ORIGINAL ARTICLE

Usefulness of *mec*-associated direct repeat unit (*dru*) typing in the epidemiological analysis of highly clonal methicillin-resistant *Staphylococcus aureus* in Scotland

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ABSTRACT

The incidence of the epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA) strains EMRSA-15 and EMRSA-16 in Scotland has increased dramatically, now accounting for *c*. 70% and *c*. 20% of isolates, respectively. Epidemiological tracking of these EMRSA strains is difficult, as *c*. 50% of EMRSA-15 and *c*. 35% of EMRSA-16 isolates are indistinguishable using pulsed-field gel electrophoresis (PFGE) and other typing methods. The usefulness of *mec*-associated direct repeat unit (*dru*) sequence analysis as a more sensitive approach to tracking the persistence and spread of these 'clonal' EMRSA strains in Scotland was evaluated. Analysis of 47 EMRSA-15 and 57 EMRSA-16 isolates (including two separately cultured isolates of the Harmony collection type strain) obtained from 22 hospital laboratories over an 8-year period (1997–2005) revealed 13 and 12 different *dru* types, respectively. Whereas some types appeared to be endemic in multiple hospitals, subtypes that may represent specific strain movement among hospitals in a given geographical region were identified in other instances. These results suggest that *mec*-associated *dru* typing may have potential for identifying and tracking specific subtypes of otherwise indistinguishable epidemic MRSA isolates such as those in Scotland.

Keywords EMRSA-15, EMRSA-16, MRSA, *Staphylococcus aureus*

INTRODUCTION

Epidemiological monitoring has identified a number of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA) strains in the UK, and two of the most important are EMRSA-15 and EMRSA-16. These strains were first reported in England in the early 1990s [1,2], and their persistence and spread has continued unabated, despite a variety of attempts at control. By the mid-1990s, these strains accounted for the majority of methicillin-resistant *S. aureus* (MRSA) in England and began to make their appearance in Scotland [3,4]. By 2001, the incidence of EMRSA-15 and EMRSA-16

in Scotland had dramatically increased to *c*. 70% and *c*. 25%, respectively, of all MRSA isolates as determined by the Scottish MRSA Reference Laboratory (SMRSARL) (Annual Report, 2000–2001; http://www.smrsarl.scot.nhs.uk/reports.asp/rpt00-01.pdf) (12th International Symposium on Staphylococci & Staphylococcal Infections, abstract P178). Pulsed-field gel electrophoresis (PFGE) is currently the most widely used molecular approach to the epidemiological analysis of problem nosocomial pathogens such as *S. aureus* [5]. However, MRSA isolates are often difficult to analyse with this method, due to their clonal nature and endemic presence in hospital environments, resulting in a limited number of different PFGE patterns. For example, *c*. 50% and *c*. 35% of Scottish EMRSA-15 and EMRSA-16 isolates, respectively, are indistinguishable using PFGE (14th European Congress of Clinical Microbiology and Infectious Diseases, abstract P582). Thus,

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although PFGE is routinely employed in the initial 'fingerprinting' of these strains, their chromosomal macrorestriction patterns are too conserved to allow further analysis, such as specific tracking of intrahospital and interhospital spread. Ryffell *et al.* [6] have identified a cluster of repeated imperfect 40-bp sequences (i.e. direct repeat units; *dru*) adjacent to IS*431* within the SCC*mec* region of *S. aureus* isolates. Although absent in a minority of MRSA isolates, when present the *dru* sequence location is constant, regardless of chromosomal SSC*mec* type. A limited number of studies have explored the use of *dru* sequences in the epidemiological analysis of MRSA, especially in the discrimination of unrelated isolates [7–9]. The utility of *mec*-associated *dru* analysis was examined in combination with PFGE for improved tracking of highly related epidemic EMRSA-15 and EMRSA-16 isolates in Scotland.

MATERIALS AND METHODS

Bacterial isolates

As summarized in Table 1, 104 isolates obtained from 22 hospital laboratories over an 8-year period (1997–2005) and previously characterized by the SMRSARL as typical EMRSA-15 or EMRSA-16 (47 and 57 isolates, respectively) were included in the study. The isolates were chosen to represent a variety of geographical locations over a multi-year period. Within each group, the selected isolates exhibited identical chromosomal macrorestriction fragment patterns when digested with the restriction enzyme *Sma*I and analysed by PFGE. For comparison, the study also included the EMRSA-15 and EMRSA-16 type strains from the Harmony collection [10].

