.

Susceptibility Tests

All strains were tested by disk diffusion assay on Mueller-Hinton agar with standardized procedures, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[11] All isolates were tested beforehand for methicillin susceptibility, and all were categorized as methicillin-susceptible *S. aureus* (MSSA). The antibiotics disks used in this study were: gentamicin (10µg); neomycin (30µg); bacitracin (10µg); mupirocin (5µg); and fusidic acid (10µg). MIC was determined for bacitracin, mupirocin and fusidic acid using Etest® (BIOMERIEUX, Marcy-l'Etoile, France); and for neomycin using the broth microdilution method, according to the CLSI protocol.[11]

We used CLSI breakpoints to define gentamicin-resistant strains according to the disk diffusion assay. Recent CLSI publications provide no interpretative criteria for antibiotics of exclusive topical use, except for mupirocin. The technique for the mupirocin disk diffusion test from the CLSI constitutes merely a screening test that utilizes 256µg disks to detect high-level mupirocin resistance in *S. aureus* strains.[11] The other test interpretations were as follows. For mupirocin and fusidic acid MICs, breakpoints available from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) publication were used.[18] Bacitracin MIC criteria were available in the document from the *Comité de l`Antibiogramme de la Société Française de Microbiologie* (SFM).[19] Neomycin MIC interpretative criteria were based on historical reports.[20] For the mupirocin 5µg disk diffusion test, breakpoints outlined in publications from the British Society for Antimicrobial Chemotherapy (BSAC) Methods for Antimicrobial Susceptibility Testing were employed.[21] There are two different inhibition zone sizes in EUCAST and BSAC publications for the Fusidic acid 10µg disk diffusion test. In accordance with both criteria, we sought to display fusidic acid resistance prevalence. No interpretative criteria were found for the neomycin 30µg and bacitracin 10µg disk diffusion test in the literature.

Statistical Analysis

Statistical analyses were conducted on SPSS software, version 17.0 (Chicago, IL, USA). Sampling was performed for a prospective study of MRSA colonization prevalence in AD patients, based on an expected frequency of 18.3%.[22] Ninety-three patients were required to estimate population prevalence with significance set at 0.05% and power at 90%. Continuous variables were summarized by mean or median values (and their respective variability measures) according to distribution symmetry, verified by Kolmogorov-Smirnov test. MIC_{50} and MIC_{90} were defined as the minimum inhibitory concentrations, encompassing 50 and 90% of isolates tested, respectively.
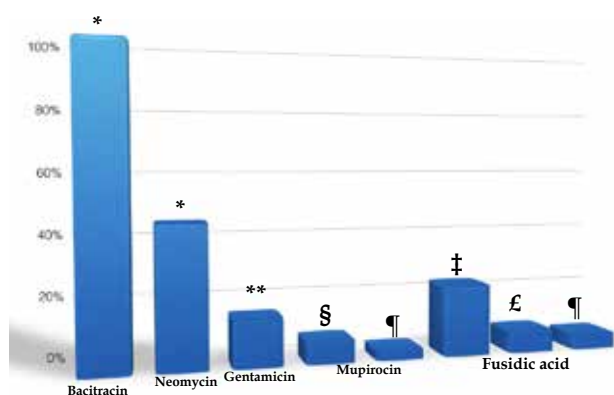
For the statistical analysis, we included one isolate from each patient with positive cultures. For patients with two positive samples, we selected the isolate with the smallest inhibition zone diameter and the highest MIC for each antibiotic. For the comparative analysis, we applied the t-Student test for continuous variables with nearly normal distribution, and the Mann-Whitney test for continuous variables with asymmetric distribution, with significance set at p<0.05. A two-sided Pearson's chi-squared analysis or Fisher's exact test, with p<0.05, was used to compare the categorical variables. The linearity relation between continuous variables was investigated via the Spearman's Correlation coefficient.

