Received: 5 July 2023 | Revised: 3 November 2023 | Accepted: 21 November 2023

DOI: 10.1002/vetr.3714

Vet Record

ORIGINAL RESEARCH

Intervention with impact: Reduced isolation of methicillin-resistant *Staphylococcus pseudintermedius* from dogs following the introduction of antimicrobial prescribing legislation in Germany

Anette Loeffler[1] | **Lee Beever**[2] | **Yu-Mei Chang**[3] | **Babette Klein**[4] | **Veit Kostka**[5] | **Cornelia Meyer**[5] | **Elisabeth Müller**[4] | **Jessica Weis**[6] | **Brett Wildermuth**[7] | **John Fishwick**[8] | **David H. Lloyd**[1]

[1]Department of Clinical Services and Science, Royal Veterinary College, Hatfield, UK

[2]School of Veterinary Science, Massey University, Palmerston North, New Zealand

[3]Department of Comparative Biomedical Sciences, Royal Veterinary College, Hatfield, UK

[4]Laboklin, Bad Kissingen, Germany

[5]SYNLAB Vet, Augsburg, Germany

[6]Biocontrol, Bioscientia Healthcare, Ingelheim, Germany

[7]Tierdermatologie Dr Wildermuth, Wiesbaden, Germany

[8]Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield, UK

Correspondence
Anette Loeffler, Department of Clinical Services and Science, Royal Veterinary College, Hatfield, UK.
Email: aloeffler@rvc.ac.uk

Abstract

Background: Legislation was introduced in Germany in 2018, requiring bacterial culture and antimicrobial susceptibility testing before the prescription of fluoroquinolones and third-generation cephalosporins to dogs. We hypothesised that, following this intervention, the number of clinical samples testing positive for methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) would reduce.

Methods: Reports of *S. pseudintermedius* isolated from canine clinical samples by three German veterinary diagnostic microbiology laboratories during the 38 months before the introduction of the legislation and the 46 months after were compared. Bacterial identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry, and antimicrobial susceptibility testing followed recognised recommendations but with changes during the study period.

Results: Among a total of 120,571 *S. pseudintermedius* isolates, MRSP accounted for 7.1% overall. Following the legislative intervention, monthly submissions yielding *S. pseudintermedius* increased at all three laboratories. The MRSP percentage was lower in the period after the intervention in two of the three laboratories ($p < 0.001$); in the third laboratory, there was no change between periods, but a year-on-year reduction in MRSP percentages occurred after the intervention ($p = 0.0004$).

Limitations: Changing susceptibility testing methods limited the direct comparison of resistance patterns among laboratories.

Conclusion: The reduction in MRSP in canine clinical samples following the introduction of this legislation suggests a positive impact of compulsory laboratory testing on reducing antimicrobial resistance.

INTRODUCTION

Antimicrobial drugs for the treatment of bacterial infections have revolutionised human and veterinary medicine. However, their use, both appropriate and inappropriate, selects for antimicrobial resistance among bacterial pathogens, presenting a major public health threat that has become a leading cause of human death around the world.[1]

Antimicrobial use in livestock has been strictly regulated in European countries for several decades and with good success. In the UK, the Veterinary Medicines Directorate has reported a fall of 55% in the use of antimicrobial agents in food-producing animals, from 62.3 mg/kg in 2014 to 28.3 mg/kg in 2021.[2] When agents categorised as 'highest priority critically important antimicrobials' (HP-CIAs) for human medicine, such as fluoroquinolones, third- and

fourth-generation cephalosporins and colistin,[3] are considered, the reduction was 83% over the same period (to the current level of 0.12 mg/kg). These reductions in food-producing animals have been achieved largely as a result of voluntary collaboration between the farming sectors, the veterinary organisations, the government (represented by the Veterinary Medicines Directorate) and the pharmaceutical industry. This has been overseen by the Responsible Use of Medicines in Agriculture Alliance (RUMA).[4]

More recently, antimicrobial prescribing in small animal practice has started to come under scrutiny, particularly for the fluoroquinolones and the third-generation cephalosporin cefovecin, for which authorised products are available for use in dogs in many countries. In order to reduce excessive and inappropriate prescribing and to promote responsible use in small animal practice, a range of voluntary and compulsory strategies are in place in different countries. Voluntary approaches include educational efforts such as national antimicrobial use guidelines,[5] app-based prescribing support[6] and disease-specific clinical practice guidelines for common bacterial infection scenarios.[7-9] In the UK, there have been various voluntary initiatives in recent years, including posters for practice use, for example, the PROTECT ME poster by the British Small Animal Veterinary Association[10] and the British Veterinary Association seven-point plan.[11] RUMA has also turned its attention to small animal practice, with the establishment of RUMA Companion Animal and Equine, with a wide range of objectives, including a commitment to try and standardise laboratory protocols for culture and susceptibility testing protocols and reporting.[12] Studies of the impact of guidelines on prescribing behaviour in human and veterinary medicine have shown promising results, with reductions in both overall prescribing and in prescribing of HP-CIAs, although mostly in hospital settings.[13-15] However, direct evidence of adherence to guidelines reducing resistance in pathogens is awaited.

