Factors influencing bacteriological cure after antibiotic therapy of clinical mastitis

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Date submitted:15/12/2015 *Date accepted: 05/02/2016* *Volume/Page(s): 69/7-14*

Abstract

Antibiotic therapy of clinical mastitis (CM) is difficult and often results in unsatisfactory outcomes. At detection of every CM case a reliable prognosis for the probability of bacteriological cure (BC) is beneficial to avoid useless application of antibiotic treatments. Therefore, factors which are associated with BC of CM have to be determined. A randomised, matched field study was conducted on 24 free-stall dairy farms located in Northern - and Central Germany. Data of CM cases receiving antibiotic treatment were recorded. A foremilk sample of the affected quarter was taken before treatment and again approximately 14 days and 21 days after the end of therapy for bacteriological examination. The BC of every CM case was determined. Animal-, pathogen-, treatment-, herd- and environment-related factors were added to every CM case and analysed statistically for associations with BC of the CM cases. The study resulted in the following findings: The overall BC rate was 74.6%. Cows with bacteriologically cured CM cases showed a lower somatic cell count, based on the seven Dairy Herd Improvement (DHI) test days before treatment (individual sum-200-7), and milk yield in the final DHI test before CM occurrence than cows with bacteriologically non-cured CM cases. The probability of BC decreased significantly if a cow had previously suffered from more than one CM case in current lactation. The likelihood of BC decreased significantly in CM cases where staphylococci were cultured in pre-treatment samples, especially due to the low BC rate of *Staphylococcus aureus* (46.7%), compared to CM cases caused by *Enterobacteriaceae*, streptococci or other pathogens. The probability of BC decreased with an increasing amount of the pathogen excreted pre-treatment.

Key words: Clinical mastitis, antibiotics, bacteriological cure, prognosis, dairy cattle

Introduction

Clinical mastitis (CM) is a common and costly disease occurring in cows on dairy farms [1, 2]. It manifests itself in visible abnormalities in milk, often with clinical symptoms of the udder quarter but also in some cases with signs of systemic illness leading to death of the cow [3]. Mastitis arises due to different causes and in approximately 70% of CM cases microorganisms are cultured in pre-treatment foremilk samples of affected udder quarters [4]. Broad-spectrum antibiotic therapy is commonly applied to treat CM on dairy farms. Since antibiotics are only able to combat microorganisms, the success of antibiotic treatment is assessed by bacteriological cure (BC) and is defined as the elimination of the mastitis-causing pathogen from the infected udder quarter [5-7]. The probability of cure varies between different CM cases [8] and therefore a reasonable prognosis for the likelihood of BC of a CM case before antibiotic treatment is needed [9].

In order to predict a prognosis, factors associated with the BC of CM cases treated with antibiotics in dairy cows have to be determined first (Table 1). Animal-related factors associated with BC of CM are age, stage of lactation, cow somatic cell count (CSCC) before onset of CM, severity of clinical signs of CM and mastitis history of the cow. Younger cows have a significantly higher chance of BC of CM than older cows [8, 10-12]. However, some other studies relativised these findings and only showed a tendency for this observation [13-15]. McDougall et al. [12, 16] described significant differences in BC for various stages of lactation in two studies and in an earlier study a significant decrease in BC with increasing days in milk (DIM) at CM occurrence [17]. The probability of BC is significantly associated with the course of CSCC before onset of CM [14, 18]. Other studies also showed that bacteriologically cured CM cases are associated with a significantly lower CSCC in a previous Dairy Herd Improvement (DHI) test before CM than bacteriologically non-cured CM cases [15, 19, 20]. The likelihood of BC increased with the severity of clinical signs at the onset of CM, where the majority of severe cases were caused by gram-negative pathogens, [21] and with elevated rectal temperature in comparison to normal rectal temperature [19]. However, BC decreased when blood was detected in milk or the udder quarter was swollen [12]. Pinzón-Sánchez and Ruegg [15] considered mastitis history and reported on a significantly higher likelihood of BC when a cow suffered from CM for the first time in lactation. Species/genus of the pathogen cultured in pre-treatment sample showed associations with BC of CM as a pathogen-related factor. CM cases where *S. aureus* was cultured prior to treatment showed a significantly lower probability of BC than cases caused by other pathogens [7, 13, 19]. Furthermore, CM cases with ß-lactamase-negative *S. aureus* strains had a significantly higher likelihood of BC than