To evaluate the discrimination power of 30µg neomycin disk inhibition zone diameter according to neomycin MIC, all *S. aureus* isolates available were investigated and the area under the ROC (Receiver Operating Characteristic) curve was determined. We established the breakpoint of inhibition zone diameter to designate neomycin resistance according to neomycin MIC by calculating the sensitivity and specificity arithmetic mean and taking the point with the highest mean.

To evaluate aminoglycoside cross-resistance likelihood between gentamicin and neomycin, a logistic regression model was run, including gentamicin resistance defined by inhibition zone size, neomycin MIC and demographic variables, like sex, age and medical history. In all analyses, the significance level was set as p<0.05.

RESULTS

A total of 91 patients were included in the study. Sixty-eight patients had at least one positive culture for *S. aureus* (48.4% of the swabs from cutaneous lesions and 61.5% from nasal cavities were positive). Topical antimicrobial resistance frequency is summarized in graph 1. All isolates were resistant to bacitracin. Concerning

aminoglycosides, 42.6% and 14.7% were resistant to neomycin and gentamicin, respectively. Five isolates exhibited resistance to mupirocin upon the 5µg disk diffusion assay, but only one (1.1%) was confirmed by MIC test. The existence of two different criteria led to significant discrepancies in interpretation of 10µg disk diffusion assay of fusidic acid. According to the BSAC[21] breakpoint, 22.1% of isolates were resistant and this proportion dropped to 7.4% when the EUCAST[18] breakpoint was used. MIC criteria (the same in both references) confirmed only four (5.9%) isolates to be resistant to fusidic acid. The MIC_{50} and MIC_{90} values for the 68 isolates available for analysis are displayed in table 1.

Demographic characteristics are shown in table 2, associated with colonization by resistant *S. aureus*. This analysis could not be performed for mupirocin due to the small number of resistant isolates. The only statistically significant association detected was between the EASI score and fusidic acid resistance (the greater the severity of AD, the higher the probability of colonization by FRSA, p=0.009).

Since testing neomycin susceptibility using a 30µg disk diffusion assay was not standardized, we performed an ROC curve analysis and set a value of ≥16mm for the inhibition zone diameter to define susceptibility (according to the MIC reference) (Graph 2). Regarding aminoglycoside cross-resistance, our logistic regression model did not demonstrate an association between gentamicin and neomycin resistance (p=0.896), adjusted by demographic and medical variables (Table 3).

DISCUSSION

This study evaluated the *in vitro* susceptibility of *S. aureus* to commonly used topical antimicrobials. Our data, from a southern Brazilian sample of AD patients, showed a low prevalence of mupirocin and fusidic acid resistance among *S. aureus* atopic eczema colonizer strains. All our strains were MSSA, but a previous study of MRSA carriers from an intensive care unit in Porto Alegre also demonstrated low levels of mupirocin resistance.[23]

MIC determination confirmed low levels of fusidic acid resistance in our sample. Clinical and laboratorial evidence support the hypothesis that the selection of naturally occurring mutant *S. aureus* is an important mechanism of fusidic acid resistance when exposed to the drug.[24] Alsterholm *et al.* conducted a study on Sweden and found high proportions of FRSA in impetigo (as high as 75%) but a significantly lower prevalence (6.1%) was detected in

Resistance was defined by: * historical MIC breakpoints; ** CLSI standard 10µg disk diffusion assay; § BSAC breakpoint on 5µg disk diffusion assay; ¶ EUCAST MIC breakpoints; ‡ BSAC breakpoint on 10µg disk diffusion assay; £ EUCAST breakpoint on 10µg disk diffusion assay.

GRAPH 1: Relative frequencies of S. aureus isolates resistant to topical antimibrobials, cultured from the nose and cutaneous lesions of AD patients (n=68)

TABLE 1: MIC50 and MIC90 of *S. aureus* isolates (n=68)

Antibiotic	MIC50 (µg/ml)	MIC90 (µg/ml)
Neomycin	1.5	17.6
Bacitracin	96	>256
Mupirocin	0.19	0.38
Fusidic acid	0.38	0.75

MIC50 and MIC90 were defined as the minimum inhibitory concentrations encompassing 50 and 90% of isolates tested, respectively.