In some countries, for instance, the Netherlands, Belgium and Denmark, the use of antimicrobials in pets is more directly steered by national policy, and a ban on some antimicrobials for use in any animal species was introduced for EU member countries in 2022.[5,16-18] A compromise 'middle way' regulation that does not restrict prescribing but legislates that bacterial culture and susceptibility results must be available or pending when using fluoroquinolones or third-generation cephalosporins came into force in Germany in March 2018 (Verordnung über Tierärztliche Hausapotheken, TÄHAV).[19]

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), a canine multidrug-resistant, opportunistic pathogen with zoonotic potential, was recently identified as one of the three most relevant multidrug-resistant bacteria in EU small animal veterinary practice.[20] MRSP isolates are *S. pseudintermedius* that have acquired a small genetic element, *mecA* or *mecC*, which confers resistance to all β-lactam antibiotics, but most MRSP isolates are also resistant to fluoroquinolones and other clinically relevant antimicrobials, likely selected for by exposure to antimicrobial drugs in veterinary patients.[21]

The current study compared canine *S. pseudintermedius*, and specifically MRSP, from clinical submissions to three veterinary microbiology laboratories in Germany before and after the introduction of the new TÄHAV legislation. We hypothesised that this legislative intervention would be associated with an increased uptake of susceptibility testing and a reduction in methicillin resistance among clinical *S. pseudintermedius* isolates from dogs.

MATERIALS AND METHODS

Laboratory reports

Databases from three major veterinary diagnostic microbiology laboratories (Biocontrol, Laboklin and SYNLAB Vet) were searched for *S. pseudintermedius*, *S. intermedius* and *S. intermedius*-group isolates from clinical samples from dogs submitted by first-opinion veterinary practices and veterinary referral centres in Germany. Data received by the investigators (A.L., L.B. and Y.M.C.) were coded without personally identifiable data of dogs or dog owners. Isolates were excluded if susceptibility testing did not report on the drugs used in the analysis.

Study period

Reports were from sample submissions made between January 2015 and December 2021 inclusive, covering 38 months before (period 1) and 46 months after (period 2) the introduction of the TÄHAV legislation on 1 March 2018.

Isolation and laboratory identification of *S. pseudintermedius*

Initial cultures from samples were assessed for colony morphology and haemolysis (complete α-haemolysis and partial β-haemolysis) typical for *S. pseudintermedius* after 18–24 hours incubating at 36 ± 2°C on Columbia agar with 5% sheep blood (Becton Dickinson and Oxoid). Presumed staphylococci were confirmed as *S. pseudintermedius* species using a Vitek 2 microbial identification system (bioMérieux) or matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Bruker).

Antimicrobial susceptibility testing

Antimicrobial susceptibilities were determined by broth microdilution testing truncated around breakpoints using methods as described by the Clinical and Laboratory Standards Institute (CLSI) Performance Standards documents, by the manufacturers of Vitek 2 (AST-GP80 cards, bioMérieux) or by MICRONAUT Software (MERLIN Gesellschaft für

mikrobiologische Diagnostika); breakpoints used were by CLSI for pathogens from animals, or, where not available (e.g., for trimethoprim-sulfamethoxazole [TMPS]), those for *S. aureus* or for *Staphylococcus* spp. from humans.[22-24] The results were reported as 'susceptible', 'intermediate' or 'resistant'. As MRSP is considered resistant to all β-lactam antibiotics, data for β-lactam antibiotics were not analysed for MRSP.[22,24,25]

Rifampicin is not routinely included in susceptibility test panels but may be tested upon request by the submitting veterinary surgeon. Assessment of rifampicin susceptibility was performed by either microbroth dilution or disk diffusion using a 5 μg disk with human-derived breakpoints for *Staphylococcus* spp.[22]

Laboratory identification of MRSP

All *S. pseudintermedius* were screened for resistance to methicillin after a 24-hour incubation at 30 ± 1°C on either Oxa Screen Agar (Becton Dickinson) or Oxoid Brilliance MRSA 2 Agar (Thermo Fisher Scientific). Minimum inhibitory concentrations (MICs) for oxacillin were determined by microtitre broth dilution, with an MRSP defined by MICs of 0.5 μg/mL or greater. If oxacillin MICs were not reported for an isolate, methicillin resistance was inferred from growth on screening agar and cefalexin and broad β-lactam resistance. Molecular confirmation of the presence of *mecA* or *mecC* was not required for clinical reporting purposes or for this study.

