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Direct Repeat Unit (*dru*) Typing of Methicillin-Resistant *Staphylococcus pseudintermedius* from Dogs and Cats

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Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has emerged in a remarkable manner as an important problem in dogs and cats. However, limited molecular epidemiological information is available. The aims of this study were to apply direct repeat unit (*dru*) typing in a large collection of well-characterized MRSP isolates and to use *dru* typing to analyze a collection of previously uncharacterized MRSP isolates. Two collections of MRSP isolates from dogs and cats were included in this study. The first collection comprised 115 well-characterized MRSP isolates from North America and Europe. The data for these isolates included multilocus sequence typing (MLST) and staphylococcal protein A gene (*spa*) typing results as well as SmaI macrorestriction patterns after pulsed-field gel electrophoresis (PFGE). The second collection was a convenience sample of 360 isolates from North America. The *dru* region was amplified by PCR, sequenced, and analyzed. For the first collection, the discriminatory indices of the typing methods were calculated. All isolates were successfully *dru* typed. The discriminatory power for *dru* typing ($D = 0.423$) was comparable to that of *spa* typing ($D = 0.445$) and of MLST ($D = 0.417$) in the first collection. Occasionally, *dru* typing was able to further discriminate between isolates that shared the same *spa* type. Among all 475 isolates, 26 different *dru* types were identified, with 2 predominant types (dt9a and dt11a) among 349 (73.4%) isolates. The results of this study underline that *dru* typing is a useful tool for MRSP typing, being an objective, standardized, sequence-based method that is relatively cost-efficient and easy to perform.

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a canine-adapted, multidrug-resistant opportunistic pathogen that has emerged and disseminated internationally in recent years (1–3). First reported in the mid 1990s as methicillin-resistant *Staphylococcus intermedius* (4), MRSP is now a leading cause of opportunistic infections, such as pyoderma, otitis, and surgical site infections, in dogs in many geographical regions (5–7).

To date, only limited molecular epidemiological information is available. For MRSP, different typing methods have been used (1, 8), with no consensus approach. Ideally, a sequence-based method that can be standardized across laboratories and that is discriminatory, repeatable, objective, economically viable, and practical would be used. Multilocus sequence typing (MLST) of *S. pseudintermedius* (9) is sometimes used, as it is standardized and provides good discriminatory power, but it is costly and time consuming. Pulsed-field gel electrophoresis (PFGE) (1, 10) is a pattern-based method that not only requires expensive equipment and laboratory skills but also has limitations in interlaboratory comparison and throughput. Sequence analysis of the X region of the staphylococcal protein A gene (*spa* typing) (11, 12) has advantages in objectivity and interlaboratory comparison, yet there are problems with its ability to type all isolates and with the current lack of an automated system to interpret sequences. More recently, sequence analysis of the direct repeat unit (*dru*), a variable-number-of-tandem-repeats region, which consists of mostly 40-bp *dru* repeats and is located downstream of the *mecA* gene and adjacent to IS*431* in SCC*mec* elements of methicillin-resistant staphylococci, has been described (13). This sequence-based method has advantages of ease of performance, low cost, interlaboratory reproducibility, objectivity, automated sequence analysis, and no need for reference strains. Therefore, it could be a useful tool for typing of MRSP.

The objectives of this study were to test the usefulness of *dru* typing of MRSP by application of this method to a well-characterized collection of MRSP isolates and to use *dru* typing for the comparative analysis of a larger collection of uncharacterized MRSP isolates.

MATERIALS AND METHODS

Bacterial isolates. Two test collections were used for this study. The first collection to investigate whether *dru* typing is a suitable typing method for MRSP comprised 115 well-characterized MRSP isolates from dogs ($n = 103$) and cats ($n = 12$). These 115 MRSP isolates from North America and Europe had previously been characterized by SCC*mec* typing, MLST, *spa* typing, and PFGE of SmaI-digested whole-cell DNA (1, 2). The second collection was a convenience sample of 360 MRSP isolates from Canada ($n = 287$) or the United States ($n = 73$). They originated from dogs ($n = 352$) or cats ($n = 8$) and were from colonizations ($n = 198$; mainly rectal [$n = 98$] or nasal [$n = 94$] swabs) or from infections ($n = 162$; mainly skin infections [$n = 105$]).