Table 1: Results of literature research: variables associated with bacteriological cure (BC) of clinical mastitis (CM)

Factors	Variables	Results	References
Animal-related factors	Age	Younger cows higher chance of BC than older cows (significance)	8, 10-12
		Tendency	13-15
	Stage of lactation	Significant differences in BC for various stage of lactation	12, 16
		Significant decrease in BC with increasing stage of lactation	17
	CSCC	BC is significantly associated with course of CSCC before CM	14, 18
		Cured cases showed significantly lower CSCC in previous DHI test before CM	15, 19, 20
	Clinical signs	BC increased with severity of clinical signs at CM onset	21
		BC increased with elevated rectal temperature at CM onset	19
		BC decreased when blood in milk or when udder quarter was swollen	12
	Mastitis history	Significantly higher BC when cow suffered from CM for first time in lactation	15
Pathogen-related factor	Species/genus of pathogen cultured in pre-treatment sample	Significantly lower BC for cases caused by *S. aureus* than other pathogens	7, 13, 19
		Significantly higher BC for ß-lactamase-negative *S. aureus* strains than for ß-lactamase-positive *S. aureus* strains	11, 14, 20
		Significantly higher BC for *Sc. uberis* than *S. aureus*, *Sc. dysgalactiae* or multiple pathogens	12
		Significantly higher BC for CNS than *S. aureus*	22
		Significantly higher BC for minor pathogens than major pathogens	16, 17
		Significantly higher BC for coliform bacteria (especially *E. coli*) than environmental streptococci or mixed infections	23
		Higher BC for *E. coli* than for *Enterobacter cloacae*, lowest BC for *Klebsiella* spp. (without indication of significance)	24
		Significantly higher BC for cases no pathogen or gram-negative pathogens were cultured than cases caused by gram-positive or other pathogens	21
		Tendency for higher BC in culture-negative cases than in culture-positive cases	15
Environment-related factor	Season	Significantly higher BC for cases occurring in winter than for cases arising in spring	19

ß-lactamase-positive *S. aureus* strains [11, 14, 20]. McDougall et al. [12] reported a significantly higher probability of BC for CM cases caused by S*treptococcus* (*Sc.*) *uberis* than cases where *S. aureus*, *Sc. dysgalactiae* or multiple pathogens were cultured. Furthermore, Taponen et al. [22] showed a significantly higher likelihood of BC for CM cases caused by coagulase-negative staphylococci (CNS) than cases caused by *S. aureus.* When microorganisms cultured prior to treatment were grouped into major pathogens (*S. aureus*, *Escherichia* (*E.*) *coli*, *Sc. uberis, Sc. agalactiae*, *Sc. dysgalactiae*) and minor pathogens (CNS, *Corynebacterium* spp.) a significantly lower probability of BC for CM cases caused by major pathogens than cases infected with minor pathogens was observed [16, 17]. CM cases where coliform bacteria (especially *E. coli*) were cultured prior to treatment appeared to be significantly more likely to be cured than cases caused by environmental streptococci or mixed infections [23]. In the group of gram-negative microorganisms Schukken et al. [24] showed a higher BC for CM cases caused by *E. coli* (73%) than by Enterobacter cloacae (61%) and *Klebsiella* spp. (44.7%) without any indication of the significance level. CM cases without bacteriological growth in pre-treatment samples were also examined for their relevance to cure. Some researchers used absence of every pathogen in all post-treatment samples to define treatment success. Using this method, Oliveira et al. [21] showed a significantly higher probability of BC in CM cases where no pathogen or gram-negative pathogens were cultured as opposed to cases caused by gram-positive pathogens or other pathogens. A tendency, that in culture-negative CM cases the probability of BC was higher than in culture-positive CM cases, was reported by Pinzón-Sánchez and Ruegg [15]. Bradley and Green [19] conducted a study in the United Kingdom, France and Germany and showed an influence of season on BC as an environment-related factor. CM cases occurring in winter had a significantly higher likelihood of BC than CM cases arising in spring.