Data extraction from laboratory reports

With resistance to oxacillin as the key determinant of the analyses in this study, oxacillin test results were further assessed by the addition of and comparison to results for cefalexin (or other first- or second-generation cephalosporins). *S. pseudintermedius* isolates resistant to cefalexin or another first- or second-generation cephalosporin were included as MRSP if no entry on oxacillin susceptibility was recorded (based on the shared resistance mechanism in staphylococci). For disparate results, the oxacillin entry was preferentially used.

For analysis of other antimicrobial resistance trends, agents were selected for their relevance to clinical small animal practice and as representatives of important antimicrobial classes (β-lactam antibiotics, lincosamides, fluoroquinolones, tetracyclines, sulfonamides, rifampicin). The results reported as 'intermediate' were grouped together with 'resistant', and isolates were categorised as susceptible or resistant for all subsequent statistical analyses.

Statistical analyses

The number of samples submitted per month was summarised using the median [25th, 75th percentiles]. The Mann–Whitney *U*-test was used to compare the medians of numbers of samples submitted per month between the two time periods (38 months for the pre-legislation period and 46 months for the post-legislation period); this comparison was carried out for each laboratory individually and combined. Frequencies and proportions were used to summarise the laboratory test results. The chi-squared test was used to compare the proportion of isolates characterised as MRSP between the pre- and post-legislation periods. Fisher's exact test was used to compare the drug resistance proportions between periods for either MRSP or methicillin-susceptible *S. pseudintermedius* (MSSP) isolates only; *p*-values were adjusted using the Benjamini and Hochberg approach to account for multiple testing within each laboratory. The Clopper–Pearson method was used to calculate the exact 95% confidence interval of a proportion. The Cochran–Armitage test for trend was employed to evaluate the linear increase or decrease in yearly resistance proportions before or after February 2018 for Laboklin, where laboratory methods had remained consistent. The chi-squared test was used to check the association between MRSP frequency and rifampicin resistance. Additionally, the homogeneous associations between MRSP and rifampicin resistance were compared between Laboklin and SYNLAB Vet using the Breslow–Day test. All analyses were carried out using R statistical software (version 4.2.2; R Core Team, 2022), summarytools (version 1.0.1), latticeExtra (version 0.6-30) and DescTools (version 0.3.4) packages. Statistical significance was set at a *p*-value of less than 0.05 (type I error rate at 5%).

RESULTS

S. pseudintermedius isolates from clinical samples overall

In total, 120,571 *S. pseudintermedius* isolates, irrespective of their methicillin resistance, were included from the three laboratories over the 7-year study period. The three laboratories combined reported a lower median number of *S. pseudintermedius* isolates submitted per month during period 1 compared to period 2 (980 [877, 1128] vs. 1796 [1556, 2016], $p < 0.0001$); this increase in the median number of isolates submitted per month was also seen for each laboratory individually (Biocontrol: 156 [127, 166] vs. 282 [254, 323], $p < 0.0001$; Laboklin: 684 [611, 804] vs. 1259 [1083, 1367], $p < 0.0001$; SYNLAB Vet: 146 [125, 164] vs. 275 [234, 304], $p < 0.0001$) (Table S1).

MRSP isolation frequency

Over the 7 years, 8589 MRSP isolates were reported from all three laboratories combined, accounting for between 4% and 11.9% of *S. pseudintermedius* isolations from the different periods and laboratories (Table 1). In 1.53% of all *S. pseudintermedius* isolates,

TABLE 1 Percentages of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) among the *S. pseudintermedius* isolates tested and changes between the two study periods

Origin	% MRSP (number of *S. pseudintermedius* isolates tested)			
	Period 1	Period 2	*p*-Value	Total
Biocontrol	11.9 (5636)	8.9 (13,177)	<0.001[a]	9.8 (18,813)
Laboklin	6.8 (26,764)	7.2 (57,518)	0.06	7.1 (84,282)
SYNLAB Vet	5.2 (5549)	4.0 (11,927)	<0.001[a]	4.4 (17,476)
Total study isolates	7.4 (37,949)	7.0 (82,622)	0.03[a]	7.1 (120,571)

Note: Isolates are from clinical samples from dogs submitted to three diagnostic microbiology laboratories in Germany. Period 1: January 2015–February 2018 inclusive. Period 2: March 2018–December 2021 inclusive.
[a]Chi-squared test, $p < 0.05$ significant.