Methods. Isolates were characterized by *dru* typing, as previously described (13, 14). In brief, performance of *dru* typing is similar to that of *spa* typing, with PCR amplification of the variable *dru* region followed by sequence analysis of the *dru* amplicon and identification of the *dru* repeat order. The *dru* repeat order determines the *dru* type. A *dru* database (http:

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TABLE 1 Characteristics of collection 1 of canine and feline methicillin-resistant *Staphylococcus pseudintermedius* isolates ($n = 115$)

dru type	*dru* cluster	No. of isolates	SCC*mec* type	MLST	*spa* type	SmaI pattern (PFGE)
dt8u[a]		1	*ccrA2* and *ccrB2* + *ccrA4* and *ccrB4* + *mec* complex B	ST69	t07	A
dt8f	9a	1	II/III	ST71	t02	R
dt9a	9a	77	II/III	ST71	t02	G ($n = 3$), H, J ($n = 66$)[b], K ($n = 2$), L, M, N ($n = 2$)[b], O[b]
dt9a	9a	2	II/III	ST71	t03	J
dt9a	9a	1	II/III	ST71	t05	J
dt9a	9a	2	II/III	ST71	t06	J
dt9a	9a	1	II/III	ST71	t06	M
dt9a	9a	1	II/III	ST106	t02	Nontypeable
dt9a	9a	1	II/III	ST118	t02	J
dt9a	9a	1	V	ST115	t021	E
dt9b	9a	1	II/III	ST71	t02	J
dt10h	11a	1	IV	ST106	t02	U
dt10h	11a	1	IV	ST111	t05	U
dt10h	11a	1	IV	ST112	t25	Q
dt10h	11a	1	IV	ST113	t06	D
dt10h	11a	1	IV	ST116	t02	W
dt10ai[a]	11a	1	IVa	ST71	t02	H
dt11a	11a	13	V	ST68	t06	C
dt11a	11a	3	VII-241	ST58	t06	F
dt11v	11a	1	II/III	ST114	t06	V
dt11y[a]	11a	1	*ccrA1* and *ccrB1* + *mec* complex A	ST5	t05	S
dt11z	11a	1	VII-241	ST73	t24	S
dt11af[a]	11a	1	V	ST100	t23	B[b]

[a] These were novel *dru* types, detected for the first time within this study.
[b] The feline MRSP isolates belonged to these types, with nine isolates showing II-III(SCC*mec*)-ST71(MLST)-t02(*spa* type)-J(SmaI-PFGE) and single isolates with the same characteristics but displaying SmaI patterns L, M, N, and O.

//dru-typing.org) was established and enables comparisons with the *dru* sequences and *dru* types stored in this database.

The discriminatory indices for the different typing methods used for the first collection of isolates were calculated as previously described (15). A minimum spanning tree (MST) was generated using BioNumerics v6.6 (Applied Maths, Austin, TX, USA) and the tandem-repeat sequence typing (TRST) plugin. Distance intervals were created using a bin distance of 1.0%. The *dru* types separated by a MST distance of ≤2 repeats (>98.5% similarity) were considered closely related and assigned to the same cluster. The root node was assigned to the multilocus sequence type (ST) with the greatest number of isolates. Descriptive statistics were applied. Categorical comparisons were performed using Fisher's exact test or the chi-square test. A P value of <0.05 was considered significant.

