Evaluation of composite and quarter-milk cultures for detecting penicillin-resistant *Staphylococcus* in dairy cows using hierarchical models

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Summary
Latent class models have become a popular tool to evaluate several diagnostic tests applied to the same samples, when no test can be considered a gold standard [1,2]. In this work we developed hierarchical latent class models to assess the test characteristics for culturing of quarter milk samples and a composite sample from all 4 quarters. The outcome of interest was the presence of antimicrobial resistant staphylococci bacteria. The study was part of a development of a herd-level diagnostic test for penicillin resistance, and offered insight into whether such a test should be based on quarter or composite samples.

Materials and methods
Quarter milk samples were collected from all lactating cows in 23 dairy herds located in a southern part of Denmark (Kongeå region), according to the recommendations of the International Dairy Federation [3]. A composite milk sample was made by pooling 1ml from each of the four quarter milk samples. Both quarter and composite milk samples were cultured on the same day as collection. Approximately, 0.01ml of each quarter milk sample and 1ml of each pooled sample were streaked onto bovine blood agar, supplemented with aesculin and incubated aerobically at 37°C overnight. From apparent pure cultures, a representative single colony of *Staphylococcus aureus* and/or coagulase negative *Staphylococcus* (CNS) was further identified based on colony morphology, microscopic examination, coagulase and hyaluronidase tests. Cultures showing more than one type of colony morphology growth were subcultivated separately and further identified. All staphylococci colonies were tested for β–lactamase production as indicative of resistance to penicillin. Samples were classified as either negative, sensitive or resistant with respect to *S. aureus* and CNS by interpreting several culture results in parallel (if both sensitive and resistant cultures were obtained, the sample was classified as resistant).

The association between the test outcomes of quarter milk and composite milk samples was explored using latent class models (the latent variables being the true status of each quarter-milk sample) involving the assumptions:

- quarter and composite sample culturing had perfect specificity,
- the number of true positive quarters in a cow followed a beta-binomial distribution with a mean probability or prevalence (p) depending on the herd and a correlation (Δ) between the four quarter values,
- herd prevalences were modelled by random effects on logistic scale, assumed to follow a normal distribution (standard deviation Φ),
- given the quarter's true infection status, the outcomes of testing different quarter samples and the composite sample were conditionally independent,

- given the quarter's true infection status, the quarter and composite tests had constant sensitivities across herds.

Two such latent class models are presented here, differing only in the specification of the sensitivity of the composite samples as a function of the number of true positive quarters. The first model assumed a fixed composite sensitivity (cSe) whenever at least one quarter was true positive (parallel interpretation). The second model assumed the composite sensitivity cSe to increase as a function of the number (n) of true positive quarters, as follows: $\text{cSe}(n \text{ pos}) = 1-(1-\text{cSe}(1 \text{ pos}))^n$, corresponding to positive quarters being overlooked independently and with the same probability. Model parameters were estimated by the maximum likelihood method using a quasi-Newton search algorithm and adaptive Gaussian quadrature for the integrals associated with the random effects ("nlmixed" procedure, SAS version 8.2). The estimation algorithm was found to be insensitive to its initial values.

Results

In total, 1816 cows from the 23 dairy herds sampled were included in the study, each with four quarter-milk samples and a composite sample. The crude proportions of samples resistant to penicillin for *S. aureus* were 1.5% and 3.7% for quarter and composite samples; for CNS, the corresponding figures were 3.3% and 5.7%. Simple sensitivity and specificity for composite testing of penicillin-resistance, assuming quarter samples to be perfect and a parallel interpretation, were 0.721 and 0.989 for *S. aureus*, and 0.335 and 0.977 for CNS (Table 1).