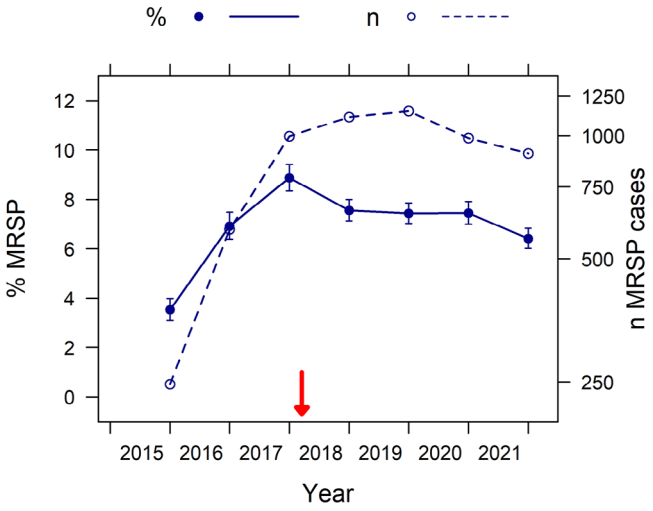

FIGURE 1 Percentages (with 95% confidence intervals) of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (solid line) and numbers (dotted line) of MRSP among 84,282 *S. pseudintermedius* isolations at Laboklin from clinical samples of dogs between 2015 and 2021. The positions of the *x*-axis ticks relate to the end of each year when total numbers of cases and percent were calculated. Arrow indicates the timing of the legislative intervention in Germany at the end of February 2018 (TÄHAV amendment)

methicillin susceptibility or resistance was inferred from cefalexin and broad β-lactam resistance.

MRSP frequencies were lower during period 2 compared to period 1 for two laboratories (Biocontrol and SYNLAB Vet, $p < 0.001$ for each) (Figures S1 and S2), while no statistically significant change was seen for Laboklin ($p = 0.06$, Table 1). For Laboklin, where laboratory methods had remained unchanged over the 7-year period, a trend analysis was performed that showed a steep increase in MRSP from relatively low levels in 2015 up to the 2018 intervention ($p < 0.0001$), followed by a decline in MRSP percentages ($p = 0.0004$) during the subsequent years (Figure 1).

Antimicrobial resistance

Changes in resistance profiles that were seen for both MRSP and MSSP isolates between period 1 and period 2 included a reduction in resistance to enro- and marbofloxacin (although no change was seen among MRSP tested at Biocontrol) and TMPS, and an increase in resistance to pradofloxacin in isolates from the two laboratories that had tested it from 2015 onwards (Tables 2 and 3).

Among the MRSP isolates, resistance to the non-β-lactam antimicrobial agents showed little change. Resistance to clindamycin remained high, above 95% at all laboratories, although with a reduction from period 1 to period 2 among SYNLAB Vet isolates; for tetracycline or doxycycline, an increase in resistance was seen in period 2 in Biocontrol and Laboklin isolates (Table 2).

In MSSP isolates, resistance was less than 10% for all β-lactams (except for ampicillin), all three fluoroquinolones and TMPS at all three laboratories and during both study periods (Table 3). For the cephalosporins (first and third generation), resistance reduced or remained unchanged between the two periods at all three laboratories. For clindamycin and tetracycline, higher resistance, up to 35% and 32%, respectively, was reported by two laboratories. Trends that were not in agreement between all three laboratories were observed for ampicillin (increasing resistance in two laboratories) and for amoxicillin-clavulanic acid and tetracycline (reducing resistance in two laboratories).

In addition, results for resistance to rifampicin were available from two laboratories for a total of 83,123 *S. pseudintermedius* isolates. Resistance at both laboratories was higher in MRSP than in MSSP (9% vs. 6% at Laboklin; 31.4% vs. 8.7% at SYNLAB Vet). Rifampicin resistance did not change between periods 1 and 2 in the 70,557 isolates tested by Laboklin ($p = 0.90$), but an increase was seen in the 12,566 isolates tested by SYNLAB Vet in both MSSPs (from 5.6% to 10.1%) and MRSPs (from 13.6% to 34.1%) (both $p < 0.0001$).

DISCUSSION

The timely decrease in MRSP and the increase in microbiology laboratory submissions following the legislative intervention introduced in Germany in February 2018 support our two hypotheses and the premise that mandatory susceptibility testing can have a beneficial impact on efforts to improve antimicrobial stewardship and reduce antimicrobial resistance in staphylococcal pathogens.

The increase in uptake of bacterial culture and susceptibility testing will have been a direct result of the TÄHAV amendment that made laboratory testing a

TABLE 2 Antimicrobial resistance (and number of isolates tested) in methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates from dogs submitted to three diagnostic microbiology laboratories in Germany during the two study periods