The aim of this present study was to identify factors associated with BC of CM in German dairy herds.

Materials and Methods

Study Design, Herds and Animals:

The randomised and matched (lactation number: 1; >1 and severity of CM: mild/moderate; severe) study was conducted from August 2010 to June 2014 on 24 free-stall dairy farms located in Northern - and Central Germany in accordance with the guidelines on good clinical practice (GCP) [44]. All farms were conventional and commercially oriented with a herd size between approximately 100 and 1,900 Holstein-Friesian dairy cows. The 305 d milk production ranged from 7,840 and 12,202 kg with bulk milk SCC (BMSCC) between 164,000 and 368,000 cells/ml. All farms were equipped with modern milking systems and used common hygiene management methods. Furthermore, every cow in Germany is registered by a unique ear tag to clearly identify every cow. The study farms participated in the German Dairy Herd Improvement (DHI) programme, which records cow data, CSCC, milk yield and milk ingredients on a monthly basis. The analysis results of these data are sent to the herd owner in written or electronic form to be used in herd management applications for further evaluation.

Lactating Holstein-Friesian dairy cows with CM signs in one or more quarters were included in the above-mentioned study. A quarter was classified as affected by a mild, moderate and severe CM if there were changes in the appearance of the milk (i.e. flaky sediments, watery appearance, discoloration) with or without clinical signs of mastitis in the quarter (i.e. swelling, heat, pain) and with or without associated general clinical signs (i.e. fever, dehydration, anorexia, depression), respectively. The identification of the CM was accomplished by trained milkers. Cows were excluded from the study if they showed significant udder, teat and teat orifice lesions, had been treated with other products in addition to the mastitis treatment or had concurrent diseases at the time of CM. As usual in Germany, cows with severe CM were allowed to receive anti-inflammatory treatment (i.e. NSAID).

Treatment:

If a cow developed CM, every affected quarter received antibiotic treatment and if necessary an anti-inflammatory agent by instructed farm staff. All used antibiotic ingredients were broad-spectrum antibiotics. In the registration of these antibiotics they claim to be effective against common mastitis pathogens in Germany. Due to no significant effect of different antibiotic treatments on BC of CM, all cases were analysed further as one group in the statistical investigations.

Sampling and Laboratory Procedure:

In the case of CM, a foremilk sample of the affected quarter was taken by trained milkers before treatment. Instructed farm staff collected quarter foremilk samples at day 14 (±2) and day 21 (±2) after the end of treatment. Ly20, containing boric acid as preserving agent, was used in test tubes. All milk samples were collected aseptically and were stored below 8°C until analysis [25]. The samples were sent to the microbiological laboratory at the University of Applied Sciences and Arts Hannover (Germany). Microbiological examinations were performed in accordance with the guidelines of the German Veterinary Association [25], which are based on National Mastitis Council recommendations [26]. Only one modification in examination procedure was performed. The clumping factor test (DiaMondiaL Staph Plus Kit, Sekisui Virotech, Germany) instead of the coagulase test was used to differentiate presumptive *S. aureus* from coagulase-negative staphylococci. The amount of colonies of the isolated microorganisms was used in the statistics as colony forming units (cfu) and divided in three groups: group 1: 1-10 cfu/0.01ml, group 2: 11-50 cfu/0.01ml, group 3: >50 cfu/0.01ml. If two pathogens were cultured, the case was included in the study and both microorganisms were documented. A milk sample was considered as contaminated when more than two pathogens were identified, except in cases where also *S. aureus*, *Sc. agalactiae*, *Sc. dysgalactiae* and *T. pyogenes* were cultured. Then only the growth of these pathogens was recorded and the cases were classified as contaminated if the samples contained more than two of these pathogens.