	S. aureus		CNS	
Observed counts:	**Composite**		**Composite**	
Quarter	Neg	Pos	Neg	Pos
0 Pos, 4 Neg	1729	19	1584	38
1 Pos, 3 Neg	14	23	111	50
2 Pos, 2 Neg	5	16	17	11
3 Pos, 1 Neg	0	10	1	4
Total	1748	68	1713	103

Table 1. Cross-classification of cows according to quarter and composite sample outcomes for resistant *Staphylococcus* bacteria. (Neg – negative; Pos – positive)

The hierarchical latent class models showed appreciable clustering in prevalence both within herds and among the cow's quarters (as indicated by the estimates of Δ and Φ and corresponding SEs in Table 2). The agreement between observed (Table 1) and predicted counts (Table 2) indicated an acceptable fit of the models; also, goodness-of-fit statistics were non-significant (not shown). Model 2 showed a better fit to the data than Model 1; the log-likelihood values were 1.5 and 1.0 units higher (for *S. aureus* and CNS, respectively). Most parameters were reasonably constant between models for the same outcome, except for the cSe parameter (sensitivity of composite testing when only 1 quarter is true positive) because of its different role in the two models. Since the data were described better by taking the composite sensitivity to be increasing with the number of true positive quarters instead of constant, the estimated sensitivities (Table 3) were based on Model 2. The comparison of composite and quarter sample testing showed similar sensitivity values for *S. aureus* resistance, with values ranging from 0.42 to 0.90. For CNS, quarter sampling was estimated to give a higher sensitivity than composite sampling, with quarter sampling sensitivities for CNS resistance ranging from 0.51 to 0.94.

	S. aureus				CNS			
Parameter	Model 1: constant cSe[a]		Model 2: increasing cSe[a]		Model 1: constant cSe[a]		Model 2: increasing cSe[a]	
prevalence *p* (SE)	0.0072 (0.0046)		0.0066 (0.0040)		0.047 (0.009)		0.052 (0.011)	
quarter Se (SE)	0.404 (0.101)		0.442 (0.090)		0.564 (0.058)		0.514 (0.067)	
composite Se[b] (SE)	0.719 (0.053)		0.418 (0.085)		0.337 (0.034)		0.248 (0.035)	
beta-binomial ρ (SE)	0.683 (0.222)		0.544 (0.172)		0.131 (0.042)		0.145 (0.049)	
herd std. dev. Φ (SE)	2.129 (0.472)		2.097 (0.464)		0.749 (0.139)		0.744 (0.138)	
Predicted counts:	**Composite**		**Composite**		**Composite**		**Composite**	
Quarter	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
0 Pos, 4 Neg	1729.92	18.99	1730.03	16.62	1583.82	37.48	1584.34	36.81
1 Pos, 3 Neg	10.31	26.42	14.02	26.47	108.23	54.96	113.68	49.77
2 Pos, 2 Neg	6.06	15.54	3.77	16.75	18.33	9.31	14.26	13.35
3 Pos, 1 Neg	2.14	5.49	0.95	6.12	2.4	1.22	1.42	2.14
4 Pos, 0 Neg	0.31	0.81	0.12	0.95	0.17	0.08	0.08	0.16

Table 2. Parameter estimates, model statistics and predicted counts for hierarchical latent class models for resistant *Staphylococcus* bacteria. ([a] =composite Se, [b] =sensitivity for 1 quarter true positive)

	S. aureus		CNS	
Positive Quarters	Composite testing Se (95% CI)	Quarter testing Se (95% CI)	Composite testing Se (95% CI)	Quarter testing Se (95% CI)
1	0.42 (0.25-0.59)	0.44 (0.27-0.62)	0.25 (0.18-0.32)	0.51 (0.38-0.64)
2	0.66 (0.44-0.83)	0.69 (0.46-0.85)	0.43 (0.33-0.53)	0.76 (0.62-0.87)
3	0.80 (0.58-0.93)	0.83 (0.60-0.94)	0.57 (0.45-0.68)	0.89 (0.77-0.96)
4	0.89 (0.68-0.97)	0.90 (0.71-0.98)	0.68 (0.54-0.78)	0.94 (0.86-0.98)

Table 3. Sensitivities for detection of resistant *Staphylocuccus* bacteria computed from hierarchical latent class model 2, based either on composite testing or quarter testing (interpreted in parallel) and given according to the number of true positive quarters in a cow for the respective bacteria.

Discussion

The latent class models enabled estimation of quarter and composite test sensitivities while taking the hierarchical structure into account, and gave a fairly good fit to the data. In line with recent research on latent class models [2], several extensions of the approach have been or are currently being investigated: allowing for more flexibility in the functional form of the composite test sensitivity, relaxing the assumptions of perfect specificity of culturing and of conditional test independence, and – within a Bayesian framework – incorporating prior information about test characteristics.

References

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