PCR and DNA sequencing

PCR and DNA sequence analysis of the *mec*-associated *dru* region was performed using the nucleotides 5′-GTTAGCA TATTACCTCTCCTTGC-3′ and 5′-GCCGATTGTGCTTGAT GAG-3′ as forward and reverse primers, respectively. PCR was performed with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. DNA sequencing was performed using an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). For purposes of sequence comparison, the consensus *dru* sequence was defined as: ATAAGAGGTA CGTTAAAAGC AGTTCTAAGT AAAAT TGCAG [8].

dru typing nomenclature

To provide a framework, not only for this, but also for future studies, in a manner similar to that applied for staphylococcal protein A gene (*spa*) typing [11], a prefix (dr; *dru* repeat) was used, combined with numbers to identify specific 40-bp repeat sequences, whereas a different prefix (dt; *dru* type), combined

Table 1. Origin, direct repeat unit (*dru*) type and year of isolation of study isolates

Laboratory	EMRSA-15			EMRSA-16		
	dru type	Number	Year	*dru* type	Number	Year
Aberdeen	10a	2	2004	9a	1	2003
				8f	1	2005
				7c	7	2003–2005
Clydebank	6d	1	2005			
Dumfries	11f	1	2001			
	10a	2	2002–2004			
	10i	2	2002–2005			
	9e	1	1998			
Dundee	10a	1	2004	8e	1	2004
	9j	1	2005	7c	7	2003–2005
Edinburgh-1				9a	1	1998
				7c	2	2002–2003
Edinburgh-2	11g	1	2002	7c	4	2002–2005
	10a	7	1998–2005	6c	1	2004
	9d	1	1998			
Fife	10a	2	2004–2005	9a	1	2004
				7b	1	2005
				7c	1	2004
Glasgow-1	10a	2	2004–2005	9a	1	2004
				9c	1	2002
				9f	1	1997
				8d	3	2002–2005
				7c	1	2003
Glasgow-2	11a	1	1998			
	11e	1	2003			
	10a	4	1998–2003			
	10g	2	2003			
Glasgow-3				8c	1	1998
				8d	2	2002–2005
				4a	1	2002
Glasgow-4				None	1	2002
Greenock	10a	1	2004	9a	1	2004
Inverness	10a	1	2005	8f	1	2003
	8a	1	2004	7c	1	2003
Kilmarnock	10a	1	2004			
Lanarkshire-1	10a	1	2004	7c	1	2005
Lanarkshire-2				9a	1	2004
Melrose	10a	1	2005			
Oban	10a	1	1998			
Orkney				7c	2	1998–2002
Perth	10a	1	1998	7c	2	2004–2005
Stirling	10a	4	1998–2002	9a	1	1998
	10g	1	2003			
Western Isles	10a	1	2005	8b	1	2000
				6c	4	2003–2005
Harmony	10h	1		9a	2	
Total		47			57	

EMRSA, epidemic methicillin-resistant *Staphylococcus aureus*.

with numbers, was used to identify specific repeat combinations. The nomenclature was modified from that of Nahvi *et al.* [8], using ATAAGAGGTA CGTTAAAAGC AGTTCTAAGT AAAATTGCAG as the consensus. As shown in Fig. 1, the 40-bp *dru* repeat sequences observed in this study, plus sequences previously published or found in GenBank, were labelled numerically according to the number of nucleotide differences from the consensus. An additional alphabetical designation was used to indicate different locations of change (for example, dr2a and dr2b both differ from the consensus by two nucleotides but at different positions within the sequence). As shown in Table 2, different combinations of repeats observed in this study, plus sequences previously published or found in GenBank, were assigned numerical *dru* type designations based on the number of repeat sequences present, with an additional alphabetical designation to indicate different tandem arrangements of specific repeats (for example, dt8a and dt8b both contained the same eight *dru* repeats but in different arrangements).