Definitions:

Only CM cases where one or two pathogens were cultured in the pre-treatment sample, in accordance with the above-mentioned definition, were eligible for statistical analysis. If pre-treatment samples showed no growth or were contaminated, the CM cases were excluded from further evaluations.

Bacteriological cure (BC) was defined as an absence of the pathogen cultured in the pre-treatment sample in both of the post-treatment samples. If a bacterial species other than the pathogen cultured pre-treatment was isolated in the post-treatment samples, the case was still defined as bacteriologically cured. In case one post-treatment sample was contaminated, the outcome of the other post-treatment sample was used to determine the BC. If two pathogens were isolated in the pre-treatment sample the case was enrolled as mixed infection and applied as bacteriologically cured if neither of the two pathogens were cultured in both of the post-treatment samples.

Data collection:

Data on all CM cases were collected using the program Excel, Office 2003 (Microsoft Corporation). Characteristics of the variables (Table 2), which were investigated for possible associations with BC of CM, were added to every case. Animal-related factors from DHI were collected by using the written DHI print or the herd management programme of the individual farms. CSCC of the seven previous monthly DHI recordings before CM occurrence were taken into account, whereby CSCC1 was the most recent value before CM case and CSCC7 the value six month before CSCC1. In case of missing values for CSCC (i.e. dry-period) an empty space was inserted. The association of CSCC before CM with BC was examined using six different factors. The origin of log CSCC1, mean logCSCC1-3 and mean log CSCC1-7 is described in Table 2. The "individual sum" is a calculated sum making a statement about the course of CSCC before CM with a higher weighting of CSCC closer to the CM case [18]. The CSCC of the previous seven months before CM were considered and different thresholds were used; for the individual sum-200-7 a threshold of 200,000 cells/ml, for the individual sum-400-7 a threshold of 400,000 cells/ml and for the individual sum-1000-7 a threshold of 1,000,000 cells/ml, respectively. A summand was calculated for every month. If the respective threshold of CSCC was not exceeded the summand amounted to 0. For an exceedance of the threshold of CSCC1 directly before CM occurred the summand was 7, this amount diminishing every subsequent month before CM. Therefore, an exceedance of CSCC2 two DHI recordings before CM occurrence was given the summand 6 and so on. CSCC7 with the highest distance from CM occurrence was given summand 1 in the case of threshold exceedance. The seven summands were added up and a sum from 0 to 28 could be expected. Thus, a number for every individual sum and CM case was determined. Information on mastitis history was collected by using the herd management programme and was added to the CM case if available. Unfortunately, not all farms recorded and entered this information into the herd management programme. Farms with available CM information recorded all cases of each cow.

Species/genus and the amount of the pathogen cultured in the pre-treatment quarter foremilk sample were determined by microbiological analysis as previously described. For the examination associated with BC, pathogens were grouped into Enterobacteriaceae, streptococci, staphylococci and other pathogens, respectively. When two pathogens were cultured, the case was included as a mixed infection into the group other pathogens. To determine the factor "amount of pathogens excreted prior to treatment" (shedding) for a mixed infection the following procedure was used: If both pathogens showed the same excretion rate the shedding was simply adopted. In case two major pathogens (*S. aureus*, *E. coli*, *Sc. uberis*, *Sc. agalactiae*, *Sc. dysgalactiae*) were cultured the amount of the highest excretion rate was used. The excretion rate of the major pathogen was used if a major pathogen was cultured with another pathogen and if two non-major pathogens were isolated the amount of the higher excreted pathogen was used.