RESULTS

MRSP collection 1. All 115 isolates were successfully *dru* typed, and 11 different *dru* types were identified: dt8u, dt8f, dt9a, dt9b, dt10h, dt10ai, dt11a, dt11v, dt11y, dt11z, and dt11af. Among them, four novel *dru* types (dt8u, dt10ai, dt11y and dt11af) were detected and added to the *dru* typing database (Table 1). In addition, dt10ai harbored two novel *dru* repeats. The *dru* types dt9a ($n = 86$; 74.8%) and dt11a ($n = 16$; 13.9%) were most common. The *dru* type dt9a was mainly detected in isolates from Europe with the characteristics II-III(SCC*mec*)-ST71(MLST)-t02(*spa* type)-J(SmaI-PFGE) ($n = 66$; 57.4%)—representing the European clone—but also were detected in single isolates with other MLST types (ST106, ST118) or *spa* types (t03, t05, t06) or PFGE patterns (G, K, H, L, M, N, O) as well as in one isolate with V-ST115-t021-E (1, 2). Single isolates belonging to the most common clone, II-III-ST71-t02-J, harbored *dru* types dt8f or dt9b. In contrast, all 13 isolates belonging to the North American clone with V-ST68-t06-C harbored dt11a. This *dru* type was only present in three additional isolates, all of which were VII-241-ST58-t06-F. The *dru* type dt10h was present in five isolates with SCC*mec* type IV elements and with different MLST, *spa*, and PFGE types (ST106-t02-U, ST111-t05-U, ST112-t25-Q, ST113-t06-D, ST116-t02-W). The remaining *dru* types were present only in single isolates with individual characteristics. The 12 MRSP isolates from cats belonged to the European clone with *dru* type dt9a ($n = 9$) or had only other PFGE types (N, O; $n = 1$ each), with the same characteristics (II-III-ST71-t02-dt9a) (2). A single feline isolate with V-ST100-t23-B had the novel *dru* type dt11af. The remaining three novel types were found in single isolates with individual characteristics, including two nontypeable SCC*mec* cassettes (*ccrA1* and *ccrB1* + *mec* type A and *ccrA2* and *ccrB2* + *ccrA4* and *ccrB4* + *mec* type B) (Table 1).

Two main *dru* clusters were identified and were designated clusters 9a and 11a, as dt9a and dt11a, respectively, formed the root nodes. Cluster 9a consisted of the types dt9a, dt9b, and dt8f, whereas six *dru* types belonged to the cluster 11a (Table 1). There was a significant association between *dru* cluster and MLST type; ST71 accounted for 85 of 88 (95.6%) cluster 9a isolates and only 1 of 27 (3.7%) noncluster 9a isolates ($P < 0.0001$). This single ST71 isolate with *dru* type dt10ai belonged to cluster 11a. There was more vari-

TABLE 2 Distribution of *dru* types among all canine and feline methicillin-resistant *Staphylococcus pseudintermedius* isolates ($n = 475$)