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dr1a    * * * * * * * * *  A * * * * * * * * *   * * * * * *  * * *   * * * * * * * * * *
dr1b    * * * * * * * * *  * * * * * * * * * *   * * * * * C  * * *   * * * * * * * * * *
dr1c    * * * * * * * * G  * * * * * * * * * *   * * * * * *  * * *   * * * * * * * * * *

dr2a    * * * * * G * * *  A * * * * * * * * *   * * * * * *  * * *   * * * * * * * * * *
dr2b    * * * * * * * * *  A * * * * * * * * *   * * * * * C  * * *   * * * * * * * * * *
dr2c    * * * * * * * * G  * * * * * * * * * *   * * * * * C  * * *   * * * * * * * * * *
dr2d    * * * * * * * * T  T * * * * * * * * *   * * * * * *  * * *   * * * * * * * * * *
dr2e    * * * * * * * * *  * * * * * * * * * C   * * * * * C  * * *   * * * * * * * * * *
dr2f    * * * * * * * * *  * * * * * * * * * *   * A * C * *  * * *   * * * * * * * * * *
dr2g    * * * * * * * * *  * * * * * * * * * *   * * * * * *  G * C   * * * * * * * * * *
dr2h    * * * * * * * * G  * * * * * * * * * *   * * * * * *  * * *   * * * * * * * T *

dr3a    * * * * * G * * *  A * * * * * * * * *   * * * * * C  * * *   * * * * * * * * * *
dr3b    * * * * * * * * G  * * * * * * * * * *   * * * * * C  * * *   * * * * * * * T *
dr3c    * * * * * * * * T  T * * * * * * * * *   * * * * * C  * * *   * * * * * * * * * *
dr3d    * * * * * * * * T  T * * * * * * * * *   * A * * * *  * * *   * * * * * * * * * *
dr3e    * * * * * * * * *  A * * * * * * * * *   * A * C * *  * * *   * * * * * * * * * *
dr3f    * * * * * * * * *  A * * * * * * * * *   * * * * * C  * * *   * * * * * A * * *
dr3g    * * * * * * * * G  * * * * * * * * * *   * A * C * *  * * *   * * * * * * * * * *
dr3h    * * * * * * * * T  T * * * * * * A *     * * * * * *  * * *   * * * * * * * * * *
dr3i    * * * * * * * * T  T * * * * * * * * *   * * * * * *  * * *   * * * * * A * * *
dr3j    * * * * * * * * *  * * * * * * * * * *   * * * * * C  T * *   * * * * * * * T *
dr3k    * * * * * G * * *  A * * * * * * * * *   * * * C * *  * * *   * * * * * * * * * *

dr4a    * * * * * * * * T  T * * * * * * * * *   * * * * * C  * * *   * * * * * * A * * *
dr4b    * * * * * * * * T  T * * * * * * * * *   * * * * * C  * * *   * * * * * * * T *
dr4c    * * * * * * * * T  T * * * * * * * * *   * * * * * *  G * C   * * * * * * * * * *
dr4d    * * * * * * * * T  T * * * * * * * * *   * A * C * *  * * *   * * * * * * * * * *
dr4e    * * * * * G * * *  A * * * * * * * * *   * A * C * *  * * *   * * * * * * * * *
dr4f    * * * * * * * * *  * * * * * * * * * *   * A * * * C  * T * C * * * * * * * * *

dr5a    * * * * * * * A *  T A G * * * * * * *   * A * * * *  * * *   * * * * * * * * *
dr5b    * * * * * * * * *  * * * * * * * * * *   * A * * * C  * T * C * * * * * * * T *

dr6a    * * * * * * * A *  T A G * * * * * * *   * A * * * *  * * *   * * * * * C * * *

dr7a    * * * * * * * * T  T * * * * * * * * *   * A * * * C  * T * C * * * * * * * T *

dr0     A T A A G A G G T A   C G T T A A A A G C   A G T T C T A A G T   A A A A T T G C A G
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Fig. 1. Direct repeat unit (*dru*) repeat sequence variations in comparison to the consensus sequence (asterisks mark agreement with consensus; dr0). Conserved nucleotide positions [8] are in bold and underlined. Sequences dr3f, dr3g and dr4d were found in this study. The remainder were from Nahvi *et al.* [8], except for dr1a [14], dr2h [15], dr3h [9], dr3i [16], dr3j, dr3k, and dr4f [17].