	% Resistance (number of MRSP isolates tested)								
	Biocontrol			Laboklin			SYNLAB Vet		
	Period 1 (670)	Period 2 (1171)	Adjusted *p*-value	Period 1 (1833)	Period 2 (4150)	Adjusted *p*-value	Period 1 (289)	Period 2 (476)	Adjusted *p*-value
Clindamycin	98.86 (88)	99.85 (663)	0.40	99.73 (1833)	99.86 (4150)	0.33	98.62 (289)	95.17 (476)	0.02[a]
Enrofloxacin	84.48 (670)	82.91 (1170)	0.40	78.78 (1833)	69.11 (4150)	<0.0001[a]	55.94 (286)	44.75 (476)	0.01[a]
Marbofloxacin	83.43 (670)	81.73 (1171)	0.40	80.80 (1833)	70.09 (4149)	<0.0001[a]	64.91 (114)	–	–
Pradofloxacin	–	–	–	14.18 (1833)	50.55 (4150)	<0.0001[b]	25.51 (98)	45.21 (303)	0.003[b]
Tetracycline	66.52 (669)	83.85 (1170)	<0.0001[b]	–	–	–	67.96 (181)	63.03 (476)	0.27
Doxycycline (excluding 2021 data)	–	–	–	11.24 (1833)	15.06 (3246)	<0.0001[b]	–	–	–
Trimethoprim-sulfamethoxazole	–	–	–	81.34 (1833)	69.18 (4150)	<0.0001[a]	50.85 (177)	39.50 (476)	0.02[a]

Note: MRSP identified based on growth on screening agar or oxacillin or first-generation cephalosporin minimum inhibitory concentrations. Period 1: January 2015–February 2018 inclusive. Period 2: March 2018–December 2021 inclusive.
[a]Fisher's exact test, $p < 0.05$ indicating a significant decrease.
[b]Fisher's exact test, $p < 0.05$ indicating a significant increase.

TABLE 3 Antimicrobial resistance (and number of isolates tested) in methicillin-susceptible *Staphylococcus pseudintermedius* (MSSP) isolates from dogs submitted to three diagnostic microbiology laboratories in Germany during the two study periods

	% Resistance (number of MSSP isolates tested)								
	Biocontrol			Laboklin			SYNLAB Vet		
	Period 1 (4966)	Period 2 (12,006)	Adjusted *p*-value	Period 1 (24,931)	Period 2 (53,368)	Adjusted *p*-value	Period 1 (5260)	Period 2 (11,451)	Adjusted *p*-value
Ampicillin	74.26 (1080)	81.64 (7189)	<0.0001[a]	64.62 (24,931)	66.00 (53,368)	0.0002[a]	77.54 (5133)	67.01 (10,908)	<0.0001[b]
Amoxicillin-clavulanic acid	1.43 (4623)	0.50 (11,818)	<0.0001[b]	4.39 (24,931)	1.32 (53,368)	<0.0001[b]	0.39 (5133)	0.84 (10,908)	0.002[a]
Cefalexin (cefazolin, cefalothin)	1.31 (4898)	0.79 (10,582)	0.004[b]	6.27 (24,930)	0.91 (53,363)	<0.0001[b]	0.00 (5132)	0.00 (10,886)	–
Cefovecin	5.69 (791)	0.51 (10,768)	<0.0001[b]	4.73 (24,928)	0.82 (53,359)	<0.0001[b]	0.02 (5129)	0.09 (11,233)	0.19
Clindamycin	9.80 (4879)	8.59 (11,768)	0.01[b]	32.4 (24,930)	30.9 (53,354)	<0.0001[b]	34.82 (5132)	32.38 (11,107)	0.003[b]
Enrofloxacin	7.31 (4897)	5.29 (11,881)	<0.0001[b]	6.46 (24,930)	4.48 (53,359)	<0.0001[b]	6.53 (5128)	5.31 (11,253)	0.003[b]
Marbofloxacin	5.69 (4900)	3.50 (11,889)	<0.0001[b]	7.37 (24,930)	5.14 (53,356)	<0.0001[b]	7.22 (1136)	3.68 (353)	0.02[b]
Pradofloxacin	–	–	–	1.44 (24,929)	3.35 (53,350)	<0.0001[a]	2.93 (2392)	4.58 (8306)	0.0007[a]
Tetracycline	31.09 (4893)	27.23 (11,857)	<0.0001[b]	–	–	–	32.4 (4375)	27.4 (11,099)	<0.0001[b]
Doxycycline (excluding 2021 data)	–	–	–	4.13 (24,930)	5.04 (40,166)	<0.0001[a]	–	–	–
Trimethoprim-sulfamethoxazole	–	–	–	6.91 (24,930)	5.42 (53,361)	<0.0001[b]	9.43 (4219)	7.03 (10,903)	<0.0001[b]

Note: Period 1: January 2015–February 2018 inclusive. Period 2: March 2018–December 2021 inclusive.
[a]Fisher's exact test, $p < 0.05$ indicating a significant increase.
[b]Fisher's exact test, $p < 0.05$ indicating a significant decrease.

legal requirement when prescribing HP-CIAs. Reasons for the reduction in MRSP are more complex as the increased sampling will inevitably have introduced selection bias since more cases with fewer refractory infections will have been sampled, increasing the number of susceptible *S. pseudintermedius* isolations. However, the legislation change is also expected to have influenced antimicrobial prescribing behaviour away from empirical prescribing towards more responsible antimicrobial use. This was recently evidenced in the results from an online survey of 303 veterinarians in Germany, which showed a 36% reduction in antimicrobial prescribing for dogs and cats after the TÄHAV amendment and a 79% reduction in HP-CIA prescribing.[26] It is therefore reasonable to assume that the declining trend in MRSP in our study is at least partially attributable to legislative intervention.