dru type	Repeats	*dru* cluster	No. by collection		Location							
					Europe		United States		Canada		Total	
			1	2	No.	%	No.	%	No.	%	No.	%
dt2e	5a-2d			2					2	0.7	2	0.4
dt5i	5a-3c-4b-4e-3e			3					3	1.0	3	0.6
dt6r	5a-2d-2g-3b-4e-3e			1					1	0.3	1	0.2
dt7d	5a-4a-0-2d-2g-3b-4e			1					1	0.3	1	0.2
dt7l	5a-5b-3a-2g-3b-4e-3e			3					3	1.0	3	0.6
dt8f	5a-2d-4a-0-2g-3b-4e-3e	9a	1	1			1	1.1	1	0.3	2	0.4
dt8u	5a-2d-4a-0-3c-2g-3b-3q		1		1	1.1					1	0.2
dt9a	5a-2d-2d-4a-0-2g-3b-4e-3e	9a	86	156	82	91.1	23	24.7	137	46.9	242	50.9
dt9b	5a-2d-2d-4a-0-2g-2c-4e-3e	9a	1		1	1.1					1	0.2
dt9au	5a-2d-4a-0-2d-5b-2a-2g-3g			1					1	0.3	1	0.2
dt10a	5a-2d-4a-0-2d-5b-3a-2g-3b-4e	11a		10			2	2.2	8	2.7	10	2.1
dt10h	5a-2d-4a-0-2d-5b-3a-2g-4b-4e	11a	5	55	2	2.2	9	9.7	49	16.8	60	12.6
dt10ai	5a-2d-4a-0-2d-5b-2l-2g-3b-3q	11a	1		1	1.1					1	0.2
dt10ak	5a-2d-4a-0-5b-3a-2g-3b-4e-3e	11a		1					1	0.3	1	0.2
dt10bm	5a-2d-4a-1d-2a-5b-3a-2g-4b-4e	11a		1			1	1.1			1	0.2
dt11a	5a-2d-4a-0-2d-5b-3a-2g-3b-4e-3e	11a	16	91			46	49.5	61	20.9	107	22.5
dt11b	5a-2d-4a-0-2d-5b-2a-4c-3b-4e-3e	11a		1			1	1.1			1	0.2
dt11o	5a-2d-4a-0-2d-5b-3a-2g-3b-4e-4e	11a		1			1	1.1			1	0.2
dt11v	5a-2d-4a-0-3c-5b-3a-2g-3b-4e-3e	11a	1	2	1	1.1			2	0.7	3	0.6
dt11y	5a-2d-4a-1b-2d-5b-3a-2g-3b-4e-3e	11a	1	2	1	1.1			2	0.7	3	0.6
dt11z	5a-2d-3i-0-2d-5b-3a-2g-3b-4e-3e	11a	1		1	1.1					1	0.2
dt11af	5a-2d-4a-0-2d-5b-2a-2g-3b-4e-3e	11a	1	22			8	8.6	15	5.1	23	4.8
dt11av	2d-4a-0-2d-5b-3a-2g-3n-4e-3e	11a		1			1	1.1			1	0.2
dt11ax	5a-2d-4a-0-2d-6f-3a-2g-3b-4e-3e	11a		1					1	0.3	1	0.2
dt11bn	5a-2d-4a-1f-2d-5b-2a-2g-3b-4e-3e	11a		3					3	1.0	3	0.6
dt11bo	5a-2d-4a-0-2d-0-2d-2g-3b-4e-3e	11a		1					1	0.3	1	0.2

ability within cluster 11a, but there was a significant association with ST68, as ST68 accounted for 13 of 26 (50.0%) cluster 11a isolates and for no noncluster 11a isolates ($P < 0.0001$).

The discriminatory power of *dru* typing was 0.423, which means that two randomly selected isolates from this test population could be assigned to different *dru* types with a probability of 42.3%. In comparison, the discriminatory indices of the other sequence-based typing methods were 0.445 for *spa* typing and 0.417 for MLST. In contrast, SCC*mec* typing had a discriminatory power of 0.397, and SmaI digestion with subsequent PFGE, 0.635.

MRSP collection 2. Among the isolates collected in North America from cats ($n = 8$) or dogs ($n = 352$), 22 different *dru* types were identified (Table 2). The *dru* types dt9a ($n = 156$; 43.3%) and dt11a ($n = 91$; 25.3%) were most frequent, and dt10h ($n = 55$; 15.3%), dt11af ($n = 22$; 6.1%), and dt10a ($n = 10$; 2.8%) accounted for other relatively common types. In contrast, few isolates had dt5i ($n = 3$), dt7l ($n = 3$), dt11bn ($n = 3$), dt2e ($n = 2$), dt11y ($n = 2$), or dt11v ($n = 2$); the remaining 11 *dru* types (dt6r, dt7d, dt8f, dt9au, dt10ak, dt10bm, dt11av, dt11ax, dt11b, dt11bo, and dt11o) were detected only in single isolates.