RESULTS

As noted above, the 104 isolates examined in this study were nearly equally divided between EM-RSA-15 and EMRSA-16 types obtained from a variety of hospital locations within Scotland over a 9-year period (Table 1). Although isolate-specific clinical information was not available, the study goal was to determine whether *dru* typing might assist in differentiating isolates within these otherwise indistinguishable EMRSA groups. As shown in Fig. 1, a total of 33 *dru* repeat sequences, most from Nahvi *et al.* [8], along with three previously unreported repeat sequences from this study (dr3f, dr3g, and dr4d),

were available for *dru* type analysis. The repeat sequences exhibited a range of from one to seven nucleotide changes from the consensus. Table 2 summarizes the different repeat sequence combinations, which constituted 58 available *dru* types. Of these, 25 were found in this study, 20 of which were previously unreported.

EMRSA-15 *dru* types

As shown in Table 1, the 47 EMRSA-15 isolates (46 from Scotland plus the Harmony EMRSA-15 type strain) exhibited 13 *dru* types containing six, eight, nine, ten or 11 *dru* repeats. The largest group (dt10a) was present throughout the

Table 2. Order of different 40-bp repeats in direct repeat unit (*dru*) types[a]

dru types	Order of *dru* repeats												Source
dt12a	5a	2d	2d	4a	0	2d	5b	3a	2g	2h	4e	3e	[15][b]
dt12b	5a	2d	2d	4a	2d	2d	5b	3a	2g	2h	4e	3e	[18][c]
dt12c	5a	2d	4a	0	2d	3j	3k	2g	3b	4e	3e	3e	[17][d]
dt11a	5a	2d	4a	0	2d	5b	3a	2g	3b	4e	3e		This study, [8]
dt11b	5a	2d	4a	0	2d	5b	2a	4c	3b	4e	3e		[8]
dt11c	5a	2d	4a	0	2d	5b	3a	2g	4b	4e	3e		[8]
dt11d	5a	2d	4a	0	3c	7a	3a	2g	3b	4e	3e		[8]
dt11e	5a	2d	4a	0	2d	5b	3a	2g	3b	3a	2g		This study
dt11f	5a	2d	4a	0	2d	2d	4f	3a	2g	3b	4e		This study
dt11g	5a	2d	4a	0	2d	2d	5b	3a	2g	3b	4e		This study
dt11h	5a	2d	4a	0	3h	5b	3a	2g	3b	4e	3e		[9]
dt11i	5a	2d	4a	0	2d	5b	3a	2g	1c	4e	3e		[14][e]
dt10a	5a	2d	4a	0	2d	5b	3a	2g	3b	4e			This study, [6], [8], [13][f]
dt10b	3d	2d	4a	0	2d	5b	3a	2g	3b	4e			[8]
dt10c	5a	2d	4a	0	2d	1b	2g	3b	4e	3e			[8]
dt10d	5a	2d	4a	0	2d	2b	2g	3b	4e	3e			[8]
dt10e	5a	2d	4a	2d	2d	5b	3a	2g	3b	4e			[8], [19]
dt10f	5a	2d	2d	2d	4a	0	2g	3b	4e	3e			[8]
dt10g	5a	2d	4a	0	2d	5b	2a	2g	3b	4e			This study[f]
dt10h	5a	2d	4a	0	2d	5b	3a	2g	4b	4e			This study
dt10i	5a	2d	4a	0	2d	4f	3a	2g	3b	4e			This study
dt10j	5a	2d	4a	0	2d	7a	3a	2g	3b	4e			[f]
dt10k	5a	2d	4a	0	3h	5b	3a	2g	3b	4e			[9]
dt10l	5a	2d	4a	0	2d	4f	3b	4e	3e	3e			[17][g]
dt10m	5a	2d	3i	0	2d	5b	3a	2g	3b	4e			[16][h]
dt9a	5a	2d	2d	4a	0	2g	3b	4e	3e				This study, [8]
dt9b	5a	2d	2d	4a	0	2g	2c	4e	3e				[8]
dt9c	5a	2d	2d	3f	0	2g	3b	4e	3e				This study
dt9d	5a	2d	4a	0	5b	3a	2g	3b	4e				This study
dt9e	2d	4a	0	2d	4f	3a	2g	3b	4e				This study
dt9f	5a	3c	2d	4a	0	2g	3b	4e	3e				This study
dt9g	5a	4a	0	2d	5b	3a	2g	3b	4e				[12]
dt9h	5a	4a	0	2d	0	2d	5b	3a	2f				[12]
dt9i	5a	4a	0	3c	5b	3a	2g	3b	4e				[i]
dt9j	5a	2d	4a	0	2d	5b	3a	2g	3b				This study
dt9k	5a	2d	4a	3h	5b	3a	2g	3b	4e				[9]
dt8a	5a	2d	4a	0	2d	2g	3b	4e					This study, [8]
dt8b	5a	2d	2d	4a	0	2g	3b	4e					This study
dt8c	5a	2d	2d	4a	0	2g	3g	3e					This study
dt8d	5a	2d	2d	4a	0	3e	3e	3e					This study
dt8e	5a	2d	2d	2d	4a	0	3e	3e					This study
dt8f	5a	2d	4a	0	2g	3b	4e	3e					This study
dt8g	5a	2d	4a	0	3h	2g	3b	4e					[9]
dt7a	5a	2d	4a	0	2d	5b	3[a]						[8]
dt7b	5a	1a	2d	4a	0	3e	3e						This study, [8], [20][j]
dt7c	5a	2d	2d	4a	0	3e	3e						This study
dt7d	5a	4a	0	2d	2g	3b	4e						[i]
dt7e	5a	4a	0	2d	2g	2c	4e						[i]
dt7f	5a	2d	5b	3a	2g	3b	4e						[21][k]
dt6a	5a	2d	2d	2g	3b	4e							[8]
dt6b	6a	2e	2g	3b	4e	2f							[8]
dt6c	5a	2d	2d	4a	0	3e							This study
dt6d	5a	2d	3c	2g	3b	4e							This study
dt6e	5a	7a	3a	2g	3b	4e							[f]
dt6f	6a	2e	2g	3b	4e	3e							[7]
dt6g	5a	2d	4a	0	2d	2f							[22][l]
dt5a	5a	2d	2g	3b	4e								[9]
dt4a	5a	2d	2d	4d									This study