Remarkably, the decline in MRSP became apparent within the first year after the intervention. A similar prompt reduction in methicillin and multidrug resistance among staphylococcal pathogens was seen following prescribing restrictions in individual hospital settings. In a small animal hospital in Japan, methicillin resistance among 196 *S. intermedius*-group isolates reduced from 41.5% to 9.3% in the years following prescribing restrictions, which entailed the hospital-wide introduction of antimicrobial selection criteria mandating susceptibility testing prior to the use of fluoroquinolones and cefovecin.[26]

The overall MRSP prevalence of 7.1% among the 120,571 clinical canine *S. pseudintermedius* isolates was surprisingly low and lower than that previously reported from continental European countries, including Germany.[27,28] Clinical canine MRSP infection was first reported in 2007 (as methicillin-resistant *S. intermedius*) in a case series of 12 dogs from a dermatology referral centre in Germany.[29] At that time, 23% of all *S. (pseud)intermedius* submissions from the same centre were found to be resistant to at least five classes of antimicrobials, and were likely MRSP. Higher rates were also reported from Italy (32%)[30] and from Finland (14%).[31] Even higher rates were seen in Southern China and a university hospital in the United States, with 47% and 50% of clinical *S. pseudintermedius* isolates, respectively, being MRSP.[32,33] However, these prevalence data were either from dermatology clinics or referral hospitals, which typically treat patients with more risk factors for multidrug resistance than those expected in our current large population of mainly general practice-derived isolates.[33,34]

While the reduction in resistance to enrofloxacin and marbofloxacin was striking and encouraging, the increase in pradofloxacin resistance seen in both MRSP and MSSP from the two laboratories that tested for pradofloxacin is interesting but difficult to rationalise. With enro- and marbofloxacin preferentially targeting topoisomerase IV in staphylococci and pradofloxacin targeting topoisomerases IV and II, isolates resistant to pradofloxacin would be expected to also show resistance to the other two fluoroquinolones. Molecular analyses of MRSP reported as pradofloxacin resistant in this study are needed to explore whether unique molecular changes are emerging or whether testing recommendations need to be reviewed.

Interestingly, resistance to rifampicin was seen in a substantial number of MRSP (and also MSSP) isolates despite its very infrequent use. Rifampicin, categorised by the WHO as critically important for humans, is not authorised for dogs and carries a high risk of hepatotoxicity, but it may be considered as a last resort, off-license drug for some cases of deep MRSP infection in dogs.[35] Our results add to the currently sparse data on resistance to this drug and highlight the importance of specific susceptibility testing for rifampicin prior to use, as treatment may be ineffective in up to 30% of cases.

The main limitations of the current study are related to its retrospective nature, the variation in methods between laboratories over time and the above-mentioned inherent selection bias post-intervention. While differences in bacterial identification were likely minimal due to all three laboratories using validated automated methods, methods for antimicrobial susceptibility testing varied. CLSI laboratory standards documents detailing methods to be used for susceptibility testing tend to change little over time, and the same CLSI standards were used by all three laboratories. However, clinical breakpoints are continuously being reviewed, and new breakpoints for animal pathogens are being developed by CLSI and the veterinary section of the European Committee on Antimicrobial Susceptibility Testing.[36] Furthermore, the selection of antimicrobials tested or included in reports to clinicians varied over time. Decisions by microbiology laboratories to exclude or include certain drugs can support clinicians in responsible antimicrobial prescribing, but evidence-based consensus guidance on diagnostic stewardship is urgently needed. Additionally, Simpson's paradox is a known statistical phenomenon where combining data from multiple strata can sometimes cause the apparent association between two variables to change. In order to present a better picture of the data structure from the three laboratories, our results were presented stratified and combined, which may have increased our type I error rate. It is therefore essential to consider all relevant information (total number of isolates tested, frequency of resistance and proportions and the *p*-value for statistical comparison) when interpreting the analyses.

CONCLUSIONS

The decrease in MRSP in clinical isolates seen at all three independent laboratories after the legislative intervention suggests that mandatory laboratory testing when prescribing HP-CIAs has a positive, measurable impact on reducing multidrug resistance in clinical staphylococci. By preserving the prescribing freedom for clinicians, and thus supporting animal welfare, while fostering rational antimicrobial use, the TÄHAV intervention presents an excellent example of

an effective antimicrobial stewardship policy[37] worthy of consideration by other countries.

AUTHOR CONTRIBUTIONS

Conceptualisation, methodology, analysis, writing, review and editing: Anette Loeffler, Brett Wildermuth and David Lloyd. *Methodology, analysis, review and editing*: Lee Beever. *Methodology, analysis, writing, review and editing*: Yu-Mei Chang. *Data curation, methodology, manuscript review and editing*: Babette Klein, Veit Kostka, Cornelia Meyer, Elisabeth Mueller and Jessica Weis. *Conceptualisation, writing, review and editing*: John Fishwick.