The "new inflammation rate" was investigated as a herd-related factor. This is a monthly parameter on herd level derived from the CSCC change between two DHI test results. The new inflammation rate was calculated for every month as the percentage of cows having a CSCC over 100,000 cells/ml in the current DHI test from those cows which had a CSCC less than or equal to 100,000 cells/ml in the previous DHI test. For our study, a new inflammation rate was calculated for every

Table 2: Variables examined for association with bacteriological cure (BC) of clinical mastitis (CM)		
	Variable	**Groups, explanation, units**
Animal-related factors	Quarter location	Front, rear
	Lactation number	1, 2, >2 lactations
	DIM	0-100, 101-200, > 200 days in milk
	Log CSCC1	Log transformed CSCC last DHI before CM occurred
	Mean Log CSCC1-3	Mean of log transformed CSCC last 3 DHI before CM occurred
	Mean Log CSCC1-7	Mean of log transformed CSCC last 7 DHI before CM occurred
	Individual sum-200-7	Explained above (CSCC last 7 DHI before CM occurred included, threshold 200,000 cells/ml, weighted sum of the exceedances of the threshold was determined)
	Individual sum-400-7	Explained above (CSCC last 7 DHI before CM occurred included, threshold 400,000 cells/ml, weighted sum of the exceedances of the threshold was determined)
	Individual sum-1000-7	Explained above (CSCC last 7 DHI before CM occurred included, threshold 1,000,000 cells/ml, weighted sum of the exceedances of the threshold was determined)
	Milk yield	Kg, last DHI sampling before CM occurred
	Number of CM in current lactation	0, 1, >1, no information
	CM in last lactation	Yes, No, no information
Pathogen-related factors	Pathogen cultured pre-treatment	*Enterobacteriaceae*, streptococci, staphylococci, other pathogen (include mixed infection)
	Amount of pathogen excreted pre-treatment (shedding)	1: 1-10 cfu/0.01ml; 2: 11-50 cfu/0.01ml; 3: >50 cfu/0.01ml
Herd-related factor	New inflammation rate	Explained below 0-20%, 20-30%, >30%
Environment-related factor	Season	Spring, summer, autumn, winter

month a CM occurred on the farms. In 2015 this parameter is also issued by DHI analyses in all German federal states and therefore it was used for the investigations in the study. Finally, the seasons, spring (March to May), summer (June to August), autumn (September to November) and winter (December to February) were examined as environment-related factor.

Statistical Analysis:

The data were collected and analysed using Excel, Office 2003 (Microsoft Corporation) and SPSS (IBM SPSS 23.0.0.0, Armonk, USA). The statistical unit was the CM case of an udder quarter. For every CM case BC or no BC (encoded as 1 or 0, respectively) was determined according to the aforementioned definition, constituting the binary dichotomous-dependent variable. With logistic regression procedures [28] the associations between BC and animal-, pathogen-, treatment-, herd- and environment-related factors were analysed. First, associations between explanatory variables and BC were analysed at univariate level using $\chi 2$-analysis, Student´s t test or one-way ANOVA depending on the variable. Variables related to the BC at $P < 0.10$ were included in a reverse, stepwise, binary logistic regression model with BC as the binary outcome using the likelihood ratio as the inclusion/exclusion criteria. As clustering was present in the design (i.e. gland within cow, and cow within herd) a generalised estimating equation (GEE) model was used with those main effects included in the final logistic model. A random cow in herd effect was involved in the model, but had no relevant influence. Statistical significance was assumed at $\alpha = 0.05$. Predictors showing a strong correlation with each other ($r > 0.7$) had to be excluded from the model to avoid multicollinearity. Hosmer-Lemeshow goodness of fit statistical test [29] was used to assess goodness of fit of models. A rescaled pseudo R^2 with a maximum of 1.0 was performed to measure the predictive power of a model. Finally, the odds ratio (OR) was determined with 95% confidence intervals (95% CI). The linear predictor was calculated as

*Logit (BC) = pathogen + shedding + individual sum-200-7 + milk yield + number of CM in current lactation + herd*cow (random).*

Results

In total, 1270 CM cases were recorded. After excluding those cases with no growth in the pre-treatment sample, contamination of the pre-treatment sample or both post-treatment samples and missing samples (354, 94 and 98 cases, respectively), 724 CM cases remained for determining BC. An additional 133 cases were excluded as cows had received no antibiotic treatment. Finally, 591 CM cases were eligible for statistical analyses.