**TABLE 2:** Sample demographic data according to *S. aureus* antimicrobial resistance

Demographic data	All patients n=91	Gentamicin* R (n=10)	S (n=58)	p	Neomycin** R (n=29)	S (n=39)	p	Fusidic acid** R (n=4)	S (n=64)	p
Male Sex – n(%)	48 (52,7)	5 (50,0)	33 (56,9)	0,740‡	15 (51,7)	23 (59,0)	0,625¶	2 (50,0)	36 (56,3)	1,000‡
Age (years)				0,674§			0,312§			0,900§
Range	0,3 - 30									
Mean ± SD	7,0±5,2	6,6±2,2	7,4±5,9		8,1±5,9	6,7±5,3		7,0±3,8	7,4±5,7	
Median (IQR)	6,0 (2,6-10,0)	7,0 (0,5-10,0)	6,0 (0,5-30,0)		7,0 (0,5-30,0)	6,0 (0,6-26,0)		8,0 (2,0-10,0)	6,5 (0,5-30,0)	
Topical antibiotic use – n(%)	9,0 (9,9)	0 (0,0)	8 (13,8)	0,593‡	3 (10,3)	5 (12,8)	1,000‡	0 (0,0)	8 (12,5)	0,975‡
Previous skin infection – n(%)	30 (33,0)	4 (40,0)	20 (34,5)	0,733‡	12 (41,4)	12 (30,8)	0,516¶	0 (0,0)	24 (37,5)	0,289‡
Recurrent infections– n(%)	24 (26,4)	3 (30,0)	16 (27,6)	1,000‡	10 (34,5)	9 (23,1)	0,445¶	0 (0,0)	19 (29,7)	0,570‡
Systemic antibiotic use – n(%)	27 (29,7)	2 (20,0)	19 (32,8)	0,250‡	9 (31,0)	12 (30,8)	1,000	2 (50,0)	19 (29,7)	0,658‡
Hospitalization – n(%)	7 (7,7)	1 (10,0)	5 (8,6)	1,000‡	2 (6,9)	4 (10,3)	1,000‡	1 (25,0)	5 (7,8)	0,315‡
Disease duration (years) £				0,896§			0,121§			0,444§
range	(0,1–30,0)									
mean ± SD	4,7 ± 4,7	5,2±2,7	4,9±5,4		6,1±6,2	4,1±3,8		6,8±4,0	4,8±5,1	
median (IQR)	3,0 (1,5 – 6,3)	5,6 (0,5-10,0)	3,0 (0,3-30,0)		5,0 (0,3-30,0)	2,5 (0,3-1,7)		8,0 (1,5-10,0)	4,0 (0,3-30,0)	
EASI score £				0,965§			0,437§			0,009§
range	(0,4–40,5)									
mean ± SD	7,8 ± 8,1	9,1±11,3	9,0±8,3		9,9±8,1	8,3±9,2		20,5±17,1	8,3±7,6	
median (IQR)	5,4 (2,0 – 9,9)	5,7 (1,2-40,5)	6,2 (0,6-32,2)		7,3 (0,6-32,2)	4,9 (0,6-40,5)		17,8 (6,0-40,5)	5,7 (0,6-32,2)	

§: Mann Whitney Test; ¶: Pearson's Chi-square Test with continuity correction; ‡: Fisher's Exact Test; £ asymmetric variable; * defined by disk diffusion technique; ** defined by MIC breakpoints. R: resistant; S: sensible; SD: standard deviation; IQR: interquartile range (the values between the 75th percentile and the 25th percentile).