EMRSA, epidemic methicillin-resistant *Staphylococcus aureus*.
[a]*dru* types were assigned to different combinations of repeat sequences observed in this study plus previously published sequences as indicated.
[b]GenBank accession number AY894415.
[c]GenBank accession number AB353125.
[d]GenBank accession number AF142103.
[e]GenBank accession number AM292304.
[f]17th European Congress of Clinical Microbiology and Infectious Diseases, abstract P1306.
[g]GenBank accession number AF142101.
[h]GenBank accession number AB245470.
[i]17th European Congress of Clinical Microbiology and Infectious Diseases, abstract P1573.
[j]*S. aureus* strain 252 (EMRSA-16); GenBank accession number BX571856.
[k]*S. aureus* strain MW2; GenBank accession number NC_003923.
[l]GenBank accession number AY271717.

country from 1998 to 2005, and contained 32 of the 46 Scottish isolates (70%), consistent with the clonal nature of this epidemic strain. However, 12 additional minor *dru* types were also observed, including the Harmony strain, which was the only dt10h isolate in the dataset. These minor *dru*

types were found in a limited number of isolates from geographically distinct hospitals. The one exception to this observation (dt10g) was interesting, with isolates found not only in Glasgow but also in nearby Stirling, potentially indicating interhospital spread.