The various antibiotic treatments applied in this study showed no significant difference in BC rate of CM, therefore all antibiotics were used as one group in the data presentation.

The median of lactation number for all CM cases amounted to 3 (minimum 1; maximum 11), of DIM 135 (minimum 1; maximum 851) and of CSCC last DHI before CM onset 453,000 cells/ml (minimum 7,000 cells/ml; maximum 16,053,000 cells/ml). In 298 cases the front quarters and in 293 cases the rear quarters suffered from CM. The results of bacteriological culture are presented in Table 3. The pathogen mostly cultured from pre-treatment sample was *Sc. uberis* (34.7%), followed by *E. coli* (16.9%), CNS (9.3%) and *S. aureus* (7.6%), respectively. 441 CM cases were bacteriologically cured (74.6%) and 150 cases showed no BC (25.4%). Animal-related factors with significant association with BC of CM were individual sum-200-7, milk yield of last DHI before CM onset and the number of CM in current lactation (Table 4). Also, the pathogen cultured in the pre-treatment sample and the shedding rate were significantly associated with BC of CM. With increasing individual sum-200-7 the probability of BC decreased (OR=0.963, 95% CI 0.937-0.991; P=0.01). The likelihood of BC decreased with the increasing milk yield in the last DHI before CM onset (OR=0.965, 95% CI 0.943-0.987; P=0.002). Cows suffering from more than one CM case before CM occurrence showed the lowest BC rate of 50% (OR=0.316,

Table 3: Pathogens cultured from pre-treatment sample

Pathogen	No BC; no. (%)	BC; no. (%)	Total; no.
Enterobacteriaceae	**18 (13.2)**	**118 (86.8)**	**136**
E. coli	10 (10.0)	90 (90.0)	100
Coliforms other than *E. coli* and *Klebsiella* spp.	6 (19.4)	25 (80.6)	31
Klebsiella spp.	2 (40.0)	3 (60.0)	5
Streptococci	**64 (26.3)**	**179 (73.7)**	**243**
Sc. uberis	60 (29.3)	145 (70.7)	205
Sc. dysgalactiae	3 (8.8)	31 (91.2)	34
Other streptococci	1 (25.0)	3 (75.0)	4
Staphylococci	**40 (40.0)**	**60 (60.0)**	**100**
S. aureus	24 (53.3)	21 (46.7)	45
CNS	16 (29.1)	39 (70.9)	55
Other pathogens	**28 (25.0)**	**84 (75.0)**	**112**
Enterococci	4 (14.8)	23 (85.2)	27
Mixed infections	10 (50.0)	10 (50.0)	20
T. pyogenes	7 (46.7)	8 (53.3)	15
Bacillus spp.	1 (6.7)	14 (93.3)	15
Coryneforms	1 (6.3)	15 (93.7)	16
Yeasts	2 (20.0)	8 (80.0)	10
Pseudomonas spp.	1 (16.7)	5 (83.3)	6
Prototheca spp.	2 (66.7)	1 (33.3)	3

95% CI 0.152-0.658; P=0.002) compared with CM cases where no data were available, or in cases having zero CM cases and one CM case (BC rate of 74.5%, 78.7% and 75.6%, respectively, shown in Table 5). CM cases caused by staphylococci, including *S. aureus* with the lowest BC rate (46.7%) and CNS (BC rate of 70.9%), showed a significantly lower

Table 5: Count and percentage of bacteriological cure results by the clinical mastitis cases history in the current lactation

Number of CM in current lactation	No BC; no. (%)	BC; no. (%)	Total; no.
0 CM	54 (21.3)	199 (78.7)	253
1 CM	10 (24.4)	31 (75.6)	41
>1 CM	21 (50.0)	21 (50.0)	42
No information	65 (25.5)	190 (74.5)	255