Combined collections. When looking at isolates from both collections, 26 different *dru* types were identified, with two predominant types (dt9a and dt11a) accounting for 349 (73.4%) of the 475 isolates. The two *dru* clusters, 9a and 11a, accounted for 460 (96.9%) isolates (Fig. 1). There were significant differences in the distribution of *dru* types between regions, with cluster 9a predominating in Europe, cluster 11a predominating in the United States, and a similar distribution of the two clusters in Canada (Fig. 2). The second-most-common *dru* type in cluster 11a was dt10h, a *dru* type that was overrepresented in Canada. Forty-nine of 60 (81.7%) dt10h isolates were from Canada, with nine from the United States and two from Europe. The prevalence of this *dru* type in Canada (49 of 292; 16.8%) far exceeded that elsewhere (11 of 183; 6.0%; $P < 0.001$). Except for a single *dru* type (dt9au), the major *dru* types seen in canine isolates, dt11a and dt9a, were also present among the feline MRSP isolates ($n = 5$ and $n = 2$, respectively). No significant differences between the *dru* types found in dogs versus cats were detectable ($P = 0.17$).

DISCUSSION

Molecular typing is an important tool for studying the emergence and dissemination of pathogens, from local to international levels. Various typing methods are available for bacterial pathogens, with differences in discriminatory power, reproducibility, cost, ease of performance, and objectivity. An ideal method would be a rapid, low-cost method that is able to type all isolates, provides repeatable results, is objective (e.g., does not rely on visual assessment of DNA fragment patterns), and allows multiple laboratories to generate the same results without the need to exchange reference strains. Based on the results of this study, *dru* typing appears to be a convenient, objective, and discriminatory typing method for MRSP. All isolates were successfully typed, allowing for good discriminatory power, with 26 different *dru* types identified in the entire study population.

Although much higher discriminatory indices (*D*) were recommended by Hunter and Gaston (15), the comparison of results