ACKNOWLEDGEMENTS

We are grateful to Christine Simon and Alexander Pankraz (Biocontrol) for their help with the data collection.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

FUNDING INFORMATION

Self-funded.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

No animals were used in this study. No personally identifiable data were used. Data transfer agreements were in place with all three laboratories.

ORCID

Anette Loeffler https://orcid.org/0000-0002-0446-3729

John Fishwick https://orcid.org/0000-0001-6296-951X

REFERENCES

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55. Erratum in: Lancet. 2022;400(10358):1102.
2. Veterinary Medicines Directorate. Veterinary antimicrobial resistance and sales surveillance (VARSS). 2021. Available from: https://www.gov.uk/government/publications/veterinary-antimicrobial-resistance-and-sales-surveillance-2021. Accessed 2 July 2023.
3. World Health Organization (WHO). Critically important antimicrobials for human medicine, 6th revision. Geneva: World Health Organization; 2019.
4. Responsible Use of Medicines in Agriculture Alliance (RUMA). RUMA targets task force 2: two years on. 2022. Available from: https://www.ruma.org.uk/wp-content/uploads/2022/11/RUMA-TTF-Report-2022-FINAL.pdf. Accessed 2 July 2023.
5. Schmerold I, van Geijlswijk I, Gehring R. European regulations on the use of antibiotics in veterinary medicine. Eur J Pharm Sci. 2023;189:106473.
6. Canadian Veterinary Medical Association (CVMA). CVMA veterinary guidelines for AMU—firstline app. 2023. Available from: https://www.canadianveterinarians.net/veterinary-resources/antimicrobial-stewardship-resources/cvma-veterinary-guidelines-for-amu-firstline-app/. Accessed 21 June 2023.
7. Bundestierärztekammer. Leitlinien für den sorgfältigen Umgang mit antibakteriell wirksamen Tierarzneimitteln. Dt TÄBl. 2015;58:2.
8. Australasian Infectious Diseases Advisory Panel (AIDAP). Antibiotic prescribing detailed guidelines. 2nd ed. 2022. Available from: https://www.amrvetcollective.com/assets/guidelines/2022-AIDAP-Prescribing-Guidelines-2nd-edition-Final.pdf. Accessed 16 June 2023.
9. World Veterinary Association. Global repository of available guidelines for responsible use of antimicrobials in animal health. 2019. Available from: https://worldvet.org/uploads/news/docs/list_of_available_guidelines_on_amu_-aug2019.pdf. Accessed 9 June 2023.
10. British Small Animal Veterinary Association (BSAVA). PROTECT ME poster. Available from: https://Users/anetteloeffler/Downloads/bsava_samsoc_protectmeposter_011118web%20(1).pdf
11. British Veterinary Association (BVA). Responsible use of antimicrobials in veterinary practice: the 7-point-plan. Available from: https://www.vettimes.co.uk/app/uploads/2019/05/20190508-BVA-AMR-poster-2019-v4.pdf. Accessed 2 July 2023.
12. Responsible Use of Medicines in Agriculture Alliance (RUMA). RUMA companion animal and equine—annual progress report. 2022. Available from: https://rumacae.org.uk/wp-content/uploads/2022/12/RUMA-CAE-Annual-Progress-Report-December-2022-FINAL.pdf. Accessed 2 July 2023.
13. Allerton F, Prior C, Bagcigil AF, Broens E, Callens B, Damborg P, et al. Overview and evaluation of existing guidelines for rational antimicrobial use in small-animal veterinary practice in Europe. Antibiotics. 2021;10(4):409.
14. Gerber JS, Prasad PA, Fiks AG, Localio AR, Grundmeier RW, Bell LM, et al. Effect of an outpatient antimicrobial stewardship intervention on broad-spectrum antibiotic prescribing by primary care pediatricians: a randomized trial. JAMA. 2013;309(22):2345–52.
15. Lehner C, Hubbuch A, Schmitt K, Schuepbach-Regula G, Willi B, Mevissen M, et al. Effect of antimicrobial stewardship on antimicrobial prescriptions for selected diseases of dogs in Switzerland. J Vet Intern Med. 2020;34(6):2418–31.
16. European Commission. Commission Implementing Regulation (EU) 2022/1255. Designating antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6 of the European Parliament and of the Council (text with EEA relevance). 2022. Off J Eur Union L 191/58. Available from: https://eur-lex.europa.eu/eli/reg_impl/2022/1255/oj. Accessed 28 June 2023.
17. Finnish Food Safety Authority, Evira, and the Faculty of Veterinary Medicine at the University of Helsinki. Recommendations for the use of antimicrobials in the treatment of the most significant infectious and contagious diseases in animals. 2018. Available from: https://www.ruokavirasto.fi/globalassets/viljelijat/elaintenpito/elainten-laakitseminen/hallittu_laakekekaytto/mikrobilaakekaytonperiaatteet/mikrobilaakkeiden_kayttosuositukset_en.pdf. Accessed 9 June 2023.
18. Public Health Agency of Sweden. Swedish work on containment of antibiotic resistance: tools, methods and experiences. 2014. Available from: https://www.folkhalsomyndigheten.se/contentassets/dae82c7afd424a57b57ec81818793346/swedish-work-on-containment-of-antibiotic-resistance.pdf. Accessed 9 June 2023.
19. Bundestierärztekammer. Die neue TÄHAV ist in Kraft. Dt TÄBl. 2018;66(4):484–89.
20. EFSA Panel on Animal Health and Welfare (AHAW), Nielsen SS, Bicout DJ, Calistri P, Canali E, Drewe JA, et al. Assessment of animal diseases caused by bacteria resistant to antimicrobials: dogs and cats. EFSA J. 2021;19(6):e06680.
21. Morris DO, Loeffler A, Davis MF, Guardabassi L, Weese JS. Recommendations for approaches to methicillin-resistant staphylococcal infections of small animals: diagnosis, therapeutic considerations and preventative measures: clinical consensus

guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2017;28:304-e69.
22. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 3rd ed. CLSI supplement VET01S. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
23. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
24. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 5th ed. CLSI standard VET01. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
25. Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, MacGowan AP, et al. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. 2013;19(2):141–60.
26. Moerer M, Merle R, Bäumer W. A cross-sectional study of veterinarians in Germany on the impact of the TÄHAV amendment 2018 on antimicrobial use and development of antimicrobial resistance in dogs and cats. Antibiotics. 2022;11(4):484.
27. Kurita G, Tsuyuki Y, Murata Y, Takahashi T. Veterinary Infection Control Association (VICA) AMR Working Group. Reduced rates of antimicrobial resistance in *Staphylococcus intermedius* group and *Escherichia coli* isolated from diseased companion animals in an animal hospital after restriction of antimicrobial use. J Infect Chemother. 2019;25(7):531–36.
28. GERMAP 2015. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Paul-Ehrlich-Gesellschaft für Chemotherapie e.V. Bericht über den Antibiotikaverbrauch und die Verbreitung von Antibiotikaresistenzen in der Human- und Veterinärmedizin in Deutschland. Antiinfectives Intelligence, Rheinbach. Infektionen des Respirationstraktes/Infektionen von Haut. Ohr, Maul; Hund/Katze. 2016. pp. 146–47.
29. Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JM, Winkler M, et al. First report of multiresistant, *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol. 2007;18(6):412–21.
30. Menandro ML, Dotto G, Mondin A, Martini M, Ceglie L, Pasotto D. Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* from symptomatic companion animals in Northern Italy: clonal diversity and novel sequence types. Comp Immunol Microbiol Infect Dis. 2019;66:101331.
31. Grönthal T, Eklund M, Thomson K, Piiparinen H, Sironen T, Rantala M. Antimicrobial resistance in *Staphylococcus pseudintermedius* and the molecular epidemiology of methicillin-resistant *S. pseudintermedius* in small animals in Finland. J Antimicrob Chemother. 2017;72(4):1021–130. Erratum in: J Antimicrob Chemother. 2017;72(7):2141.
32. Feng Y, Tian W, Lin D, Luo Q, Zhou Y, Yang T, et al. Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China. Vet Microbiol. 2012;160(3–4):517–24.
33. Burke M, Santoro D. Prevalence of multidrug-resistant coagulase-positive staphylococci in canine and feline dermatological patients over a 10-year period: a retrospective study. Microbiology. 2023;169(2):001300.
34. Beever L, Bond R, Graham PA, Jackson B, Lloyd DH, Loeffler A. Increasing antimicrobial resistance in clinical isolates of *Staphylococcus intermedius* group bacteria and emergence of MRSP in the UK. Vet Rec. 2015;176(7):172.
35. Papich MG. Selection of antibiotics for methicillin-resistant *Staphylococcus pseudintermedius*: time to revisit some old drugs? Vet Dermatol. 2012;23(4):352–60.
36. VetCAST. Veterinary committee on antimicrobial susceptibility testing. 2019. Available from: https://www.eucast.org/ast_of_veterinary_pathogens. Accessed 10 June 23.
37. Lloyd DH, Page SW. Antimicrobial stewardship in veterinary medicine. Microbiol Spectr. 2018;6(3).

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Loeffler A, Beever L, Chang Y-M, Klein B, Kostka V, Meyer C, et al. Intervention with impact: Reduced isolation of methicillin-resistant *Staphylococcus pseudintermedius* from dogs following the introduction of antimicrobial prescribing legislation in Germany. Vet Rec. 2023;e3714. https://doi.org/10.1002/vetr.3714