TABLE 3: Cross-resistance between the aminoglycosides gentamicin and neomycin, adjusted by demographic variables (logistic regression model)

Independent variables	Gentamicin resistant Coefficient B	SE	Risk OR	IC95%	p
Factor					
Neomycin MIC	1,18	0,89	1,19	0,87-2,93	0,258
Covariables					
Male Sex	0,77	0,82	2,15	0,43-10,81	0,352
Female Sex	0,31	0,12	0,87	0,11-2,11	0,352
Age (years)	0,16	0,17	0,12	0,84-1,66	0,334
Topical antibiotics use	19,5	14,62	0,99	0,21-3,03	0,999
Previous skin Infection	-0,15	1,12	0,86	0,09-7,71	0,894
Recurrent cutaneous infections	-0,40	1,29	0,67	0,053-8,427	0,757
Systemic antibiotics use	2,15	1,54	3,29	0,88-18,54	0,214
Hospitalization	0,88	1,37	2,41	0,16-35,31	0,520
Disease duration (years) £	-0,121	0,17	0,88	0,63-1,24	0,483
EASI score (points) £	-0,01	0,04	0,99	0,91-1,01	0,828

SE: standard error. OR: odds ratio. £: model parameters: Pseudo-R2=0.237; -2 log Likelihood=46.750; Hosmer and Lemeshow (p=0.896).
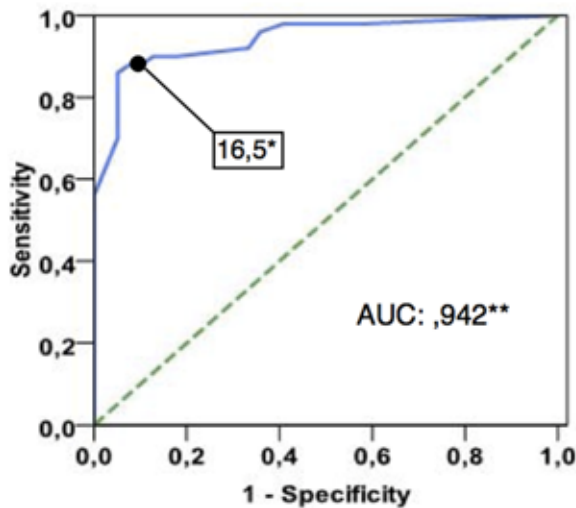
A value of 16.5mm of inhibition zone diameter corresponded to the best breakpoint regarding sensitivity and specificity to define *S. aureus* susceptibility to neomycin by disk diffusion technique, using a 30µg neomycin disk. ** the Area Under Curve (AUC) was calculated as 0.942 (CI 95% 0.895 to 0.989. p<.0001)

Graph 2: ROC curve analysis to define best inhibition zone size to define susceptibility to neomycin (according to MIC reference)

infected atopic dermatitis.[25] Some authors hypothesized that the impetigo clone, described as being responsible for impetigo outbreaks in Europe, is not a frequent atopic dermatitis colonizer.[26] In In Brazil, this low prevalence of resistance can also be explained by low use of topical mupirocin and fusidic acid. Products containing these drugs are relatively expensive and unavailable in the Brazilian public health system. In contrast, an ointment containing neomycin and bacitracin is a cheap and highly popular product, widely used as self-prescription medication for small wounds, insect bites and a variety of cutaneous infectious and inflammatory conditions, including AD. This can explain our high levels of neomycin and bacitracin resistance. All strains in our study were resistant to bacitracin according to a historical breakpoint and the MIC_{50} was considerably high (96 µg/ml). Our results also corroborate the literature about the absence of cross-resistance between neomycin and gentamicin, although an *in vitro* study has demonstrated that aminoglycoside resistance stability in *S. aureus* is closely linked to the resistance of strains to neomycin. [27,28]