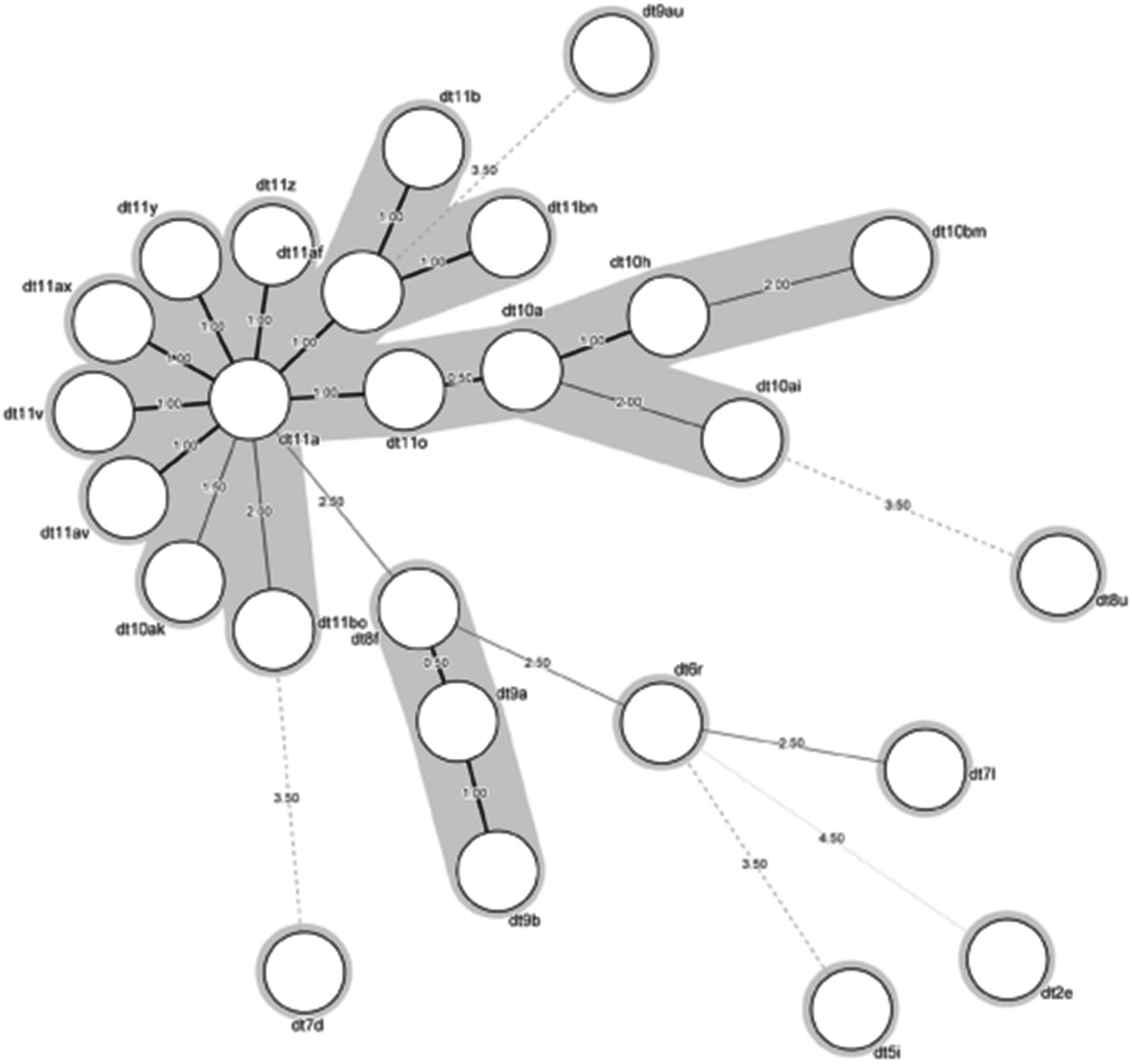

FIG 1 Minimum spanning tree (MST) of the 26 *dru* types identified in methicillin-resistant *Staphylococcus pseudintermedius* from dogs and cats ($n = 475$).

with other methods is important both to compare discriminatory power and to identify discrepancies that might indicate the potential for misleading results. This is of particular concern as the target of *dru* typing is part of a mobile genetic element, although the mobile element in this case (SCC*mec*) does not actually appear to be highly mobile given its large size and the apparent clonal dissemination of MRSP. In this study, the *dru* type dt9a was identified mainly with SCC*mec* type II-III and in a single MRSP isolate with SCC*mec* type V, whereas the same *dru* type was reported to be present with SCC*mec* type V in *Staphylococcus aureus* (16). In addition, dt11a was present solely in methicillin-resistant *S. aureus* with SCC*mec* type V (17, 18); while it was predominantly found in MRSP with SCC*mec* type V, it was also present in three MRSP isolates with SCC*mec* type VII-241.

The presence of two main clones is consistent with recent reports (1-3, 19), and, although there were clear differences in the regional distribution, data indicate that designating separate North American and European clones (1) may not be completely accurate. While these data indicate that the ST71-associated 9a cluster is dominant among European isolates, the ST68-associated 11a cluster was not equally dominant in North America. Indeed, clusters 11a and 9a were equally prevalent in Canada, with the Canadian distribution representing a hybrid of American-dominant and European-dominant strains. Considering the widespread distribution of the ST71-associated *dru* cluster 9a, it may be more appropriately termed an international clone rather than a European clone.