EMRSA-16 *dru* types

The 57 EMRSA-16 isolates (55 from Scotland plus two separately cultured isolates of the Harmony EMRSA-16 type strain) exhibited 12 *dru* types containing four, six, seven, eight or nine *dru* repeats. Similar to EMRSA-15, the largest EMRSA-16 group (dt7c) was also distributed throughout the country over the multi-year period and contained 28 of the 55 (51%) Scottish isolates examined. However, unlike EMRSA-15, the EMRSA-16 isolates exhibited additional smaller multi-hospital clusters, with dt9a, dt8d and dt6c containing 13%, 5% and 5% of Scottish isolates, respectively. Interestingly, the two cultures of the Harmony EMRSA-16 isolate, obtained from separate sources, were both also dt9a, whereas the sequenced EMRSA-16 strain 252 was dt7b (Table 2). As with EMRSA-15, the eight remaining minor *dru* types were found in geographically distinct hospitals, with the exception of dt8f, which was found in both Aberdeen and Inverness, again raising the possibility of spread among hospitals.

DISCUSSION

The problem faced by the SMRSARL and similar institutions in other countries is typified by the isolates examined in this study, for which, aside from analyses leading to their EMRSA designation, no test is available for differentiation, especially with regard to epidemiological tracking. This study was not designed to assess potential correlations between *dru* types and isolate epidemiology, as specific clinical information was not available. However, the goal was to determine, by general survey, whether *dru* analysis might yield subtypes with the potential for future epidemiological application. In this regard, the results appear promising. Although this study was not specifically designed to examine specific *dru* type stability over time, the fact that dt9a was found in two independently obtained EMRSA-16 isolates from the Harmony collection supports the notion

that *dru* sequences are stable enough to have strain-associated significance. Both EMRSA-15 and EMRSA-16 isolates were of primary *dru* types, which would be expected, given the length of time for which these strains (and perhaps specific subtypes) have been present in Scotland. However, both also exhibited numerous minor *dru* types, which might be epidemiologically useful in appropriate future investigational settings, given their low incidence and apparent geographical restriction. The fact that some minor *dru* types were found in the oldest isolates (e.g. EMRSA-15 dt11a, dt9d and dt9e, and EMRSA-16 dt8c) also raises interesting questions as to why these subtypes have not spread and increased in predominance. Thus, at the very least, *dru* typing appears to provide a measure of discrimination that could prove useful in identifying and monitoring the persistence and spread of at least some subtypes of these highly uniform strains. In this regard, even isolates such as the single EMRSA-16 lacking the *dru* region are informative, as they represent a distinct identifiable strain type.

Beyond this potential epidemiological usefulness, *dru* analysis revealed interesting differences between the EMRSA-15 and EMRSA-16 isolates. Although obviously influenced by our specific isolate dataset, EMRSA-15 *dru* types tended to be more clustered than EMRSA-16 (i.e. one vs. several predominant *dru* types, respectively). In addition, EMRSA-15 *dru* types generally contained larger numbers of repeats (i.e. primarily ten; range 6–11) than EMRSA-16, which, in this study, never contained more than nine (with a range down to as few as four). In addition, whereas the EMRSA-15 and EMRSA-16 isolates both exhibited *dru* types containing nine, eight or six repeats, no instances of shared *dru* types were found. It would be interesting in future studies to explore how these observations might further relate to EMRSA strain and perhaps SCC*mec* differences (for example, EMRSA-15 and EMRSA-16 strains carry SCC*mec* type IV and type II, respectively). In this regard, it is interesting to note that whereas EMRSA-15 isolates exhibited a variety of *dru* types, the well-known community-associated USA300 MRSA strain (also SCC*mec* type IV) appears to be highly conserved with respect to dt9g [12], a *dru* type not observed in these EMRSA-15 isolates, and the common European community-associated ST80-MRSA-IV strain appears to be conserved with respect to dt10a

[13], which we found to be the major *dru* type in Scottish EMRSA-15. However, *dru* types may not be SCC*mec*-specific, as they are known to be shared between MRSA and methicilllin-resistant coagulase-negative staphylococci [8]. Nevertheless, taken together, these results suggest *mec*-associated *dru* analysis as an approach worth further study with regard to potential usefulness in the epidemiological analysis of highly clonal EMRSA isolates for which, at the moment, no other means of subtyping are available and, perhaps additionally, as an internal 'marker' in studies specifically regarding SCC*mec*.

TRANSPARENCY DECLARATION

The authors declare that they have no conflicting interests in relation to this work.

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