For many years, the utility and importance of topical antimicrobials has been underestimated. With the emergence of multidrug resistant strains, like community acquired MRSA infections (CA-MRSA), any agent with an antistaphylococcal activity plays an important role in the limited armamentarium against these microorganisms, especially if the drug can also combat severe systemic infections, as fusidic acid does.[12,29] In the literature, there is growing evidence of numerous clinical benefits of topical applied antibiotics (apart from impetigo treatment), notably *S. aureus* decolonization. In AD patients, despite a recent randomized placebo controlled study that suggested the efficacy of antiseptics and mupirocin in de-

creasing the clinical severity of the disease, pooled evidence failed to demonstrate that antimicrobial administration (topical or systemic) aimed at reducing *S. aureus* colonization is clinically useful.[30-34] However, the benefit of mupirocin use for *S. aureus* decolonization in other clinical circumstances has been demonstrated by many other studies. Intranasal mupirocin was associated with a significant reduction in surgical-site infections and proven to be a cost-effective approach in prosthetic orthopedic surgery.[35,36] A systematic review showed a significant reduction in *S. aureus* nosocomial infections associated with intranasal mupirocin decolonization.[37] Nonetheless, reports of *S. aureus* mupirocin resistance associated with eradication treatment have raised concerns over continued clinical efficacy.[38] Clinical evidence demonstrates that judicious use to decolonize identified nasal carriers without *S. aureus* active infection should reduce resistance levels.[39] As *S. aureus* decolonization clinical benefits become relevant and mupirocin becomes less active, studies of alternatives are necessary. Resistance to neomycin, bacitracin and polymyxin B was found to be rare in the USA, despite its long period of over-the-counter sales.[27] A small study described successful MRSA decolonization using a triple compound (bacitracin, polymyxin B and gramicidin) in 9 out of 11 patients.[40]

The clinical significance of this *in vitro* study of topical antimicrobial resistance is uncertain and debate persists about appropriate breakpoints for susceptibility in laboratory testing. A topical product with a widely published breakpoint is mupirocin - susceptible at MIC≤1µg/mL and high-level resistance at MIC≥256µg/mL.[18] Experts believe that only high-level mupirocin resistance may have clinical significance, and this breakpoint corresponds to an approximate 1:100 dilution of the marketed product.[27] However, initial evidence shows that low-level mupirocin resistance combined with a genotypic chlorhexidine resistance significantly increases the failure of MRSA decolonization therapy.[41] With the exception of mupirocin, breakpoints used for topical antibiotic susceptibility testing are not widely available. Neomycin and bacitracin historical breakpoints used in this study were selected to predict success in treating systemic infections at lower in vivo concentrations, not for treating superficial infections at higher concentrations.[19,20] Bacitracin employment in systemic infections proved limited early due to its high nephrotoxicity, while neomycin was withdrawn following the development of less toxic aminoglycosides.[1,20] This lack of standardization compromises topical antimicrobial resistance research and monitoring. In the absence of standardization, we considered neomycin historical MIC breakpoints and proposed an inhibition zone diameter of 16mm to define resistance by using a 30µg neomycin disk to routinely monitor neomycin resistance in laboratory centers. Due to the 100% bacitracin resistance we found, it was not possible to establish a breakpoint for the bacitracin disk diffusion routine test.

CONCLUSIONS

Our data, from a southern Brazilian sample of AD patients, showed a low prevalence of mupirocin and fusidic acid resistance of *S. aureus* atopic eczema colonizer strains. However, for neomycin and bacitracin, commonly used topical antimicrobial drugs in Brazil, high levels of resistance were found. Although based on the an-

tibiograms of local strains, our results raise an important discussion about topical antimicrobial resistance. Given the role of these drugs in decolonization and their potential use to prevent serious systemic infections due to prevalent endemic MRSA strains in any healthcare setting, further studies are necessary to determinate the efficacy and safety of antimicrobials other than mupirocin. The use of *in vitro* surveillance information and more restrictions regarding the use of these antimicrobials should lead to a decrease in resistant strains.❑

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MAILING ADDRESS:
Giancarlo Rezende Bessa
Ambulatório do Serviço de Dermatologia da UFCSPA
Rua Capitão Montanha ,27
Centro
90010-040 Porto Alegre, RS.
E-mail: gianmed@yahoo.com