The difference between Canadian and American isolates is in-

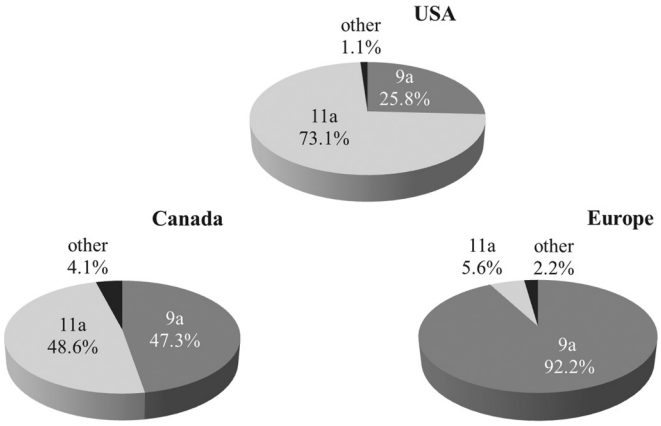

FIG 2 Assignment to the two main *dru* clusters among the methicillin-resistant *Staphylococcus pseudintermedius* isolates from Canada ($n = 292$), the United States ($n = 93$), and Europe ($n = 90$).

teresting, and more differences are noted when *dru* types within cluster 11a are evaluated individually, based on the commonness of dt10h (49 of 292; 16.8%) and the relatively low prevalence of dt11a (61 of 292; 20.9%) in Canada compared to the United States (46 of 93; 49.5%). Type dt10h is somewhat of an outlier in cluster 11a, being only approximately 97.5% related to dt11a and the majority of cluster 11a *dru* types, and one could argue that dt10h, along with dt10a and dt11o, should be classified as a separate group. If more discriminatory MST settings are applied (e.g., MST distance of 1) or if the population is assessed without the intermediary dt10a/dt11o group, dt10h is removed from the 11a cluster. The variable MLST results from the small number of dt10h isolates that were tested also suggest that this type could be considered separate from the 11a cluster.

It is interesting to see how widely the *dru* cluster 9a was disseminated among isolates from Canada, the United States, Denmark, Germany, Switzerland, Italy, the Netherlands, and Sweden. While less widely distributed, isolates of the *dru* cluster 11a were found in Canada, the United States, Denmark, the Netherlands, Germany, and Switzerland, and, with a recent report, from Australia (20). The apparent greater genetic variability among cluster 11a isolates than cluster 9a isolates was interesting, with 13 different *dru* types among 207 cluster 11a isolates from six countries compared to only three *dru* types among 246 cluster 9a isolates from eight countries. The evolutionary biology of MRSP has received limited investigation, but a broader study of the population structure of *S. pseudintermedius* indicated that ST71 resided within a group of linked STs, while ST68 was a singleton not closely related to any other ST (9). This suggests that ST68 may be a rather distinct and perhaps more genetically stable type compared to ST71, although a broader study of the population structure and evolutionary biology of MRSP is needed.

While MLST was only performed on a subset of isolates, there was good agreement between *dru* typing and MLST. To determine the role of *dru* typing, broader studies incorporating epidemiological data need to be performed. However, this study shows that *dru* typing may be a potentially useful tool for MRSP typing, being an objective, standardized, sequence-based method that is relatively easy and cost-effective to perform.

This study is subject to some limitations. A large international collection of isolates was studied, but, nonetheless, this is a population of convenience, and it is unclear whether it truly represents the broader population. Only a subset of the additional North American isolates was typed by MLST; however, adequate numbers were evaluated to demonstrate an association between ST68 and *dru* cluster 11a, and between ST71 and *dru* cluster 9a.

Conclusion. The critical importance of MRSP as a canine pathogen, its apparently rapid international emergence, and the potential (albeit perhaps low) for zoonotic transmission (21–23) indicate a need for good molecular epidemiological studies at local, regional, and international levels. This requires a rapid, objective, high-throughput, and cost-effective approach that can be applied in different laboratories without the need to exchange reference strains. The combination of the ability to successfully type all isolates, good discriminatory power, relative ease in performance, low cost, and objectivity (as a sequence-based method) makes *dru* typing a potentially useful tool for MRSP research.

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