BC than cases caused by Enterobacteriaceae, streptococci or other pathogens (OR=0.367, 95% CI 0.188-0.716; P=0.003). A significantly higher probability of BC was found for CM cases with a low shedding rate of pathogens pre-treatment than cases with a high shedding rate (OR=2.535, 95% CI 1.234-5.206; P=0.011; Tables 4 and 6). For all other variables no relationship to BC of CM was found (P > 0.05) or they were excluded due to strong correlation (i.e. CSCC variables).

Discussion

The different antibiotic therapies applied in this study did not result in various BC rates of CM cases. Many researchers compared the efficacy of diverse antibiotic ingredients in field trials and also reported no significant differences in BC rates [12, 16, 31, 32]. Reasons could be that all antibiotics used in this study were broad spectrum antibiotics and that the registration of these antibiotics claimed to be effective against common mastitis pathogens in Germany. Therefore, an overall BC rate of 74.6% was reached and this being in accordance with the BC rates determined by Krömker et al. [5], McDougall et al. [16] and Wraight [32]. Thus, the aim of the present study was to determine factors associated with BC of CM cases receiving an effective antibiotic treatment. Animal- and pathogen-related factors with a significant relationship with BC were identified. Several variables calculated from the CSCC values before CM onset were analysed. The individual sum-200-7 showed the highest association with BC. Other researchers

Table 4: Final linear mixed model with variables significantly affecting bacteriological cure (BC)

Variable	Coefficient		OR	95% CI	P-value
	X	SE			
Individual-sum-200-7	-0.037	0.014	0.963	0.937-0.991	0.010
Milk yield	-0.036	0.012	0.965	0.943-0.987	0.002
Number of CM in current lactation					
0 CM	-0.010	0.275	0.990	0.577-1.698	0.971
1 CM	-0.119	0.418	0.888	0.390-2.021	0.777
>1 CM	-1.153	0.373	0.316	0.152-0.658	0.002
No information (reference)	0				
Pathogen cultured pre-treatment					
Enterobacteriaceae	0.672	0.365	1.958	0.956-4.014	0.066
Streptococci	0.025	0.295	1.025	0.574-1.831	0.932
Staphylococci	-1.003	0.340	0.367	0.188-0.716	0.003
Other (reference)	0				
Shedding					
1-10 cfu/0.01 ml	0.930	0.366	2.535	1.234-5.206	0.011
11-50 cfu/0.01 ml	0.253	0.301	1.289	0.713-2.328	0.400
>50 cfu/0.01 ml (reference)	0				

Table 6: Count and percentage of bacteriological cure results by the shedding rate of pathogens cultured prior to treatment

Shedding	No BC; no. (%)	BC; no. (%)	Total; no.
1-10 cfu/0.01 ml	13 (15.3)	72 (84.7)	85
11-50 cfu/0.01 ml	21 (21.0)	79 (79.0)	100
>50 cfu/0.01 ml	116 (28.6)	290 (71.4)	406

also reported a significant relationship between the course of CSCC before onset of CM and the BC [14, 18] and a significantly lower CSCC at the DHI test prior to CM for bacteriologically cured cases than for non-cured cases [15, 19, 20]. The variable individual sum-200-7 considered the threshold 200,000 cells/ml which distinguished between healthy and diseased cows [33]. In addition, the course of CSCC before CM with a higher weighting of CSCC was taken into account which was closer in terms of time to the CM case. Cows having CSCC values above 200,000 cells/ml in DHI tests shortly before occurrence of the CM case received a higher individual sum-200-7. With an increasing individual sum-200-7 a decrease in the probability of BC was shown. If CSCC increased several months before CM onset it could be an indication of a longer duration of infection with a subclinical period and therefore a decreased likelihood of cure could be expected [8, 34, 35].

The milk yield in the last DHI test before CM onset was also significantly associated with BC. The probability of BC decreased with a rising milk yield. An explanation for this could be the flushing effect of higher milk yield on the intramammary applied drugs. Therefore, ingredients reach lower concentrations in the milk compartment of the udder quarter and the antibiotic efficacy decreases. In contrast to our findings, Pinzón-Sánchez and Ruegg [15] reported no significant associations of milk production prior to the case on BC. However, it should be borne in mind that they also included culture-negative cases pre-treatment and used another outcome. Treatment success was defined as the absence of growth of any pathogen in post-treatment samples [15].

Cows that had already suffered from more than one CM case in the current lactation prior to the case being considered, showed a significantly lower probability of BC. This is in accordance with Pinzón-Sánchez and Ruegg [15] who reported a significantly higher BC rate if cows were affected by a CM case for the very first time compared to cows which had already had a CM in the current lactation. Recurrent CM cases may refer to chronic udder infection with subclinical and clinical periods caused by pathogens with characteristics of persisting in the udder quarter (i.e. ability to form a biofilm) or of facilitating new infections due to tissue damage of previous CM cases [36]. Therefore, persisting pathogens and tissue damage in the udder quarter could be reasons for a decreased BC. Due to the influence of mastitis history on BC of CM cases farmers should include information on occurrence and treatment of any CM case in the data of the individual cow.

As pathogen-related factor, species/genus of the pathogen cultured in the pre-treatment sample showed significant associations with BC. The likelihood of BC for CM cases caused by staphylococci in comparison to cases caused by other pathogens was decreased. The staphylococci group contains CNS with a BC of 70.9% (55 cases), which is in the range of other pathogens, and *S. aureus* with a markedly lower BC of 46.7% (45 cases). So the decreased BC is related to *S. aureus* and in accordance with other investigators, which reported a lower probability of BC for CM cases where *S. aureus* was cultured pre-treatment than cases caused by other pathogens [7, 13, 19]. In the case of *S. aureus* the ability to form biofilms, whereby it is more difficult for immune cells and chemical substances to achieve the microorganisms, was described [37, 38], penetrating into epithelial cells of the mammary gland [39, 40] and inhibiting neutrophil bacterial activities [41] which result in negative effects on physiological defence mechanisms of the host and antibiotic efficacy. BC rates varied between the different pathogens and therefore the importance of laboratory examinations or rapid on-farm cultures of quarter foremilk samples was confirmed to estimate the likelihood of BC occurrence.

The amount of pathogen excreted pre-treatment was significantly associated with BC. CM cases with lower shedding of pathogen showed a higher likelihood of BC. In contrast to our results, Swinkels et al. [20] found no significant relationship of shedding rate on BC for CM cases caused by *S. aureus*. However, examinations performed by Dingwell et al. [42] on *S. aureus* infection in the dry period and by Deluyker et al. [43] on subclinical mastitis cases proved a decreased probability of BC with a rising shedding rate. It is well known that the shedding of *S. aureus* is cyclic and therefore this can influence results of different investigations. Possible explanations could be a poor immune defence mechanism of the cow or strong virulence factors of the pathogen which enable the reproduction and continuance of the causative microorganism.

Conclusions

In this study, different factors associated with BC of CM were determined and consideration of the detected variables aid in estimating the likelihood of curing a CM case before antibiotic treatment. CM cases in cows with a high individual sum-200-7 and milk yield in the last DHI test before the onset of mastitis showed a decreased probability of BC. Moreover, a reduced likelihood of BC was found if the cow had suffered from more than one previous CM case in the current lactation. CM cases caused by *S. aureus* were determined as having the lowest BC rate. A high amount of pathogen excreted pre-treatment showed a diminished probability of BC. We recommend consideration of the factors determined in this study for estimation of a reliable prognosis before treatment decision to ensure an effective use of antibiotics.

Acknowledgements

The authors thank dairy owners, farm personnel and veterinarians who participated in this study.

Conflict of interest

The authors declare no conflict of interest.

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